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Contents

Section (i) Botany

Sr.No	Title	Pages
1	**Yoon Myat Noe**, *Comparison of Purified Dna by Using Five Purification Methods From The Leaves of *Capsicum Annuum* L. (Nga-Yoke)	1
2	**Hnin Ei Hlaing**, *By-Products of Peanut Oil Mill for the Production of Thazin Blooms and Various Food	13
3	**Win Naing**, *Effect of Paddy Straw Substrates On the Growth and Yield of *Pleurotus Ostreatus* (Jacq. Ex. Fr.) Kummer (Ngwe-Hnin-Hmo)	23
4	**Moe Sandar Shein**, *Preliminary Study on Some Wild Orchids at Nat Ma Taung Wildlife Sanctuary, Kan Pet Let Township in Southern Chin State	29
5	**Su Su Hlaing**, Investigation on Cultivation of Four Perennial Rice Cultivars in Field Under Mandalay Climate	45
6	**Su Hlaing Htwe**, Taxonomic Study on Ten Wild Mushrooms from Ngalaik Reserved Forest in Naypyitaw Union Territory	57
7	**Theint Thandar Htet**, Mangrove Vegetation Diversity In Two Villages of Kyauktan Township, Yangon Region	67
8	**San Win**, *In Vitro* Seed Germination and Seedling Development of the Orchid *Pholidota Articulata* Lindl.	79
9	**Myint Myint Khaing**, Effects of Hormones and Coconut Water on Clonal Multiplication of Potato (*Solanum Tuberosum* L.)	89
10	**Khin Myo Win**, Distribution of Blue Green Algae From Paddy Field in Bo Taug Kone Village of Patheingyi Township, Mandalay Region	101
11	**Hnin Yu Maw**, A Study on Pollen Morphology of Sapindales and Brassicales From Mandalay and Bago Regions	111
12	**Phyo Wai Khaing**, Effect of Culture Condition on Protease Production of Proteolytic Fungi from Bean Crop Soil and Paddy Mill Soil	121
13	**May Myat Theingi Kyaw**, Isolation of Endophytic Fungi from the Leaves of *Stephanotis Volubilis* (L.F.) S. Ruess, Liede & Meve and the Studies of Antimicrobial Activities & Some Fermentation Parameters	133
14	**Zin Nwe Aye**, Isolation and Identification of Soil Fungi from Patheingyi Township, Mandalay Region	145
15	**Eaindra Chan Myae**, Isolation and Characterization of Endophytic Fungi from *Globba Arracanensis* Kurz.	153

Sr.No	Title	Pages
16	**Wint Yati**, Fermentation Studies of Rhizoctonia Sp. Isolated from the Leaves of *Combretum Indicum* (L.) R. A. Defilipps.	165
17	**Khaing Khaing**, Effect of Different Fertilizers on Germination and Seedling Growth of *Vicia Faba* L. (Fava Bean) in Laboratory Experiment	177
18	**Yee Yee Nwe**, Antimicrobial activity of Endophytes Strain Isolated from Different Parts of *Nypa Fruticans* (Wurmb) Thunb.	187
19	**Kyu Kyu**, Growth and Yield of Chilli as Affected by Organic and Inorganic Liquid Fertilizer Application in Hot Season of Taungoo Township	203
20	**Myo Win**, Effect of Chromolaena Odorata as Organic Fertilizer on *Glycine Max* (L.) Merrill.	215
21	**Thwe Thwe Oo**, Isolation of Endophytic Fungi from *Vitex Trifolia* L. and Characterization of Bioactive Compounds	225
22	**Phyo Phyo Soe San**, Isolation of Endophytic Fungi from The Leaves of *Muntingia Calabura* L. and their Antibacterial Activities	237
23	**Seng Nan**, Taxonomic Study on Fifteen Species from Mansi Township Banmaw District, Kachin State	245
24	**Hnin Yu Hlaing**, Study on the Isolation of Soil Fungi Producing Antimicrobial Metabolite from Tu Myaung Village In Labutta Township	257
25	**Tin Lae Win**, Morphological and Histological Studies of *Artocarpus Lamellosus* Blanco (Leaves)	269

Section (ii) Geology

Sr.No	Title	Pages
1	**Aung Zaw Myint**, *Genesis of Sn-W Mineralization At Mosakhee, Mawchi Sn-W Region, Myanmar: Constraints from Mineralogy and Geochemistry	279
2	**Toe Naing Oo**, Geological Prospecting and Ore Minealization of Epithermal Deposit in The Shwebontha Area, Monywa District, Central Myanmar	287
3	**Ye Zaw**, Petrogenesis and Geochemistry of Granitic Rocks in Zaw Pho Kyin Mine, Dawei Township, Tanintharyi Region	303
4	**Hnin Min Soe**, The Study on Natural Resources Related to the Micro, Small, Medium and Heavy Enterprises Around Kyaukse Area, Kyaukse District, Mandalay Region	313
5	**Min Han Nyein**, Assessment of The Groundwater Quality in Nyaung U Township, Mandalay Region	335

Sr.No	Title	Pages
6	**Aung Myo Zaw**, Review on the Pre-Paleozoic to Early Mesozoic Stratigraphy of Ye-Ngan Township, Southern Shan State, Myanmar	349
7	**Hnin Ei Hlaing**, Comparison of Aquifer Characteristics in the Part of Central Dry Zone, Myanmar	367
8	**Nay Nwe Myo**, Groundwater Quality Analysis of the Phyu Township, Bago Region	375
9	**Me Me Aung**, Petrogenesis of Igneous Rocks Exposed around the Road Section from Washaung to Sadon Village, Waingmaw Township, Kachin State	387

COMPARISON OF PURIFIED DNA BY USING FIVE PURIFICATION METHODS FROM THE LEAVES OF *CAPSICUM ANNUUM* L. (NGA-YOKE)*

Yoon Myat Noe[1], Swe Swe Aye[2]

Abstract

DNA extraction is one of the most important parts of molecular genetics. In this study, the genomic DNA were extracted from the tender leaves of *C. annuum* L by using modified Dellaporta method (Sharma *et al.*, 2000). Then, the quality of extracted genomic DNA was amplified by Polymerase Chain Reaction (PCR) technique and checked by gel electrophoresis and NanoDrop spectrophotometer analysis. The result of NanoDrop spectrophotometer revealed that DNA was yielded 336.7 ng/µL concentration with A_{260}/A_{280} ratio of 1.97 and with A_{260}/A_{230} ratio of 2.26. That isolated DNA has no protein and others impurities contaminations. The gel electrophoresis gave the intact DNA band and PCR product had the expected band (~500 bp). Moreover, the isolated DNA was purified by five different methods. It was revealed that protocol 1 (PEG 8000) obtained 166.32 ng/µL with 1.78 ratio of A_{260}/A_{280} that indicate the good quality of DNA from the tender leaves of *C. annuum* L.

Keywords: DNA, PCR, NanoDrop spectrophotometer, PEG 8000, *Capiscum annuum* L.

Introduction

The extraction of deoxyribonucleic acid (DNA) and purified DNA are the basic steps for many biotechnological techniques, such as molecular markers and genetic engineering. Basically, it is difficult to extract and purify high-quality DNA from certain plants, because of secondary metabolites (tannins, alkaloids, and polyphenols), polysaccharides and proteins. These compounds can interfere DNA, thus degrading its quality and reducing yields. The best quality DNA from mature leaf is problematic, particularly, due to the presence of phenolic compounds and polysaccharides. Mature leaves have higher quantities of polyphenols, tannins, and polysaccharides that can contaminate DNA during isolation. Some researchers have been reported the rapid and reliable procedure for extracting good quality and high quantity of genomic DNA for PCR and molecular analysis (Roomi *et al.,* 2013).

The concentration and quality of extracted genomic DNA can be evaluated by using NanoDrop spectrophotometer, gel electrophoresis and PCR. The quality of DNA concentration and purity is important to downstream processes like sequencing, restriction enzyme digestions, PCR and qPCR (Olson and Morrow, 2012).

PCR technology is based on DNA sequence, to be amplified with the synthetic DNA two chain end complementary two oligonucleotide primers, *in vitro* to be detected DNA sequences (template) were amplified in enzymatic action. The primer can be used rbcL, matK and psbA-trnH depending on the using template (Yu *et al*., 2017). For sequencing, DNA can be purified either from PCR product or from gel slice. The PCR product can be purified using polyethylene glycol (PEG), sodium acetate (NaOAc) and isopropanol, ethanol etc. There are many methods for purification of gel slice by using ethanol precipitation (Oswald, 2007; Michael and Sambrook, 2016), spin filtration (Grey and Brendel, 1992) and home spin column or freeze squeeze methods

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instead of commercial kits. The excised gel should as small as possible to avoid diluting the recovered DNA. The presence of gel after purification can interfere downstream processing (Abraham *et al.,* 2017). All these methods have their advantages and disadvantages. A fast and simple methods result in low yield and poor DNA quality; while methods that result in pure DNA are time-consuming, laborious, and expensive (Kurien and Scofield, 2002).

Recovery of DNA fragments from agarose gel with TAE buffer is one of the most common methods in molecular biology laboratories for downstream applications. If the purification of small fragments of DNA (<100 bp), can use TBE (Tris-Boric acid-EDTA) buffer (Santos *et al.,* 2017). To date, many methods have been published to extract the desired DNA band from the agarose gel. The commercial kits include binding resins such as silica, Silica-based methods are fast, but low yield and high cost, which may interfere in sequencing. Cost is a serious limitation, especially in developing countries (Abraham *et al.,* 2017). Therefore, the present investigation was to establish using inexpensive and un-harmful chemicals, to safe and reduce time-consuming protocol for DNA purification, to obtain good quality of DNA in a relatively purified form which can be used for further investigations.

Materials and Methods

Collection of Samples

Capsicum annuum L. is one of the Capsicum species used for genomic DNA extraction and purification. The samples were collected from the Vegetable and Fruit Research and Development Center, Hlegu Township, Yangon Division.

Extraction of genomic DNA (Sharma *et al.*, 2000)

Apparatus: 1.5 ml Eppendorf tube, Pipette, Mortar and pestle, Vortex, Refrigerated Centrifuge, Microcentrifuge, Beaker, Water bath, Freezer, Refrigerator

Required chemicals: Sodium Dodecyl Sulfate (SDS), Sodium chloride (NaCl), Tris hydroxymethyl aminomethane (Tris), Ethylenediaminetetraacetic acid (EDTA), Potassium acetate (KOAc), Polyvinylpyrrolidone (PVP)

Reagents and solutions

DNA extraction medium: (1 M tris, pH 8.0; 500 mM EDTA, pH 8.0; 5 M NaCl; 10 % SDS), 5 M potassium acetate buffer: pH 4.8, 20% SDS, cold isopropanol, cold 70% ethanol, RNase A, TE buffer (10 mM Tris,1 mM EDTA (pH 8.0)

Procedure

The sample 0.13 g of harvested leaf stored in -20°C was ground firstly with PVP powder and the addition of 600 µL of extraction buffer and the mixture was transferred into 1.5 ml sterilized microcentrifuge tube and vortexed. The tube was vortexed briefly for 5 mins to mix the contents well and added 100 µL of 5 M potassium acetate and 100 µL of 20% SDS. After that vortex about 1 min, the tube was incubated the tube at 65°C for 30 mins. The sample was cooled for 10 mins at room temperature and centrifuged at 15,000 rpm for 30 mins at 4°C. Amount of 200 µL of supernatant was transferred into the new tube and added 180 µL of isopropanol and then inverted the tube. The tube was incubated at room temperature for 10 mins and centrifuged at 15,000 rpm for 60 mins at 20°C to pellet out and was observed at the bottom of the tube. The supernatant was discarded and the pellet was washed with 500 µL of cold 70% ethanol and centrifuged at 15,000

rpm for 10 mins at 20°C. The step 6 was repeated. The pellet was air dried and dissolved in 50-100 µL of TE buffer and 0.5 µL of RNase A was added and incubated for 1hr at 37°C to remove the RNA. Finally, the DNA sample was stored at -20°C.

Purification Protocols

Protocol (1) PCR purification using PEG (Mikheyev, 2009**)**

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Micro centrifuge, Beaker, Water bath, Freezer, Refrigerator

Required chemicals: Polyethylene glycol (PEG) 8000, NaCl

Reagents and solutions: 3 g PEG 8000 and 2.19 g NaCl up to 15 ml with DW and chilled absolute ethanol. Lasts for at least 30 days at 4°C, 80% ethanol, nuclease free water

Procedure

The amount 120 µL of PCR product was taken to the tube and added 1:1 volume of PEG to PCR product. The tube was briefly vortexed the tube. The tube was warmed at 37°C for 15 mins. The tube was centrifuged at 15,000 rpm for 15 mins at 25°C. The supernatant was discarded carefully. The pellet was washed with excess 150 µL of 80% cold ethanol. Then, the tube was centrifuged at 15,000 rpm for 10 mins at 25°C. The supernatant was discarded and washed with 300 µL of 95% cold ethanol. Then, the tube was centrifuged at 15,000 rpm for 10 mins at 25°C. The supernatant was pipetted off and air-dried the pellet about 5-10 mins at room temperature depending on the pellet out. The pellet was dissolved in 30 µL of nuclease free water and incubated at 4°C or -20°C.

Protocol (2) PCR purification using 3M sodium acetate and chilled absolute ethanol

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Micro centrifuge, Beaker, Water bath, Freezer, Refrigerator

Required chemicals: Sodium acetate (NaOAc)

Reagents and solutions: 3 M sodium acetate (NaOAc), pH 4.5, chilled absolute ethanol, 70% ethanol, nuclease free water

Procedure

The tube containing 120 µL of PCR product was added 120 µL of 3M sodium acetate (NaOAc), pH 4.5 and 240 µL of chilled absolute ethanol. The tube was vortexed and then stored it at -20°C for 30 mins. The tube was centrifuged at 14,000 rpm for 30 mins at 25°C. The supernatant was removed carefully and don't disturb the pellet. The pellet was washed with 300 µL of 70% ethanol and centrifuged at 14,000 rpm for 5 mins at 25°C. The supernatant was discarded and then air-dried the pellet at room temperature. Re-suspend the pellet in 30 µL in nuclease free water and incubated at 4°C or -20°C.

Protocol (3) PCR purification by using Combination of PEG and sodium acetate (Lis, 1980)

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Micro centrifuge, Beaker, Water bath, Freezer, Refrigerator, Ice pack

Required chemicals: Polyethylene glycol (PEG) 8000, NaCl, Sodium acetate (NaOAc)

Reagents and solution: 5.0 M NaCl, 22% PEG 8000, 0.3 M sodium acetate (NaOAc)95% ethanol, 70%, ethanol, nuclease free water

Procedure

After running PCR, the 120 µL of PCR product was precipitated with 1:3 volume of 95% ethanol. The tube was incubated on ice for 10 mins and centrifuged at 15,000 rpm for 15 mins at 20°C. The supernatant was discarded and air-dried the pellet. The precipitated fragment was dissolved in 32 µL of nuclease free water. The tube was added 8 µL of 5.0 M NaCl and 40 µL of 22% PEG 8000 and mixed the tube (11% PEG will precipitate all DNA fragments larger than 180 bp). The tube was incubated on ice at least 30 mins and then centrifuged at 4°C for 5 to 10 mins. The supernatant was discarded the supernatant. The pellet was dissolved in 20 µL 0.3 M NaOAc, and added 2.5 volumes of 95% ethanol and mixed, on ice for about 15 mins, then centrifuged the tube at 15,000 rpm for 15 mins at 4°C. Carefully the supernatant was aspirated and discarded. The pellet was rinsed with 300 µL of 70% ethanol and centrifuged at 15,000 rpm for 5 mins at 4°C. The supernatant was removed and then, air-dried the pellet for 3 mins at room temperature. The pellet was dissolved in 20 µL of nuclease free water, and stored at 4°C or -20°C.

Protocol (4) Gel purification using home spin column (Abraham *et. al.*, 2017)

Apparatus: Syringe needle, Cotton, 1.5 mL Eppendorf tube, 2 mL Eppendorf tube Microwave oven, Microcentrifuge, Omni Doc Gel Documentation System**,** Centrifuge, Beaker, Tweezer

Reagents: 0.5X TAE buffer

Procedure

To purify the selected DNA bands from agarose gels, a 1.5 mL Eppendorf tube punctured in the center bottom with a syringe needle and packed with a small tassel of cotton was used. The cotton was embedded with 0.5X TAE buffer and squeezed until no liquid came out. The tube was placed into another 2 mL Eppendorf tube. The piece of DNA-containing gel was laid on the cotton filter in the tube and then centrifuged at 5,000 rpm for 7 min at room temperature. The DNA in the collected liquid was stored at 4°C or -20°C.

Protocol (5) Gel purification using GeneJet Gel Extraction Kit (Thermo Fisher Scientific)

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Microcentrifuge, Beaker**,** Water bath, Freezer, Refrigerator, Microwave oven, Surgical blade, Omni Doc Gel Documentation System, Tweezer

Required chemicals: Agarose gel

Reagents and solution: 1X TAE buffer, SYBR Safe DNA gel stain, Binding buffer, Wash buffer, Elution buffer, Isopropanol

Procedure

After running gel electrophoresis, the gel containing the DNA fragment was excised using a clean scalpel or razor blade. Cutting was made as close to the DNA as possible to minimize the gel volume. The gel slice was placed into a pre-weighed 1.5 mL tube and weighed again. The weight of the gel slice was recorded. The ratio 1:1 volume of Binding Buffer was added to the gel slice (volume: weight) (e.g., add 100 µL of Binding Buffer for every 100 mg of agarose gel). The gel mixture was incubated at 50-60°C for 10 min or until the gel slice was completely dissolved.

The tube was mixed by inversion every few minutes to facilitate the melting process to ensure the gel was completely dissolved. The gel mixture was vortex briefly before loading on the column. The solubilized gel solution was transferred up to 800 µL to the GeneJET purification column. The column was centrifuged at 12,000 rpm for 1 min at 25°C. Discarded the flow-through and placed the column back into the same collection tube. The column was added 100 µL of Binding Buffer. The column was centrifuged at 12,000 rpm for 1 min at 25°C. Discarded the flow-through and placed the column back into the same collection tube. The column was added 700 µL of Wash Buffer. The column was centrifuged at 12,000 rpm for 1 min at 25°C. Discarded the flow-through and placed the column back into the same collection tube. The empty GeneJET purification column was centrifuged for an additional 1 min to completely remove residual wash buffer. The GeneJET purification column was transferred into a clean 1.5 mL microcentrifuge tube. The column was added 30 µL of Elution Buffer to the center of the purification column membrane. The column was centrifuged at 12,000 rpm for 1 min at 25°C. and stored the purified DNA at -20 °C.

Analysis of DNA using NanoDrop spectrophotometer

The amount of 1µL of DNA sample from extraction was used to measure the nucleic acid concentration (Quantity) and DNA quality by means of measuring the absorbance ratio of A_{260}/A_{280} for protein contamination and that of A_{260}/A_{230} for other impurities involved in the sample. For DNA, the range of A_{260}/A_{280} must be between 1.7- 2.0 and that of A_{260}/A_{230} must be greater than 2.

Preparation for Polymerase Chain Reaction (PCR)

EmeraldAmp® Max PCR Master Mix was used to isolate part of the psbA-trnH gene (~400 bp) for amplification. The sample of DNA from extraction was diluted to 5 ng/ µL. The PCR buffer was prepared by adding 9.5 µL of dH2O, 1 µL of each forward primer (psbA-F(5' to 3'): GTTATGCATGAACGTAATGCTC) and reverse primer (trnH-R (3' to 5'): CGCGCATGGTGGATTCACAATCC) and 12.5 µL of enzyme mixture. The amount of 24 µL of this PCR buffer was needed for 1 µL of DNA Template for PCR amplification. As a PCR thermal cycler, a TechneTM PCRmax Alpha Cycler 1 PCR Machine (PCR max, UK) was used. PCR condition must be at 95°C for 5 minutes for initial temperature, at 95°C for 30s for denaturing, at 55°C for 30s for annealing Temperature, at 72°C for 40s for extension, at 72°C for 10 minutes for final extension and for hold (10°C for infinity). The reaction was repeatedly cycled for 35 times. The psbA (forward primer) and trnH (reversed primer) were intergenic spacer regions and also universal primers (~400bp). These primers possess conserved flanking sites, short sequences and discriminate between species. So, it was suitable for running of PCR. (Kress and Erickson, 2012)

Preparation of TAE Buffer for Gel Electrophoresis

Firstly, a 50X concentration of TAE stock solution was made. For this, 242 g of Tris was dissolved in 500 mL of distilled water. The amount of 18.61 g of EDTA (pH- 8) was weighed and dissolved in 100 mL of distilled water and stirred with magnetic stirrer and then added some NaOH up to pH- 8. These two solutions were combined and 57.1 mL of acetic acid was added to it. Then distilled water was added to bring the final volume to 1 L.

Preparation for Gel electrophoresis

Amount 0.17 g of agarose gel (1% agarose) for a small casting tray was weighed and put in a 200 mL beaker and then 17 mL of TAE buffer was added and then the dissolved in the microwave for about 5 min until the agarose powder completely dissolved. After that, 0.6 µL of

SYBR Safe DNA gel stain (Thermo Fisher Scientific, USA) was added and slowly shook the beaker. Then the gel solution was poured into the gel tray and the comb was put and then waited for about 30 min. The comb was then removed and the prepared gel tray was placed on the gel bed of the migration tank containing the 1X TAE buffer. For genomic DNA, 2.5 µL of DNA sample with 1 µL of loading dye was loaded into the wells for protocols. For PCR products, each 2.5 µL of PCR products was loaded into the well of an agarose gel containing SYBR stain. Then, the 100bp marker (Thermo Fisher Scientific, USA) was placed into the well for comparing with the size of the samples and run for 15 min at 100 voltages. And then, the gel was placed in the Omni Doc Gel Documentation System which is already connected with the computer. The exposure was adjusted to 3 and switched to UV light then took the photo of the gel.

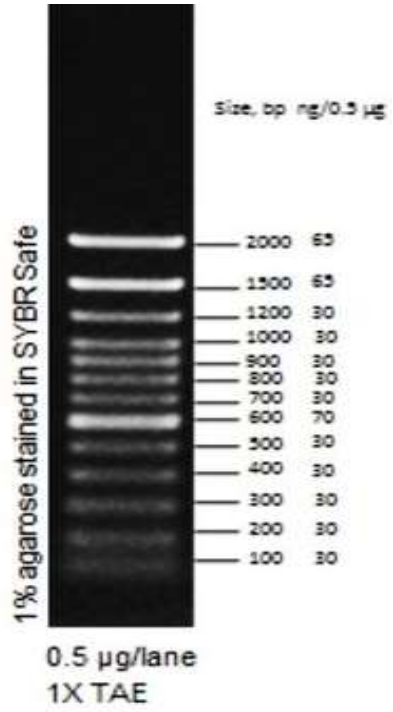

Figure 1.100 bp ladder (Invitrogen, Thermo Fisher Scientific)

Results

The species *Capsicum annuum* (cv) was collected from the Vegetable and Fruit Research and Development Center, Hlegu Township, Yangon Division during the flowering period of June - August, 2019.

Outstanding Characters of *C. annuum* (cv)

Annual or perennial herbs; pubescence of simple hairs. Stems branched. Leaves solitary or paired, petiolate; leaf blade simple, entire or sinuate. Inflorescences solitary; peduncle absent. Flowers are nodding or erect, actinomorphic. Pedicle erect or nodding. Calyx broadly campanulate to cup-shaped, denticulate, sometimes slightly enlarged. Corolla white, campanulate or rotate, divided halfway or more. Stamens are inserted near the distal end of the corolla tube; filaments are slender; anthers are yellow or purplish, ovoid, and dehisce longitudinally. Ovary 2- (-3)- locular; ovules numerous. Style slender; stigma small, capitate. Fruit is a moist berry, sometimes large, erect, nodding or reflexed. Seeds are yellowish, discoid; embryo coiled, subperipheral.

Figure 2. Habit of Capsicum annuum (cv)

Genomic DNA

i. Quality and Quantity Assessment Using NanoDrop Spectrophotometer

The genomic DNA of tender leaves of *C. annuum* L. was extracted by using five different methods in plant analytical laboratory. The quality and quantity assessment of extracted DNA was evaluated by the NanoDrop spectrophotometer, gel electrophoresis and PCR. According to the result of the NanoDrop spectrophotometer, the genomic DNA was yielded a 336.7 ng/µL concentration with A_{260}/A_{280} ratio of 1.97 and with A_{260}/A_{230} ratio of 2.26. The DNA has no protein but a little other impurity contamination.

ii. Quality and Quantity Assessment Using Gel Electrophoresis and PCR

According to the results of gel electrophoresis, the whole genomic DNA of *Capsicum* sp. might be between 3-3.5 Gb. The result showed that the genomic DNA was free from protein contamination and a little degraded. The Gel electrophoresis result were shown in Figure 3.

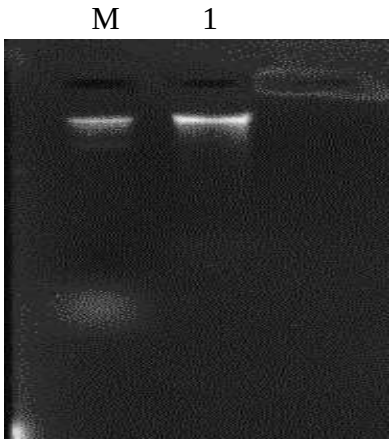

Figure 3. Checking Genomic DNA Quantity by using Gel Electrophoresis. M (λ DNA/Hind III Fragments), Lane 1. DNA sample was loaded 2.5 µL per lane.

In addition, the result of PCR product demonstrated that the DNA concentration diluted to 50 ng/µL had expected band (~500 bp) were shown in Figure 4. The result of PCR product by NanoDrop spectrophotometer was 1154.43 ng/µL concentration with A 260/280 ratio of 1.88 and with A_{260}/A_{230} ratio 1.93.

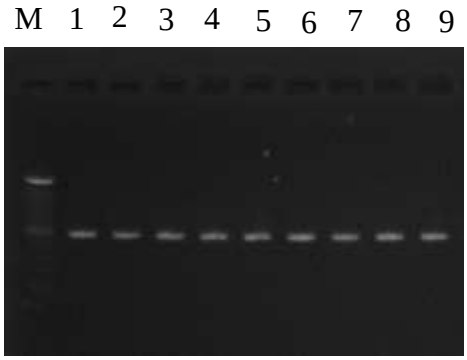

Figure 4. Checking of PCR products of genomic DNA quantity by using PCR. M (100bp ladder), Lane 1 to 9. DNA sample was loaded 5 ng per lane

Purified DNA

i. Quality and Quantity Assessment Using NanoDrop Spectrophotometer

The purified extracted DNA can be used for various types of molecular analysis such as sequencing, electrophoresis, PCR and molecular cloning. At first, the quality, purity and concentration of purified DNA must be taken by using NanoDrop spectrophotometer which measure A_{260}/A_{280} and A_{260}/A_{230}. The A_{260}/A_{280} ratio is used as an indicator of DNA purity. Weising *et al.*, 2005 stated that A_{260}/A_{280} ratio should be between 1.7 and 2.0 that means free from ethanol and other chemicals contamination (DNA purification, Promega corporation). According to the NanoDrop spectrophotometer, the purified DNA obtained by protocol 1 was 166.32 ng/µL with 1.78 the ratio of A_{260}/A_{280} and 2.33 ratio of A_{260}/A_{230}. This protocol was free from ethanol and other chemicals contamination. Protocol 2 gave 198.14 ng/µL with the ratio of A_{260}/A_{280} was 1.65 but the ratio of A_{260}/A_{230} was 1.25 of the purified DNA. The protocol 2 was a little contamination. Purified DNA obtained from protocol 3 was 126.43 ng/µL with 1.72 of the ratio of A_{260}/A_{280} and 1.51 ratio of A_{260}/A_{230} protocol 3 was better than protocol 2 and protocol 4 because of free from chemicals contamination. DNA from protocol 4 gave 37.98 ng/µL with 1.45 ratio of A_{260}/A_{280} that means many contamination and 0.5 ratio of A_{260}/A_{230}. Purified DNA yielded from protocol 5 gave 46.91 ng/µL with 1.93 ratio of A_{260}/A_{280} and 0.37 ratio of A_{260}/A_{230}. This protocol was free from contamination. The NanoDrop result of all protocols were as shown in Table 1.

Table 1. Purified DNA Concentration and Quality Using NanoDrop Spectrophotometer

Protocol	DNA Concentration (ng/µL)	Indication Ratio for Protein Contamination (A_{260}/A_{280})	Indication Ratio for Impurities Contamination (A_{260}/A_{230})
1	166.32	1.78	2.33
2	198.14	1.65	1.25
3	126.43	1.72	1.51
4	37.98	1.45	0.5
5	46.91	1.93	0.37

(All DNA concentration, ratios for protein contamination and impurities contamination were average values.)

ii. Quality and Quantity Assessment Using Gel Electrophoresis and PCR

The results of protocol 1, 2, 3 and 5 demonstrated that the purified DNA by using gel electrophoresis was free from chemical contamination. The DNA of protocol 4 was contaminated with other impurities. And the protocol 1 gave intact DNA band. The result of protocols was shown in Figure 5.

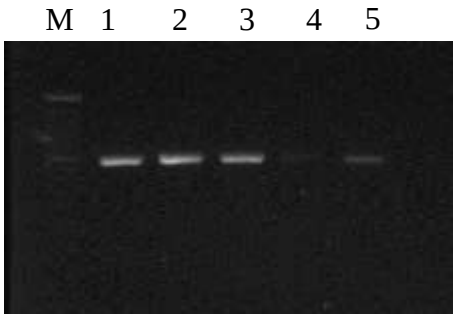

Figure 5. Checking the purified DNA Quantity by using Gel electrophoresis. M (100 bp ladder), Lane 1 (P1), Lane 2 (P2), Lane 3 (P3), Lane 4 (P4) and Lane 5 (P5). DNA sample was loaded 2.5 µL per lane

In addition, the result of PCR product revealed that 5 ng/µL dilution of all protocols had expected band (~500 bp) were shown in Figure 6.

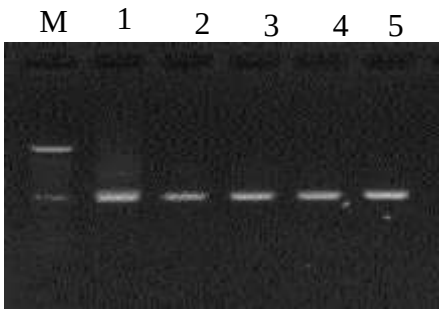

Figure 6. Checking the PCR product of purified DNA quantity by using PCR. M (100 bp ladder), Lane 1 (P1), Lane 2 (P2), Lane 3 (P3), Lane 4 (P4) and Lane 5 (P5). DNA sample was loaded 5 ng per lane.

The estimate cost on purification processes of *C. annuum* (cv) was shown in Table 2.

Table 2. Comparison of yield and quality of DNA recovered with different methods

Protocol	PEG	NaOAc	PEG+ NaOAc	Home spin column	GeneJet Gel extraction kit
Time for purification (minutes)	40	70	105	7	15
Total DNA amount (ng/µL)	1154.43	1154.43	1154.43	1154.43	1154.43
Final DNA concentration obtained (ng/µL)	166.32	198.14	126.43	37.98	46.91
Volume obtained (µL)	30	30	30	99	30
Total DNA after purification (ng)	4989.6	5944.2	3792.9	1253.34	1407.3

Protocol	PEG	NaOAc	PEG+ NaOAc	Home spin column	GeneJet Gel extraction kit
PCR results	Good	Good	Good	Good	Good
Cost per sample (MMK)	25	25	23	21	2400

Discussion and Conclusion

This research was conducted with the objectives to develop a simple and rapid method to purified the extracted DNA from leaves of *C. annuum* (cv). It was also intended to provide information for the best DNA purification protocol from plants with inexpensive, safe and reducing time consuming. The quality of extracted DNA sample was verified spectrophotometrically using a NanoDrop instrument, gel electrophoresis and amplified by PCR. The NanoDrop absorbance profile was useful for detection of contaminants such as protein, salts and polysaccharides, which can inhabit and interfere in DNA purification. The A_{260}/A_{280} nm ratio of 1.97 and the A_{260}/A_{230} nm of 2.26 indicated that extracted DNA had high purity with absence of proteins and phenols. According to the Sharma *et al.*, 2000; Dehestani and Kazemi. 2007; Doyle and Doyle, 1987, the A_{260}/A_{280} nm ratio varied between 1.7 and 1.9, 1.69-1.91, 1.76-1.93 respectively. The PCR product demonstrated that the DNA had the expected band (~500 bp). Lavanya and Arun., 2019 reported that the DNA of *Capsicum* sp. had the expected band was ~530 bp.

The protocol 1, polyethylene glycol (PEG) 8000 method produced the purified DNA with the yield of 141.2 to 186.2 ng/µL A_{260}/A_{280} ratio in this study was found to be in a range of 1.77 to 1.79 whereas the protocol 2 (NaOAc), the protocol 3 (PEG+NaOAc), the protocol 4 (home-spin method) gave the DNA with the yield of 37.98 to 198.14 ng/µL, the ratio obtained varied from 1.45 to 1.72 respectively.

Weising *et al.*, 2005, Lis, 1980 and Mikheyev, 2009 reported that the absorbance ratio obtained varied from 1.6 to 1.8 indicating that the isolated DNA was free from contamination. The DNA yielded from protocol 5 (GeneJET Gel Extraction Kit method) was 46.91 ng/µL with 1.93 ratio of A_{260}/A_{280}. The advantages of this method was less time consuming. The gel electrophoresis results of protocol 1,2 ,3 and 5 gave the intact DNA and the PCR results of all protocols had the expected band (~500 bp). According to the Abraham *et. al.*, 2017 that the expected PCR product was 312 bp in microorganism of banana leaf. The DNA extracted with optimized protocol presented a reduced degradation and an excellent overall quality. The developed procedure was fast and reproducible.

In conclusion, DNA extraction method isolate high quality of DNA. The result showed that DNA extraction using modified boiling, centrifugation time as well as percent of chemical used was more effective. From the result of purification of DNA, it can be concluded that the GeneJET Gel Extraction Kit using procedure, which was easy and rapid, could be applied for the isolation of analytical quality DNA from *C. annuum* (cv). Also the protocol 1 used PEG 8000 for DNA purification gave better yield of large DNA fragment, safe, inexpensive, no harmful chemicals. Thus, PEG method can be used instead of commercial kit as the kits are expensive for developing countries. Therefore, the PEG method can be also recommended for the isolation of analytical quality DNA from *C. annuum* L.

Acknowledgements

I would like to express my deep and sincere gratitude to Dr. Thida Oo, Professor and Head of Botany Department, University of Yangon, for his kind permission, warm encouragement and kind supports for all the needs for research. My grateful thanks and appreciation go to Dr. Thet Thet Mar Win, Professors of Botany Department, University of Yangon, and Dr. Swe Swe Aye, Associate Professor, Taungoo University for their careful suggestions and kind encouragement. Also giving me the chance to explore a new knowledge of mine as well as for giving me precious advices. I have no valuable words to express my thanks, but my heart is still full of the favours received from every person.

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BY-PRODUCTS OF PEANUT OIL MILL FOR THE PRODUCTION OF THAZIN BLOOMS AND VARIOUS FOOD*

Hnin Ei Hlaing[1]

Abstract

Nowadays, the prices of peanut oil and other food for human consumptions are increasing gradually. So, to fill the needs, a survey has been made to Tun Tauk Naing Co., Ltd., Ngwe Thazin Min Peanut Oil Mill situated at Industrial Zone (1), Hlaing Thar Yar Industrial Zone, Yangon Region by using questionnaires method. Questionnaires concerning with oil seed collection, storage and drying were made to the manager and the responsible staff. Peanut oil processing had observed and recorded by using the photographs. There are many by-products such as peanut hull, peanut skin and peanut cake are produced from peanut oil processing. In this research, the application of major by-product peanut cake can be used in many ways: such as biofertilizers for the germination of Thazin bulbs to bloom; as human consumption for crispy peanut cake, peanut cake jam and sour peanut cake; and also as animals feed for ruminant, fish and prawn. Further research can be carried out by using by-product of peanut cake in preparation of various confectionaries. In this way, various edible products production can be enhanced by using by-products from peanut oil mills. Finally, the transformation of by-products from peanut oil mill can be produced as bio-fertilizer for plants, as various confectionaries for human and also as food for ruminants and aquatic animals.

Keywords: by-product; peanut oil; Thazin; peanut cake.

Introduction

In 21st century, people are facing many waste issues in their surroundings. The waste issues also come out from agriculture products such as rice, wheat, corn, sesame seeds and peanut which are abundantly produced in Myanmar. One of the issues was found out during the survey of Ngwe Thazin Min Oil Mill, Tun Tauk Naing Co. Ltd. in Hlaing Thar Yar Township, Yangon Region. After interviewing, taking photograph and surveying of that oil mill, that research has been undertaken. The by-product of peanut oil is peanut hull; peanut skin and peanut cakes that are produced from peanut oil processing. Peanut hull and peanut skin can be used as burning fuel instead of charcoal and wood for cooking. Peanut cake can be used in biofertilizer as well as flourished for angiosperms and edible plants; many ways if human consumption such as fermented peanut cake, peanut flour, peanut butter, peanut cookies, peanut ice-cream, peanut waffle, etc., and animal feed. This research can be carried out by using by-product of peanut cake in preparation of various confectionaries. In this way, various edible products production can be enhanced by using by-product from peanut oil mill. All the literature survey was reviewed from the internet source and also shown in references.

Materials and methods

Facts concerning with the by-product of peanut oil (peanut cake) were collected from Tun Tauk Naing Co., Ltd., Ngwe Thazin Min Peanut Oil Mill. Questionnaires and interviewing to the manager and the responsible staff of Tun Tauk Naing Co., Ltd., Ngwe Thazin Min Oil Mill were made. Yes or No Question type with Recording and Surveying in the peanut oil mill by taking photographs had been undertaken.

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* Second Prize (2023)

[1] Department of Botany, University of Yangon

Plantation of Thazin by using peanut cake biofertilizer

According to the interviewing survey, there are 85 poles of Thazin in the area of 30x70 square feet. A ready-made 7 feet long pole covered with coconut fibers from mesocarp and tied around with coconut ropes having 5 feet long has been selected for plantation of Thazin bulbs. Each pole has planted with 50 ticals to 75 ticals of Thazin bulbs. One viss of Thazin bulb price is 1 lakhs or 80,000 Myanmar kyats during these days. One spray of Thazin bloom cost 40,000 Myanmar kyats nowadays. After selling of Thazin sprays or blooming period of Thazin, new Thazin bulbs can be planted on a new pole. The peanut cakes are placed in the netted container and hang on the pole as a biofertilizer, after planting the bulbs on the pole. When the bulbs are sprayed with water or seasonal rain can wash down that peanut cakes biofertilizer onto the growing bulbs. In this way, the Thazin bulbs are growing very well. Finally, the elegant sprays of Thazin are blooming again in the next season.

Preparation of Three Types of Food from Peanut Cake

According to laboratory scale, three types of food from peanut cake were made; the paste of peanut cake was prepared from 0.5 L of water and 150 g peanut cake heated on the oven during 20 minutes. After that, three types of food from peanut cake were made by the following procedures: -

(1) Crispy peanut cake

Materials, Apparatus and Procedures

Sticky rice powder; peanut cake paste; salt; Shwe-leik-pyar frying powder**;** peanut oil; balance; stove. 50 g Sticky Rice Powder, 50 g of Peanut cake paste, one tea spoon of salt, one tea spoon of Shwe-leik-pyar frying powder were mixed properly. Then, made a ball and fried it with peanut oil on the stove at a temperature of 150ºC for 5 minutes. When the crispy peanut cakes are ready, pick up from the oil and cool on sieve rack and cool down. Now, it is ready to eat.

(2) Peanut cake jam

Materials, Apparatus and Procedures

Sugar; Peanut cake paste; balance; stove. 50 g of Sugar and 50 g of Peanut cake paste were mixed properly. Then, stirred it and heated on the stove at a temperature of 100ºC for 20 minutes. Cool down and use as peanut cake jam.

(3) Sour peanut cake salad

Materials, Apparatus and Procedures

Peanut cake; peanut oil; onions; chilies; salt and sugar**;** balance; refrigerator. 50 g of Peanut cake was soaked in 1 liter of water for two days or more to get the sour taste. When the peanut cake paste changed into sour taste, put it in the dried bottle or airtight container and kept in the refrigerator for long term use. Then, 25 g of sour peanut cake paste, five tea spoons of peanut oil, three sliced onions, five cut chilies, salt and sugar were mixed together and can get the salad of sour peanut cake.

Laboratory Analysis Report of moisture, protein and crude fat of peanut cake

The laboratory Analysis Report of moisture, protein and crude fat tests of peanut cake for year 2022 and 2023 from Myanmar Food Processors and Exporters Association (MFPEA) Food Industries Development Supporting Laboratory (FIDSL), UMFCCI was also recorded by taking photographs.

Results

After interviewing from Ngwe Thazin Min Peanut Oil Mill, taking the photographs of the processing of peanut oil mill (Fig. 1-10), the results of by-products of the peanut oil, biofertilizer for good Thazin plantation, by making the products in laboratory scale, three types of food from peanut cake were carried out and the tests of moisture, protein and fat were recorded**.**

Figure. 1. Different Advertisement and Logo of Ngwe Thazin Min Oil Mill

Figure. 2. Systematically Storing of peanut

Figure. 3. Storage for 3 months peanut

Figure. 4. Storage for 6 months peanut

Figure. 5. Measuring tools for moisture

Figure. 6. Testing tool for

Figure. 7. Machines used for the production of peanut oil

Figure. 8. Standard Packaging Room for Peanut Oil

Figure. 9. Different types of Peanut Oil Storing in the showcase for shelf life

Figure. 10. Packaging of Peanut Cake

By-products of the peanut oil

The by-products of peanut oil are peanut hull; peanut skin and peanut meal or peanut cakes are produced from peanut oil processing were recorded by photograph (Fig. 11).

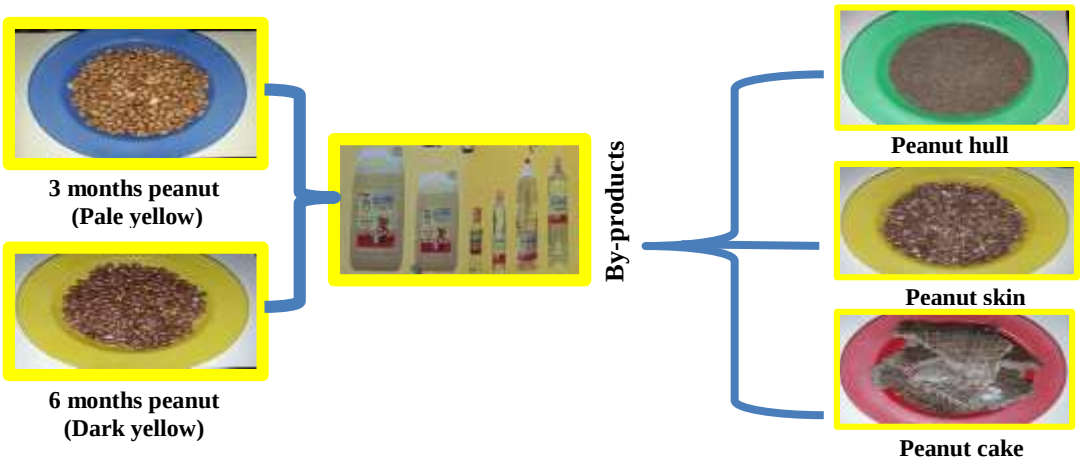


Figure. 11. Flow Diagram of the Peanut to by-products of the Peanut Oil

Biofertilizer for Thazin plantation

According to use interviewing and taking photomicrographs, peanut cake can be used as biofertilizer for good Thazin plantation (Fig. 12-16).

Figure. 12. Thazin bulbs for plantation

Figure. 13. Plantation of Thazin with the Peanut cake fertilizer

Figure. 14. New Thazin bulb with leaves

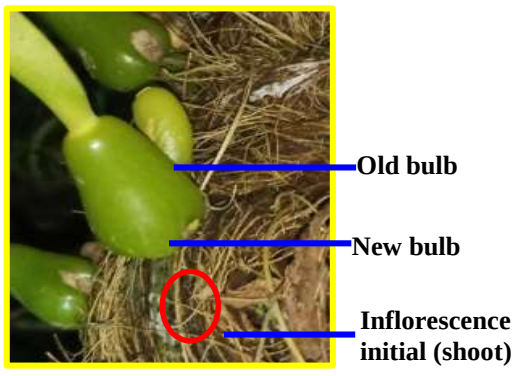


Figure. 15. New Thazin bulb with leave and shoot

Figure. 16. Transformation Stages of young Thazin spray to elegant Thazin spray is blooming on the bulb

Three Types of Food from Peanut Cake

The procedure was described in materials and methods. The photographs of three types of food from peanut meal or peanut cake were shown in (Fig. 20-23).

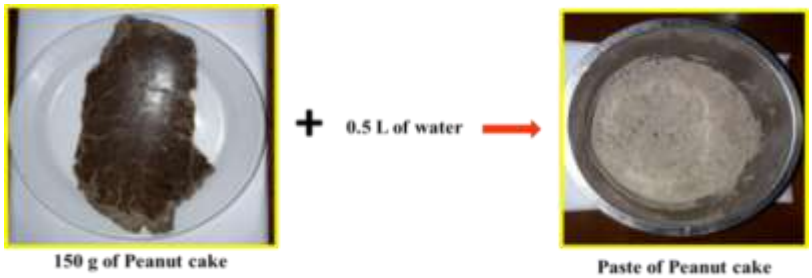


Figure. 20. Preparation of Peanut cake to paste of Peanut cake

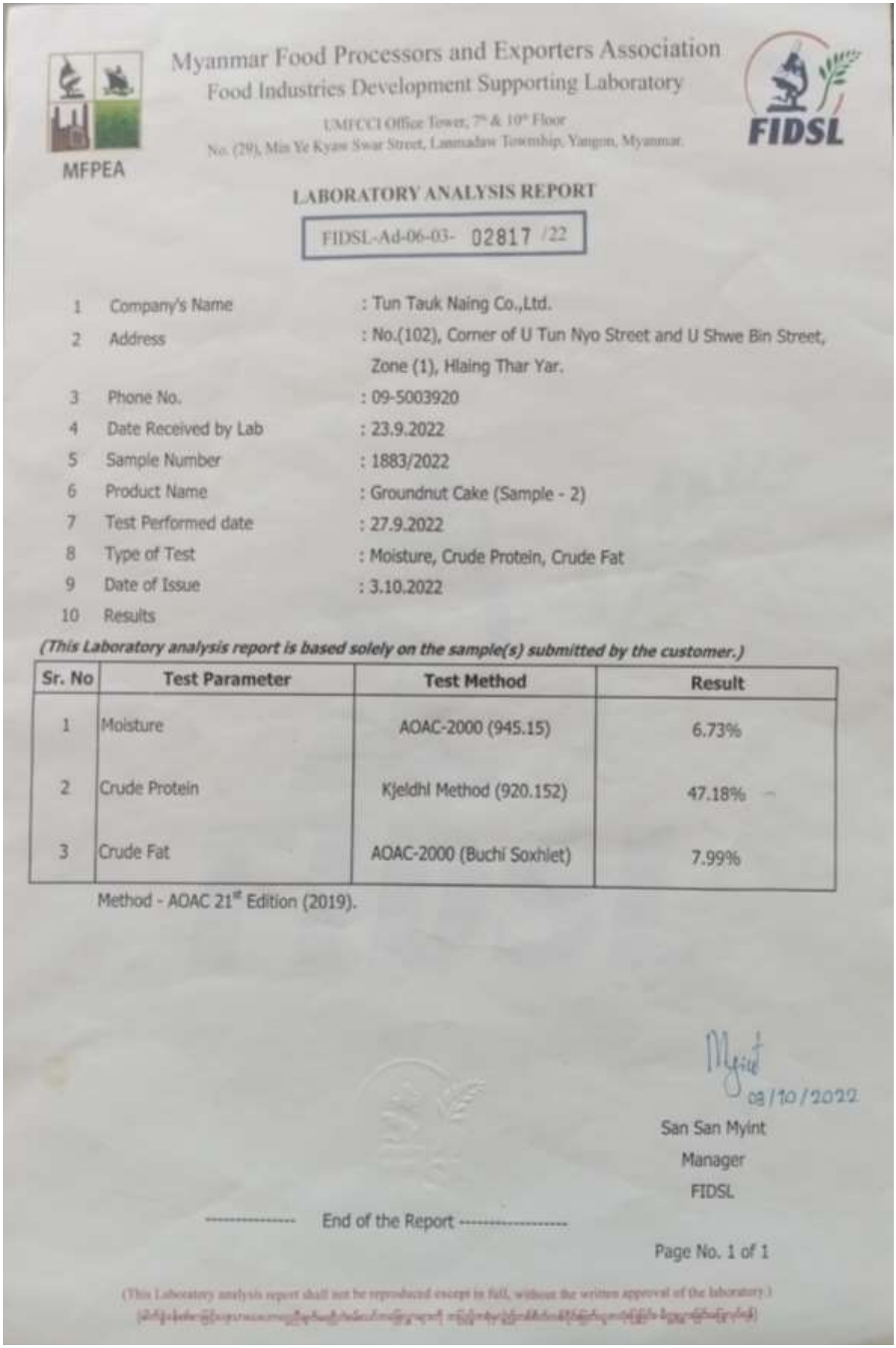
Figure. 21. Preparation of the paste of Peanut cake to Peanut cake Crispy

Figure. 22. Preparation of the paste of Peanut cake to Peanut cake Jam

Figure. 23. Preparation of the paste of Peanut cake to Salad of sour Peanut cake

Tests of Moisture, Protein and Fat

The results of moisture content, protein test and crude fat with ether extract were recorded and compared from 2022 to 2023. In the year 2022, the result of moisture content is 6.73% and 7.75% in May and 9.73% in September of the year 2023; the results of protein test are 47.18% in the year 2022 and 50.24% in May and 47.99% in September of the year 2023; the results of crude fat with ether extract are 7.99% in the year 2022 and 6.47% in May and 6.63% in September of the year 2023 (Fig. 17-19). The by-products from peanut oil mill, peanut cake had been used for animal feed such as ruminant, fish and prawns. As such, tests of moisture, protein and fat are vitally important to support the nutritional values of these animals.



Myanmar Food Processors and Exporters Association
Food Industries Development Supporting Laboratory
UMFCCI Office Tower, 7th & 10th Floor
No. (29), Min Ye Kyaw Swar Street, Lanmadaw Township, Yangon, Myanmar.

MFPEA

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-03- 02817 /22

1	Company's Name	: Tun Tauk Naing Co.,Ltd.
2	Address	: No.(102), Corner of U Tun Nyo Street and U Shwe Bin Street, Zone (1), Hlaing Thar Yar.
3	Phone No.	: 09-5003920
4	Date Received by Lab	: 23.9.2022
5	Sample Number	: 1883/2022
6	Product Name	: Groundnut Cake (Sample - 2)
7	Test Performed date	: 27.9.2022
8	Type of Test	: Moisture, Crude Protein, Crude Fat
9	Date of Issue	: 3.10.2022
10	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC-2000 (945.15)	6.73%
2	Crude Protein	Kjeldhl Method (920.152)	47.18%
3	Crude Fat	AOAC-2000 (Buchi Soxhlet)	7.99%

Method - AOAC 21st Edition (2019).

03/10/2022

San San Myint
Manager
FIDSL

---------- End of the Report ----------

Page No. 1 of 1

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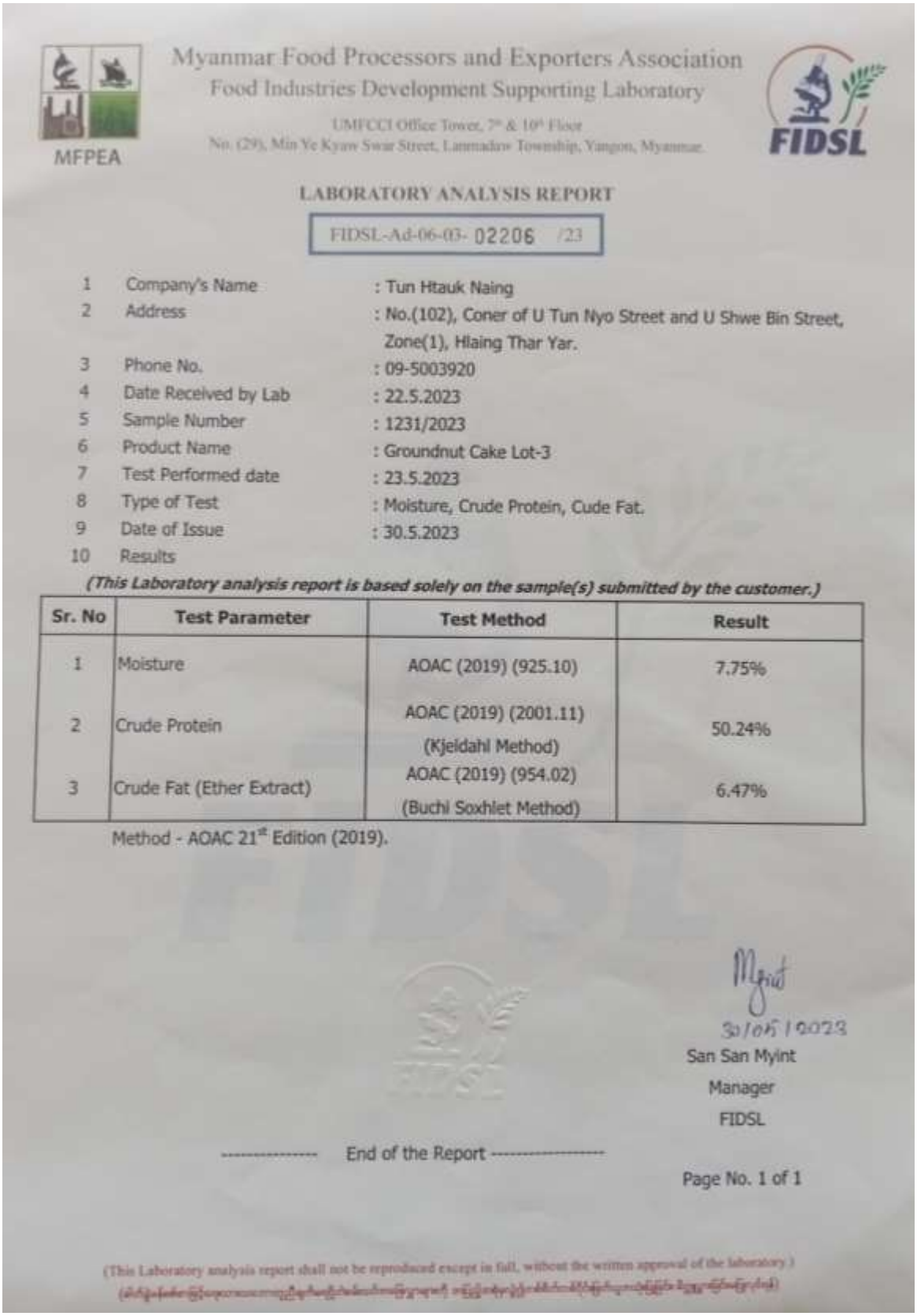
Figure. 17. Result of Protein test for 2022



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MFPEA

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-03- 02206 /23

1	Company's Name	: Tun Htauk Naing
2	Address	: No.(102), Coner of U Tun Nyo Street and U Shwe Bin Street, Zone(1), Hlaing Thar Yar.
3	Phone No.	: 09-5003920
4	Date Received by Lab	: 22.5.2023
5	Sample Number	: 1231/2023
6	Product Name	: Groundnut Cake Lot-3
7	Test Performed date	: 23.5.2023
8	Type of Test	: Moisture, Crude Protein, Cude Fat.
9	Date of Issue	: 30.5.2023
10	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr. No	Test Parameter	Test Method	Result
1	Moisture	AOAC (2019) (925.10)	7.75%
2	Crude Protein	AOAC (2019) (2001.11) (Kjeldahl Method)	50.24%
3	Crude Fat (Ether Extract)	AOAC (2019) (954.02) (Buchi Soxhlet Method)	6.47%

Method - AOAC 21st Edition (2019).

30/05/2023
San San Mylnt
Manager
FIDSL

---------- End of the Report ----------

Page No. 1 of 1

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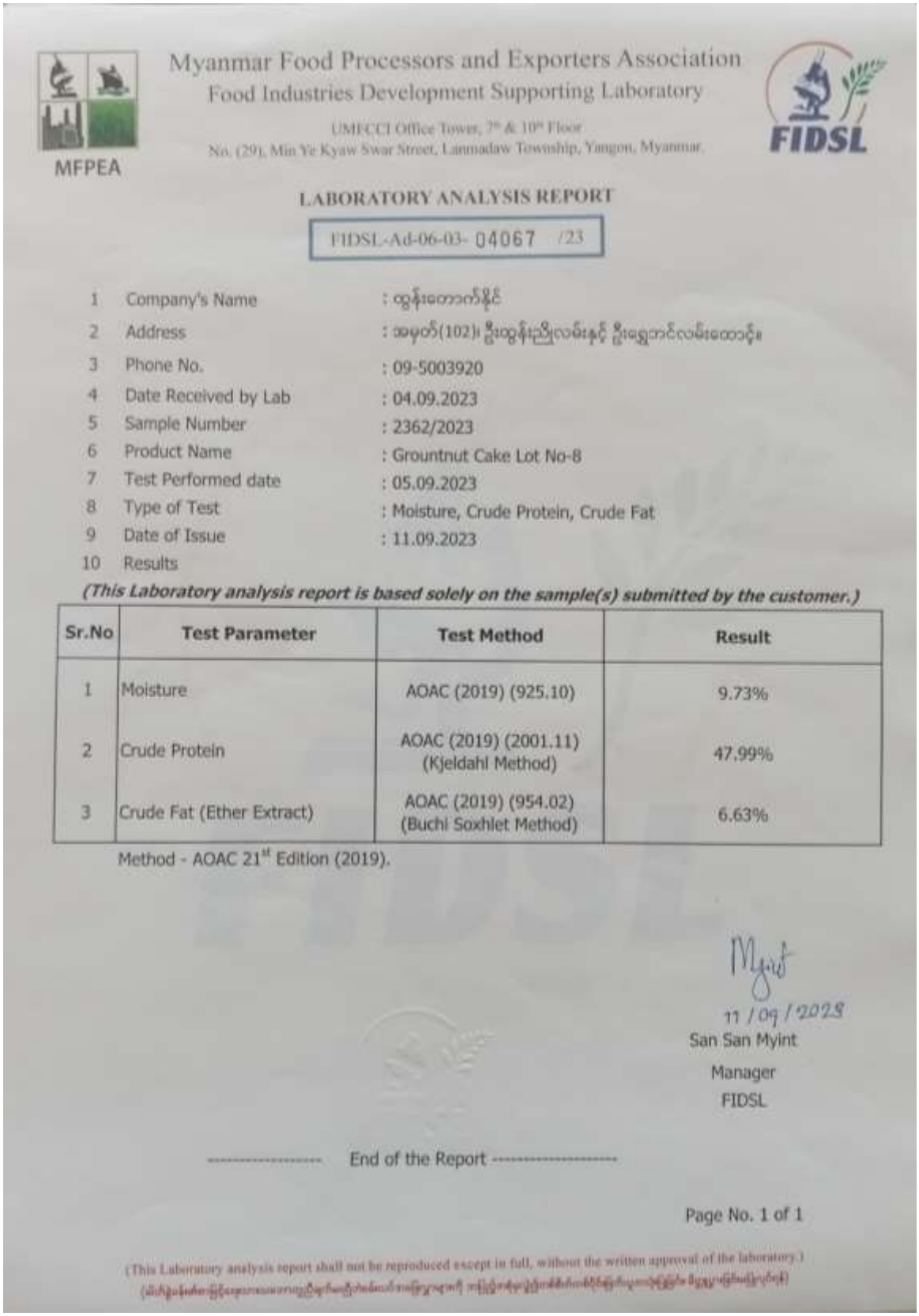
Figure. 18. Result of Protein test for May, 2023



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MFPEA

FIDSL

LABORATORY ANALYSIS REPORT

FIDSL-Ad-06-03- 04067 /23

1	Company's Name	: ထွန်းတောက်နိုင်
2	Address	: အမှတ်(102)၊ မြေထွန်းညီလေးနှင့် မြေရွှေဘင်လမ်းတောင်း
3	Phone No.	: 09-5003920
4	Date Received by Lab	: 04.09.2023
5	Sample Number	: 2362/2023
6	Product Name	: Grountnut Cake Lot No-8
7	Test Performed date	: 05.09.2023
8	Type of Test	: Moisture, Crude Protein, Crude Fat
9	Date of Issue	: 11.09.2023
10	Results	

(This Laboratory analysis report is based solely on the sample(s) submitted by the customer.)

Sr.No	Test Parameter	Test Method	Result
1	Moisture	AOAC (2019) (925.10)	9.73%
2	Crude Protein	AOAC (2019) (2001.11) (Kjeldahl Method)	47.99%
3	Crude Fat (Ether Extract)	AOAC (2019) (954.02) (Buchi Soxhlet Method)	6.63%

Method - AOAC 21st Edition (2019).

11 / 09 / 2023
San San Myint
Manager
FIDSL

---------- End of the Report ----------

Page No. 1 of 1

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Figure. 19. Result of Protein test for September, 2023

Discussion and conclusion

This survey research has been done by interviewing with questionnaires, taking photographs and making the food in laboratory scale. Treatment with peanut cake biofertilizer for Thazin plantation, showed the positive results of the bulb, the new bud and the inflorescences. According to the Food Industry Development Laboratory analysis report of moisture, crude protein and crude fat with ether extract from Ngwe Thazin Min Peanut Oil Mill gave positive results which were matched with the standard results from internet sources. After critical thinking of the food and reviewing of the literature survey, three types of food namely crispy peanut cake, peanut cake jam and sour peanut cake salad were made by laboratory scale. Many types of healthy food from peanut cake can be made for human consumption. As the different kinds of human and animal needs can be produced by the by-products of peanut oil, it can reduce the price of peanut oil. After reducing the price of peanut oil, people can be used peanut oil cheaply for their healthy life style. Further research can be carried out by the transformation of by-products from peanut oil mill can be produced as biofertilizer for plant, as various confectionaries for human and as animal feed for cow and aquatic animals like fish, crabs and prawns.

Future plan of research

According to the above research, the future research plan will be carried out to make the peanut cake powder or liquid biofertilizer for plantation; it will upgrade the peanut cake powder or liquid biofertilizer for export; it will produce snacks from by-product of peanut cake; it will find out more various food from by-products of peanut oil for human consumptions and it will also transform the peanut cake powder for animals' feed.

Acknowledgements

I would like to thank my gratitude to the owner and the manager of Tun Tauk Naing Co., Ltd., Ngwe Thazin Min Oil Mill, for their kind permission to use the data from the Mill. I also express my heartfelt gratitude are due to Professor Dr. Aye Kyi, Member of National Curriculum Committee (NCC), for her suggestion of the topics, invaluable guidance, overall supervision and her advice in preparation of this work. My thanks are due to Dr. Thidar Oo, Professor and Head of Botany, University of Yangon, for allowing me to present this research paper. I also want to show my gratitude to Dr. Thet Thet Mar Win, Professor of Botany, University of Yangon, for sharing the message of this conference.

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EFFECT OF PADDY STRAW SUBSTRATES ON THE GROWTH AND YIELD OF *PLEUROTUS OSTREATUS* (JACQ. EX. FR.) KUMMER (NGWE-HNIN-HMO)*

Win Naing [1], Ei Ei Moe [2]

Abstract

Mushroom is a crop which is cultivated in many countries using different agricultural wastes. This study was carried out to investigate the effects of paddy straw substrates on growth of *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer. The oyster mushroom, *P. ostreatus* (Jacq. ex. Fr.) Kummer was cultivated on paddy straw in polythene bags (containing 500 g paddy straw on dry weight basis per bag) using sorghum grain spawn. In this investigation, the substrate paddy straw yields a product average of 416g per bag. Therefore, it can be concluded that paddy straw is most suitable substrate for yield of oyster mushroom (Ngwe-Hnin-Hmo).

Keywords; Oyster mushroom *P. ostreatus*, paddy straw substrates

Introduction

Mushrooms are one kind of edible fungi belonging to the genus *Pleurotus* under the class Basidiomycetes. *Pleurotus ostreatus* mushroom have excellent flavor and taste. *Pleurotus* species are very popular and widely cultivated throughout the world mostly in Asia, Africa and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane *et al.*, 2007).

Oyster mushroom cultivation has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and harvested all over the year (Amin *et al.*, 2007).

Oyster mushroom have the ability to excrete hydrolyzing and oxidizing enzymes which have capable of utilizing complex organic compounds that occurred agricultural wastes and industrial by-products with broad adaptability varied agro-climatic conditions (Jandaik *et al.*, 1995).

Oyster mushrooms are rich in proteins (30.5%), fat (2.3%), carbohydrates (57.7%), fiber (8.8%) and ash (9.7%) with 346 K (cal) energy value on 100 g dry weight basis; while vitamins such as thiamin (4.9 mg), riboflavin (4.8 mg) and niacin (108.6 mg), minerals like calcium (97 mg), phosphorus (475 mg), ferrous (8.6 mg) and sodium (60 mg) on 100 g dry weight basis, are also found present (Pandey and Ghosh, 1996).

The aim of the present study is to investigate the growth performance of edible mushroom on paddy straw substrates, to produce chemical free edible mushrooms and to know the importance of edible mushroom for human being.

Materials and Methods

The experiment was carried out at the Department of Botany, Yadanabon University. Specimen were collected from P.M.K Mushroom house, for tissue culture. Oyster mushrooms (*Pleurotus ostreatus*) are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates.

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* Third Prize (2023)

[1] Department of Botany, Yadanabon University

[2] Department of Botany, Yadanabon University

Sterilization Procedure

In the laboratory, all of the apparatuses, equipment, metallic instruments, glassware and culture media were sterilized in the autoclave at 121°C about 1 hour at 1.5 kg/cm^2 pressure strictly for maintaining sterility. The culture room of the laboratory was cleaned by gently washing with detergent followed by 70% ethyl alcohol regularly. Before inoculation, laminar airflow cabinet was sterilized using ultra violet light for 30 minute keeping blower active. All inoculation measures were carried out in the laminar airflow cabinet to avoid contamination. The cabinet was exposed on the UV light for 30 minutes before use. All the instruments and equipment used were sterilized with alcohol before use.

Production of Oyster Mushroom (*Pleurotus ostreatus*)

Preparation of PDA Media

At first, 250 g potatoes were washed, peeled and sliced to prepare 1000 mL PDA media. Then peeled and sliced potatoes were boiled in water to make them soft and also filtered through a cheese cloth and further water was added to get 1000 mL media. After adding 18 g agar and 20 g dextrose, it was heated and stirred for about 45 minutes. Then 10 mL media was taken into each of test tube and mouths of the test tubes were plugged with cotton and brown paper. After that all the test tubes were sterilized in an autoclave for 20 minutes at 121°C and 1.5 kg/cm^2 and kept in slanting position for having maximum space for the organism in pure culture to proliferate.

Tissue Culture

To obtain pure culture, a small piece of tissue was collected from the fruiting body of mushroom, *Pleurotus ostreatus* and placed on the sterilized PDA medium under aseptic condition in a laminar air flow cabinet. It was then kept for 7-10 days in an incubator under 25°C for sufficient mycelial growth. These pure cultures were used for the entire experiment.

Preparation of Mother Spawn

Mother culture substrate was prepared by using sorghum grain. Sorghum grain were sieved and sun dried. The mother culture substrate was prepared by sorghum grain and wheat bran in 2:1 ratio with 0.1% calcium carbonate. Then it was mixed thoroughly with hands and maintained 55% moisture content by adding sufficient water. Then 200 g of mixture were placed into flat bottle. The neck of the bottles were plugged with cotton and covered with brown paper placing rubber band to hold it tightly in place. The flat bottles were sterilized for 1 hour at 121°C with 1.5 kg/cm^2 pressures in an autoclave and kept them for cooling. Then inoculums from pure culture were placed aseptically to the mother spawn packets. The packets (flat bottle) after inoculation were again plugged with cotton and were kept at 20-22°C for spawn run. The whole packet containing substrate became white due to fungal mycelia proliferation within 15-20 days and thus ready for spawning the substrate.

Preparation of Spawn Packets

Spawn packets using paddy straw, wheat bran and $CaCO_3$ were in ratio 69:30:1 respectively. The mixed substrates were filled into 10×12 inch polypropylene bag. The spawn packets preparation, sterilization, inoculation and incubation were done using the method described by Sarker *et al*., 2007. The weight of each spawn packet was 500 g. Moisture was measured by using the moisture meter and adjusted the moisture content at 65%. Therefore, the packets were sterilized about 1h and then these were kept for cooling. After cooling, 5 g mother spawn were

inoculated into the packets in the laminar airflow cabinet and were kept at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Then this spawn packets were transferred to the culture house.

Cultivation of Spawn Packet

Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22- 25°C.

Harvesting of Mushroom

Oyster mushrooms matured within 2-3 days after primordia initiation. The matured fruiting body was identified by curl margin of the cap, as described by Ruhul Amin, 2002. Mushrooms were harvested by twisting to uproot from the base. Mushrooms were harvested 2 times from a packet. After completing the first harvest again the packets were scraped at the place where the 'D' shaped cut had been done and were soaked in a bucket for five minutes and then placed in the culture house and water was sprayed regularly. The primordia appeared within 9-10 days after first harvest and 7-8 days after second harvest. Water spraying was continued until the mushrooms were ready to be harvested.

Results

Morphological features

According to the research *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer, Oyster mushroom can be divided into two parts; mycelium and fruit body. The fruit body, which is made up of pileus and stipe, is for eating. Pileus convex at first, expending to broadly convex, eventually flat and even upturned in age, 5.0-20.0 cm in diameter, white, grayish white to gray-brown. The entire pileus margin undulates like oyster shell. Color varies according to the strain, lighting and temperature conditions. Stipes are typically eccentrically attached to the pileus.

Table 1. Yield of Oyster mushroom on paddy straw substrates

Yield	Replicates	Weight of mushroom on first harvested	Weight of mushroom on second harvested	Total
Yield Obtained	Replicate 1	210 g	205 g	415 g
	Replicate 2	200 g	198 g	398 g
	Replicate 3	220 g	207 g	427 g
	Replicate 4	208 g	200 g	408 g
	Replicate 5	220 g	213 g	433 g
Average				**416 g**

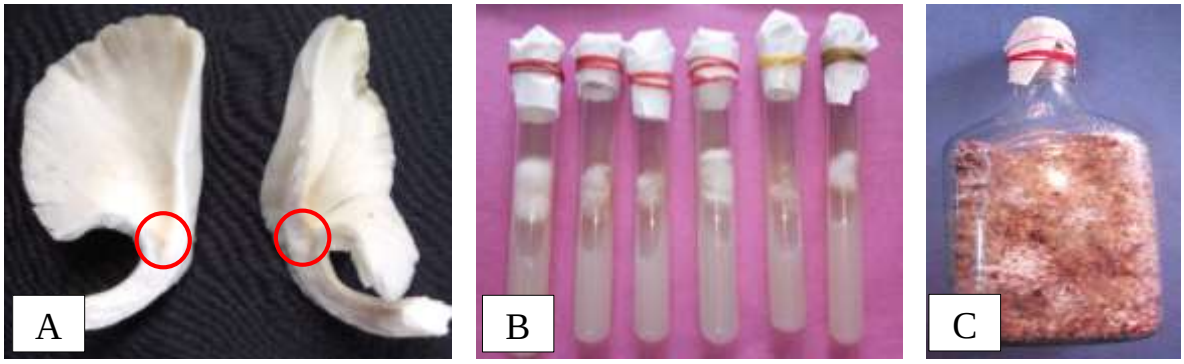

Figure. 1. Cultivation of oyster mushroom (*Pleurotus ostreatus*)

A. Small piece of specimen was taken for Tissue culture
B. Tissue were cultured in test tubes with PDA medium
C. Commercial spawn with sorghum grain

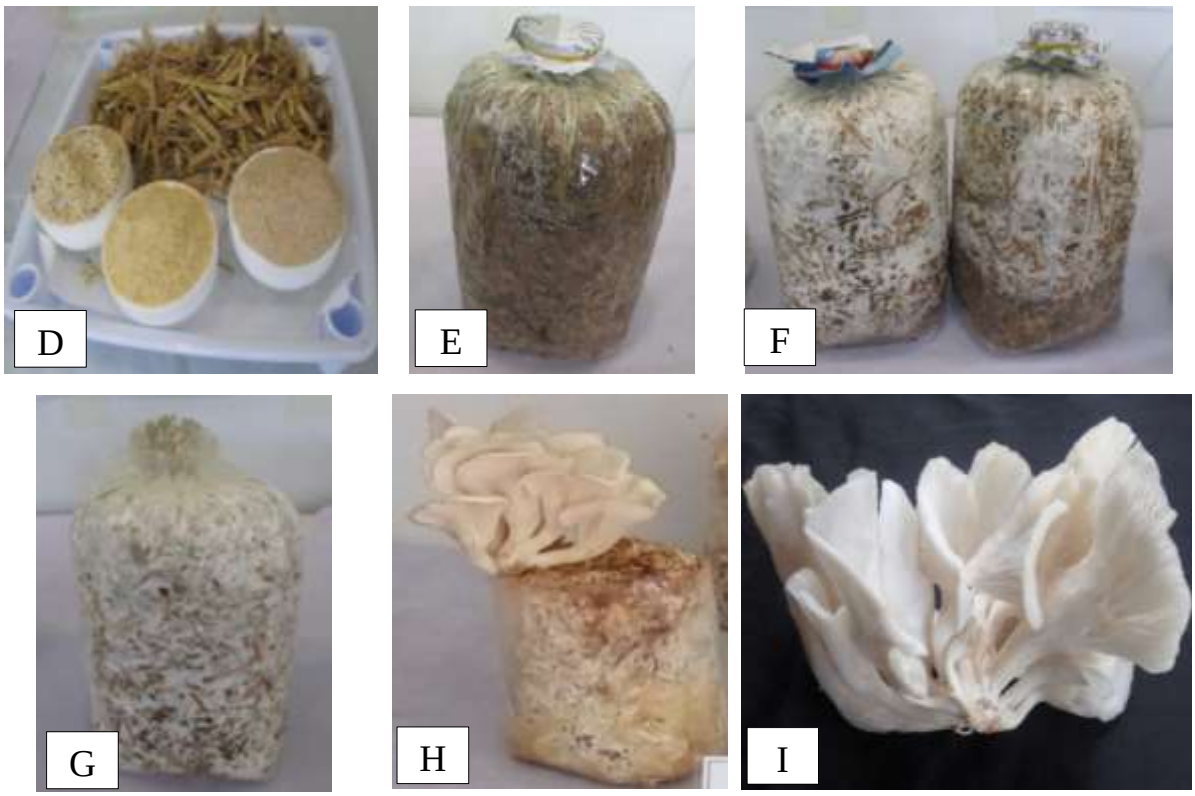

Figure. 2. Cultivation of oyster mushroom (*Pleurotus ostreatus*)

D. Material for cultivation
E. Preparation of cultivation bag with paddy straw materials
F. Cultivation bags with growing mycelium
G. Early maturing phase of growing mushroom
H. Growing mushroom before harvesting
I. After harvesting

Discussion and Conclusion

Pleurotus ostreatus is an edible mushroom which is prepared by the various agro-based products such as sawdust, cotton waste, wheat straw, etc. (Dinesh Babu, 2010). In this study, paddy straw has been used as a substrate. Oyster mushroom is grown on sterilized substrate bag cultivation.

In this study, the growth and yield of mushroom were better on paddy straw substrate. In this investigation, oyster mushroom yields a product average of 416g per bag from 500 g paddy straw substrates bags. According to Paul *et. al.*, 2014, cultivation of *Pleurotus ostreatus* on saw dust substrates were found average of 373.4 g per bag from 500 g. It may also offer economic incentives for agribusiness to examine these residues as valuable resources and develop new enterprises to use them to produce nutritious mushroom products.

Therefore, the mushroom cultivation may become one of the most profitable agribusiness that could produce food products from paddy straw substrates and help to dispose them in an environment friendly manner.

From the findings of this study, it is evident that treatment paddy straw substrates gave maximum yield of the *Pleurotus ostreatus*.

Acknowledgements

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PRELIMINARY STUDY ON SOME WILD ORCHIDS AT NAT MA TAUNG WILDLIFE SANCTUARY, KAN PET LET TOWNSHIP IN SOUTHERN CHIN STATE*

Moe Sandar Shein[1] , Khin Swe Swe Htun[2]

Abstract

This research paper was concerned with some wild orchids of natural habitat in Nat Ma Tung Wildlife Sanctuary in Kan Pet Let Township. These wildlife Sanctuary was located in Kan Pet Let Township of Min Tet District in Southern Chin State and also east part and 2.5 miles distance aways from Kan Pet Let city. In this study (9) genera and (10) species were recorded in study area. Nat Ma Taung is hill evergreen forest and rain forest type. Various wild orchids are stretch out around the Nat Ma Taung Montain hill and rich in diversed flora and fauna. All collected species are epiphyte, terrestrial and lithophyte. Collected genera namely ***Anthogonium, Arundina, Cymbidium, Dendrobium, Habenaria, Obeionia, Paniisea, Peristylis, Pleione*** and ***Spathoglottis,*** were recorded and have been taken photographs in natural habitat of orchids. The collected specimens were classified, identified and described with color photographs also found unknown species in study area. The morphological characters have been emphasized and artificial keys from the tribe to the species have been constructed and location was mention by Global Positioning System (GPS).

Keyword: Nat Ma Taung Wildlife Sanctuary, Chin State, wild orchids, unknown species, rich in biodiversity, artificial key.

Introduction

The family Orchidaceae are largest family among Angiospermae, Monocotyledonae. Some botanist estimated about 35000 orchids among flowering plants. (Seidenfaden, 1992) Orchidaceae grow well throughout the world. They can thrive in tropical, subtropical and temperate regions except in ice capped regions and deserts. The most wild orchids have distributed various regions of Myanmar that is tropical, subtropical and temperate regions, especially they have grown in temperate regions (Dassanayake, 1981). Nat Ma Taung National Park is highest mountain in Chin State within the Eastern Himalayas.Chin State lies in the north -western mountainous region of Myanmar. The Chin Hills are home to the temperate and alpine species typical of the Himalaya Mountain Range. The Mount Victoria (Nat Ma Taung in local language) is the highest mountain in Chin State and one of the most widely distributed pine in Asia. The flora and fauna of Mount Victoria is extremely rich. Nat Ma Taung National Park (NTNP) was established in 1994. It was also designated as an ASEAN Heritage Park. In addition, many medicinal orchids and other endermic plant species have been extracted from the (NTNP) and exported to China. It has reached a critical state in which precious native orchids biodiversity within the NMNP might be lost. Now the study area is Nat Ma Taung National Park of Kenpatlat Township in Mindat district of Southern Chin State. Kanpetlet Township is located on the east by Saw Township, on the west by Platwa Township, on the south by Mindat Township, on the north by Minbra Township, and it lies between North latitude 20˚69′-22˚14′ and East latitude 93˚30-94˚10′and is hill evergreen forest type. Hill evergreen forest type (The hill evergreen forest is found in the north at altitudes of over 1,000 meters above sea level. In other regions they are found area of high altitudes. This type of forest is less dense than the tropical evergreen forest because it has less large trees. This type of forest too

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* Special Award (2023)

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is quite cool because it is found at high altitudes. The hill evergreen forest is very important to the preservation of water sources. Trees are mainly shrubs mixed with some pines. Smaller plants in the forest include ground orchids and other tropical plants such as wild roses, violets and lilaes. In addition there are small plants that grown on the larger trees such as moss and orchids.(Hill evergreen forest<<pirun-Ku.ac.th>) where the wild Orchids have grown well on the various old plants which are ***Shoreaob longifolia*** Klall. (Thit-ya), ***Lagerstrmia speciera*** Pers (Thit-el).***Dipterocarpus obtusifolius*** Teysm. ex. Miq. .(Pyin-Ka-doe) .Nat Ma Taung National Park is located in Ken Pat Lat Township in Min Tet district of Southern Chin State. The area of these forest is about 176202 acres and altitude of 3200.4 m above sea level and lies between North latitude 21˚30′21˚50′and East longitude 93˚30′.94˚10′.Genus ***Anthogonium, Arundina, Cymbidium, Dendrobium, Habenaria, Obeionia, Paniisea, Pecteilis, Pleione*** and ***Spathoglottis,*** have been found in this area.

In this recent study, (3) Subfamilies belong to (5) Tribes (6) Subtribes (9) genera and (10) species have been collected from this study area including epiphyte, terrestrial and lithophytes. The classification and taxonomic description of collected specimens are provided with coloured photographic and artificial keys of Tribe, Subtribe, genera and species are also constructed. The aim of the study was to know of Myanmar wild orchids and to access the presence distribution of wild orchids in Nat Ma Taung Wildlife Sanctuary in Southern Chin State. To fulfill this aim, the collected wild orchids identified, classified and described systematically.

Methodology

The specimens were collected from Nat Ma Taung National Park of Kanplet Township of Minduct District from 2019-2021. All these specimens were colourful photographed to record their actual habitat and the nature of inflorescence. The collected specimens were classified according to Dresseler's classification Dresseler's (1927) and identified by Grant: (1966), Hooker, (1954), Chen et al (2013), Dassanayake, (1981), Seidenfaden and Wood (1992) , Nantiya Vaddhanaputi (2006), Flora of China Vol. 25 (2013) and Flora of Thailand Vol. XI & XII. Part I&II (2014) methods. Herbarium specimen well prepared and submitted to Forest Department, Nay Pyi Taw.

Arrangement of the Subfamily, Tribe, Subtribe and Genera in the present study

Class	:	Liliopsida (Monocotyledoneae)
Subclass	:	Orchidales
Family	:	**Orchidaceae**
		(I)Orchidoideae
Subfamily	:	(II) Epidendroideae
	:	(II)Vandoideae
I. Subfamily	:	Orchidoideae
Tribe		Orchideae
Subtribe:		Habenariinae
Genera		(1) ***Pecteilis***
		(2) ***Habenaria***

II. Subfamily	:	(II) Epidendroideae
Tribe	:	Arethuseae
Subtribe:		Thuniinae
Genera;		(3) ***Arundina***
		(4) ***Spathoglottis***
		(5) ***Anthogonium***
Tribe		Coelogyneae
Subtribe	:	Coelogyninae
Genera	:	(6) ***Pleione***
Tibe	:	Malaxiadeae
Genera	:	(7) ***Oberonia***
Subtribe	:	Dendrobiinae
Genera		(8) ***Dendrobium***
II. Subfamily	:	Vandoideae
Tribe	:	Vandeae
Subtribe	:	Cyrtopodiinae
Genera	:	(9) ***Cymbidium***

The classification of Subfamilies in the study is in accordance with Dresseler (1927) and the key below is cited from Seidenfaden and Wood (1992) described in "The Orchids of Indochina"

Result

In this paper (3) subfamily, (5) tribes, (6) subtribes (9) genera and (10) species have been collected from study area. According to Seidenfaden and Wood (1992).

Key to the Subfamily of family

1. Plants with characteristic tuber or root stem tuberoids. Aanther erect or reflexed at base firmly attached to the viscidium or visidia. Apex of rosetellum often protruding

between the thecae---------- (I) **Orchidoideae**

1. Plants with characteristic with often aerial root and sympodial shoot pseudobulb Anther most often distinctly incumbent. Rostellum incumbent or deflexed----------**2.**

2. Pollinia soft, waxy, without stalk or with caudiculae only rarely with stipe. Anther erect and earlier ontogeny ---------- (II) **Epidendroideae**

2. Pollinia cartilaginous or bony, usually with stipe. Anther incumbent already from earliest stages in ontogeny often strongly deflexed at maturity**.** ---(III) **Vandoideae**

Key to Tribe of Subfamily Epidandroideae

1.Terrestrial. Absent of pseudobulb. Leaves plicate. Inflorescence.Inflorescence long and erect. ----------**2**

1. Pseudobulb present and single with internode. Leaves simple and coroceous. Inflorescence suberect and too long. Flower small to medium. Column long with distinct wings and caudicle. Pollinia 2 ----------Coelogyneae

2. Plant with plicate leaf. Inflorescence erect and lateral (except *Thunia*). Pollinia8----------**Arethusea**

2. plants with conduplicate or plicate leaves. Inflorescence with many flowers----------**Malaxideae**

I. Subfamily Orchidoideae

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar name
Orchidoideae	Orchideae	Habenariiae	**Pecteilis**	*susannae*	none
			Habenaria	*sp:*	none

In this recent study two genus of *Pecteilis* and *Habanarria* was collected in subfamily Orcideae and this subfamily has not divided into tribe and subtribe.

Key to the genus of Subtribe Orchideae

1. Tall stout terrestrial orchids over 60cm or more high with large broad leaves. Flower often white. Sepals and petals are quite different ----------***Pecteilis***

1. Medium tall terrestrial orchids and about 45cm high with leaves. Scape with sessile white and yellow flowers----------***Habenaria***

(1) *Pecteilis susannae* (L) Rajin


Habitat


Inflorescence


Flower


Segment of Flower


Pedicle & column

***Pecteilis susannae* (L) Rajin**

***Orehis Uabenark* (L.) R. Brown**

Terrestrial. Inflorescence erect. Flower large white to pale greenish white about 6.00 cm long 8.00 cm wide with cylindric green spar fragrant. Dorsal and lateral sepals broadly ovate subacute, erect, with seven veined. Petals very small, suberect, linear triangular. Labellum trilobed, side- lobes preading with laciminate, mid lobe oblong, entire, 3.5 cm long and 1.00 cm wide with spur cylindric, green, 12.00 cm long 0.5 cm wide. Ovary large, green with distinct longitudinal keels. Pollinia 2.

Myanmar Name - None

Occurrence - Kan Palat Township, Near Saw-Chaung Village, Chin State. N 21º 27′ 38.6″, E 94º 10′ 32.7″

Distribution - Cambodia, India, Indonesia, Laos, Myanmar, Nepal, Malaysia, Thailand, S Vietnam (Flora of China 2013)

Ecology - Terrestrial, Dipterocarp forest, mixed deciduous forest, open grassy places, Valley, alt. 924 m

Flowering period - September- October

(2) *Habenaria sp.* Schltr, Repert.


Habit


Flower


Inflorescence


labellum


flower


Inflorescence


Curved labellum


Segment of flower

(2) *Habenaria sp.* Schltr, Repert. Spec. Nov. Regni Veg 2:82.1906

Elulophia sumarsis

Terrestrial. Inflorescence erect with lax 2-3 flowered. Flower brownish pink, medium size, 3.00 cm across. Lateral sepals similar to dorsal sepal. (reddish brown with green lip). Lip trilobed, quandencular, white with deeper purple in the center, mid lobe quandencular in shape, curved downward at the tip, margin undulate, papilose on the epichile seven fimbriate keels in center, side keels on each side shorter than the 5 middle keels. Spur slender, green, about 8.00mm long and 2.00mm wide. Operculum green.

Myanmar Name - Non

Occurrence - Myanmarm, Kan Pat let Township, Chin State, Near Chew song village. N 21º 10′ 30″, E 94º 05′ 70″

Distribution - Natmataung Wild life Century, Chin State .Myanmar (2019

Ecology - Terrestrial, Mixed deciduous forest, open grassy place valley, 924 m, alt.

Flowering period - October

II. Epidendroideae

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar name
Epdendroideae	Arethusea	*Thuniinae*	**Arundia**	*graminifolia*	မြက်သစ်ခွ
			Spathoglotis	*pubescence*	အုန်းသစ်ခွ
			Anthogonium	*glacia*	None
	Coelogyneae	Coelogyniinae	**Pleione**	*praecox*	ဖားလက်တက်
	Malaxiadeae	malaxeae	**Obeonia**	*pyrulifera*	*None*
	Epidendrae	dendrobiinae	**Dendrobium**	*longicornu*	-

Key to the genus of Subtribe Thuniinae

1. Pseudobulb absent, erect and leafy stem. Leaves two ranks, leafy sheaths at the base. Scape terminal with showy flowers. Sepal spreading nearly equal. Lip sessile, concave at the base, embracing the column.----------***Arundina***

1. Pseudobulb ovoid. Leaves with petiolate. Scape arising from the base of the pseudobulbs. Sepal not spreading. Lip not sessile----------2.

    2. Pseudobulbs ovoid. Leaves plicate, petiolate, evergreen. Scape erect with medium flowers. Sepal free and equal with petals. Lip strongly 3 lobes--------S***pathoglotis***

    2. Pseudobulb small. Leaves very narrow with petiolate. Scape branched with small flowers. Flower small. Lip adnate to the base of the column. Spur long clawed.----------***Anthogonium***

(3) *Arundina graminifolia* (D. Don) Hochr

Habit

Inflorescence

Flower

(3) ***Arundina graminifolia*** **(D. Don) Hochr., Bull. N.y. Bot. Gard. 61270,1910**

Bletia graminifolia **Don. Prods**

Arundina bambusifolia **Lindl.**

Terrestrial. Inflorescence erect, 20-30.00 cm long with few flowers, terminal flower opening in succession. Flower white with pale pinkish purple or tinged, pedicle short. Sepals elliptic lanceolate acute. Lip trilobes, side lobes rounded, embracing column, mid-lobe broadly rounded, purple with dark purple at the tip and yellow in the centre and with 3 distinct keel, margin undulate.

In this resent study only specie of genus ***Arundina*** collected from study area.

Myanmar Name	-	Wah Thit Khwa
Occurrence	-	Kan Palat Township, Near Saw-Chaung Village. N 21º 10′ 10″, E 94º 05′ 36.8″
Distribution	-	Bhutan, Cambodia, India, Indonesia, Laos, Myanmar, Nepal, Malaysia, Thailand (Flora of China, 2013)
Ecology	-	Terrestrial, Sandstone deciduous forest, alt. 105 m
Flowering period	-	May- December

(4).*Spathoglottis pubescence* Lindl.


Habit


Inflorescence


Segment of flower



Labellum & Column

4. ***Spathoglottis pubescence*** **Lindl.**

Spathoglottis fortune **Lindl.**

Distinct character

Terrestrial, Leaves linnea lanceolate, acute grass-like, glabrous, plicate. Flower greenish yellow with purple tinged, about 35 cm across with spathulate lip with brown sport. Labellum trilobed, sidelobed erect, oblong obtuse, yellow with brounish purple stipe, 8.00 mm × 5.00 mm wide, mid-lobe shortly clawed reniform, truncate with lamella-like twin-calli on the mesochile tapering toward the base. Column yellow, about 1.00 cm long and 3.oo mm wide. Rostellum whitish green, short. Ovary 8.00mm long 2.0 mm wide.

In this resent study only specie of genus ***Spathoglotis*** collected from study area.

Myanmar Name	-	Non
Occurrence	-	Near Saw-Chang Village, Kan Palat TS. N 21˚ 10′ 10″, E 94˚ 05′ 37.8″
Distribution	-	NE India, Myanmar, Thailand, China. (Seidenfaden and wood 1992). Cambodia, NE India, Laos, Myanmar, Thailand, Vietnam (Flora of China,2014,Vol-25)
Ecology	-	Terrestrial, Grassland, deciduous forest, alt 105 m
Flowering period	-	August ~ October

(5) *Anthogonium gracile.* Lindley


Habit


Inflorescence


Flower


Pedicle &Flower

(5) *Anthogonium gracile.* Lindley. in Wall. Cat 7398 Gen and Sp orchid. 426:

***A. griffithii* Rchb.f. Ic. Plant. Asiatt. 345.**

Lithophyte or terrestrial. Stem slender, 45 cm tall. pseudobulb small, Inflorescence erect with laxly 8-10 flowers, Flower white with pink tinged, nodding pedicle with ovary at right angles to the parienth. Lip wedge-shaped, cuneate, 1.00 cm long, 8.00 mm wide. Pollinia 4, without caudicle.

In this resent study only one species of genus ***Anthogonium*** collected from study area.

Myanmar Name	-	Non
Occurrence	-	Saw-Chaung village, Kan-Pat-let Township, Chin State, Myanmar. N 21º 10′ 30″, E 94º 05′ 70″
Distribution	-	Himalaya, Nepal, Naga HIll (Grant B, 1966) Bangladesh, Bhuttan, Combodia, N India, Laos, Myanmar, Nepal, Seri Lanka, Thailand, Vietnam (Flora of China, Vol 25, 2014).
Ecology	-	Terrestrial or Lithophyte, open grassy places, valley, 112 alt.
Flowering period	-	October

6. ***Pleione praecox*** **(Smith) D. Don,**


Habit


Inflorescence


Segment of Flower


Rostellum


Inflorescence


Flower


Labellum & Column

(6) ***Pleione praecox*** **(Smith) D. Don, Prodr. Fr. Nepal 37. 1825**

Epidendran praecox

Epiphyte or teretril. Pseudobulbs impulliform or turbinate. Inflorescence appearing side of the pseudobulb with one showy flower. Lip trilobed, midlobed ovate broadly, 5 distinct longitudinal keels or crest reaching near at the top, middle one scate. Column purple, about 4.5 cm long 0.4 cm wide, dentate. Pollinia 4 in pairs.

In this resent study only specie of genus ***pleione*** collected from study area.

Note:	-	(Rare orchids) (www. e Floras org Flarataron)
Myanmar Name	-	(ဖားလက်တက်) (Pha Latt.Tet)
Distribution	-	SE and SW Yunnan, Bhutan, Bangladesh, NE India, Laos, Myanmar, Nepal, N-Thailand, N- Vietnam (Flora of China Vol.25) Takangoo Tanisserim (Grant 1964), India Nepal, Myanmar, Schna, Laos, Vietnam, Shula (Flora of Thailand)
Ecology	-	Epiphyte, hill evergreen forest, terrestrial rock with mass in Mountain forest, alt-1200 m-2000 m,
Flowering period	-	September ~ October

(7) ***Oberionia pyrulifera*** Lindley.


Habit


Inflorescence


Flower

(7) ***Oberionia pyrulifera*** Lindley.

Iridorkis pyrulifera (Lindl.) Kuntze 1891.

Malaxis pyrulifera (Lindl.) Rchb,f.1861

M.verticillata **var** ***Khasiana*** (Lind.) Rchb.f

Epiphyte, stem erect, turft, short 4.5.00cm attached by fibrous root. Leaves laterally flattened, distichous. Inflorescence suberect with many flowers, whole with minute floral bract which is ovate acute. Flower pale yellow. Lip broadly oblong, protruding with deeply bilobed, small auricle on each side on hypochile. Colum short, stout. Pollinia 4 in pairs, unequal, incumbent.

Myanmar Name	-	Yet Taun Pan
Occurrence	-	Natmataung Wildlife Sanctuary. Kanpetlet Township N21˚ 13′ 39″, E 93˚ 32′ 91″
Distribution	-	Chinese Himalayas, Assam, India, Nepal, Thailand (300-2000 meters) www.orchids species). www.theplantlist.org. Bhutan, India, Thailand (Flora of China)
Ecology	-	Epiphyte, Hill Evergreen forest, Montane forest, alt2582m.
Flowering period	-	Septembre - November

(8) ***Dandrobium longicornu*** **Lindley.**


Habit


Inflorescence


Flower


Segment of Flower


Column & Labellum

(8) ***Dandrobium longicornu*** **Lindley.**

D. bulleyi **Rdfe (1913)**

D flexuosum **Griff (1851)**

Stem evergreen, erect, slender, clustered, about 7.12 cm long and 1.5 cm wide, blackish brown hair on both surfaces. Inflorescence subterminal. Flower white with orange block labellum, pendulous downward, about 3.5 cm across, mentum long and narrow, pale green. Lip rhombic, trilobed, side lobes subovate, larger than the midlobe, slightly wavy on margin, midlobe protruding downward, margin undulate and irregular toothed at apex, excurved tip, distinctly orange vein on hypochile, 3-4 longitudinal keels in the centre. Column with triangular teeth. Pollunia 4.

Myanmar Name	-	Chin Daiwy
Occurrence	-	Nat-Ma-Taung Wildlife Century, Chin State, Myanmar. N 21º 13′ 26.2″, E 93º 59′ -01.4′
Distribution	-	Bhautan, NE India, Myanmar, Nepal, N Vietnam (Flora of China 2013), E Himalayas, Assam, Bangladesh, Nepal, Bhautan, Myanmar,

Southern China, Vietnam (W3 Tropicos. Kew Monocot list, IPNI)

Ecology - Epiphyte, on tree trunk, Mountain forest, alt: 1267 m.

Flowering period - September ~ November

III. Subfamily Vandoideae

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar name
Vandoideae	Vandeae	Cyrtopodiinae	**Cymbidium**	*irridioides*	Pan Thet Shay nyo
				elegans	none

(9) *Cymbidium iridioides* D.Don Prodr. FL. Nepal 1825:36.


Habit


Flower


Segment of flower


column

***Cymbidium iridioides* D.Don Prodr. FL. Nepal 1825:36.**


labellum


Pollinia

(9). *Cymbidium iridioides* D.Don Prodr. FL. Nepal 1825:36.

***C. giganteum* Wall.ex Lindl.**

Epiphyte or terrestrial. Pseudobulb, bilaterally flattened, enclosed persistent leaf sheath Flower large, 8-10.00 cm across, fragrant. Sepals 3, dorsal sepal obovate oblong, acute, 35.00 mm long and 8.00 mm wide, yellowish green with reddish brown longitudinal stripe. Lip trilobed, side lobes creamy yellow with reddish brown stripes, ciliate, mid lobe orbicular recurved, erose and undulate, creamy yellow with reddish brown spot, 2 central callus with long papillose and red spot ending at the base of midlobe, the hairs not continuing beside of the apices of callus. Pollinia 2, yellow, subtriangular. Anther cap papillose, cap shaped.

Myanmar Name - Pan Thetshay Nyo

Occurrence - Saw-Chaung Village, Kan-Pat-let Township, Chin State, Myanmar. N 21º 27′ 39″, E 94º 12′ 32.9″

Distribution	-	NW Himalaya, eastward to SW China (Seidanfadon and Wood, 1992) NW to SE Yunnan, Bhutan, India, Myanmar, Nepal, N Vietnam (Flora of China 2013), Upper Burma (Grant 1966)
Ecology	-	Terrestrial, Epiphyte on tree trunk, Mountain forest, alt: 1267 m.
Flowering period	-	October ~ December

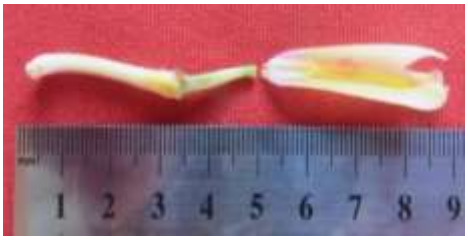
(10) *Cymbidium elegans* Lindl.


Habit


Inflorescence


flower


Segment of flower


Labellum & collum


labellum


column


Pollinia

(10) *Cymbidium elegans* Lindl. in Wall. Cat 7345: Gen and Sp. 163,1833

***C. lushuiense* (Z.h. liu, S.E. (hen and X.C. Shi) ZJ. Liu and Sc. Chen 2006)**

C densiflorum Griff, 1851

***Cyperorchis elegans* Blume Rumph.**

Epiphyte. Inflorescence pendulous and nodding, arising from the base of the flattened pseudobulb, densely flowered 20-35 flowered about 45.00 cm long with small floral bracts. Flower pendulous, peculiarity half closed, bell shaped, creamy yellow, 4.00 cm long and 1.5 cm wide, fragrant with short pedicel. Lip trilobed, midlobe small, endulate, with 2 distinct longituding yellow lamellae enduring at the base of midlobe with longitudinal channel between them, two short appendage on hypochile with densely pubescent. Pollinia 2, caudile,2.00mm wide, obovate, yellow.

* Note	-	Very rare Species (Grant, 1964)
Myanmar Name	-	Pan Thet Shay Pyar Ohn
Occurrence	-	Nat-Ma-Taung, Chin State, Myanmar. N 21º 12′ 0.9″, E 93º 56′ 32″
Distribution	-	SE Xizang Yunnan, Bahutan, India, Myanmar, Nepal (Flora of China 2013), Myanmar (Grent B, 1964). China, Himalayas, Assam India, Nepal, Bhutan, Sikkim and Myanmar (W3. Tropicas, Kew. Monocot list- IPNI)
Ecology	-	Epiphyte, on t hree, Mountain forest, alt: 2582 m.
Flowering period	-	October

IUCN status of collected species

Subfamily	Tribe	Subtribe	Genus	Species	Myanmar name	IUCN Status
Orchidoideae	Orchideae	Habenariiae	**Pecteilis**	*susannae*	none	Appendix (II)
			Habenarria	*sp:*	none	-
Epdendroideae	Arethusea	*Thuniinae*	**Arundia**	*graminifolia*	မြက်သစ်ခွ	Appendix (II)
			Spathoglotis	*pubescence*	အုန်းသစ်ခွ	rare
			Anthogonium	*glacia*	None	
	Coelogyneae	Coelogyniinae	**Pleione**	*praecox*	ဖားလက်တက်	endangered
	Malaxiadeae	malaxeae	**Obeonia**	*pyrulifera*	*None*	
	Epidendrae	dendrobiinae	**Dendrobium**	*longicornu*	ချင်းဒေဝီ	Threaten
				elegans	none	rare
Vandoideae	Vandeae	Cyrtopodiinae	**Cymbidium**	*irridioides*	ပန်းသက်ရှည်ညို့	Appendix II

Discussions and Conclusions

This paper based on some collected wild orchids specimens. The present list is (3) subfamily, (5) tribe, (6) subtribe, (9) genera and (10) species. The Subfamily Orchidoideae contains (1) Tribe (1) Subtribe and (2) genera. The subfamily Epidendroideae includes (3) tribe, (4) subtribe, (6) genera. The Subfamily Vandoideae is (1) Tribe (1) Subtribe (1) genera. Genus ***Anthogonium, Arundina, Cymbidium, Dendrobium, Habenaria, Obeionia, Paniisea, Peristylis, Pleione*** and ***Spathoglottis*** have been collected in recent study. In Subfamily Orchidoideae, two genus of Subtribe Habenariinae under Tribe Orchideae were collected in this study area. They are ***Pecteilis susannar*** (L)Rajin and ***Habenaria sp***: ***Pecteilis susannar*** (L)Rajin possess long scape with large white flower, linear triangular very small petals, trilobed labellum and spreading sidelobed with laciminate and long green cylindric spur these characters agreed with Seidenfaden and Wood (1992). ***Habenaria sp***: contain 2-3 lax flowers with erect inflorecence, brownish pink medium size flower, quandencular lip with seven fimbriate keels in the center. In the subfamily Epidendroideae, (6) genus of (3) Subtribe under (3) Tribe have collected in recent study. Three genus of Subtribe Thunninae are ***Arundina, Spathoglottis, Anthogonium***. ***Arundina graminifolia*** have medium size white flower with pale purple tinged, broadly rounded mid -lobed with yellow in the center with 3 distinct keels and undulate margin. ***Anthogonium gracile*** is lithophte, white flower with pink tinged and nodding pedicle with ovary at right angle, wedge-shaped cuneate lip and mid-lobe obovate rechrved with purple sport. ***Spathoglottis pubescence*** possess linea lanceolate plicate grass like leaves, greenish yellow flower and mid-lobe of lip shortly claw reniform with twin calli on the mesochile tapering toward the base and above three species of all characters are agree mention by Chen X., *et al.*, (2013), Henrik, *et al* (2014) and Dassanayake, (1981). One genus of Subtribe Coelogyninae is ***Pleione. Pleione praecox*** present impulliform green pseudobulbs with reddish brown blotched, purple flower and broadly ovate mid-lobed with 5 longitudinal keels and yellow in the centre. These characters are agreed by Grang (1966). Only One genus of Tribe Malaxiadeae is ***Oberonia***. ***Oberonia pyrulifera*** has suberect many flowers inflorescence and broadly oblong protruding lip with deeply bilobed and small auricle on hypochile. Above all characters of these species conform by Seidenfaden and Wood (1992). Only one genus of Subtribe ***Dendrobiinae*** is ***Dendrobium.***

Dendrobium longicornu possess white flower with orange block labellum, distinct keels on the back of sepals and 5 veins and mid-lobed rombic,distinctly orange vein on hypochile 3-4 longitudinal keels in the centre. These characters are agreed with Seidenfaden and Wood (1992). In the subfamily Vandoideae, only one genus of Subtribe Crytopodiinae under the Tribe Vandeae is *Cymbidium.* Two species genus *Cymbidium* are *C. iridioides* and *C. elegans* have been collected in this study area. *C. iridioides* contain greenish brown fragrant large flower, lip creamy yellow with reddish brown sport, orbicular recurved mid lobe with 2 centre callus, long papillose and red spot at the base of mid lobe. *C. elegants* possess pendulous and nodding inflorescence with densely flowers, peculiarity half closed flower, large side lobes of lip and small midlobe with 2 distinct longitudinal yellow lamellae and two short appendage on hypochile. All characters are conform with Henrik AE (2014) Seidenfaden and Wood (1992) and in this paper all collected species are epiphyte, lithophytes and terrestrial.

In recent study, species ***Pecteilis susannae***, ***Arundina graminifolia*** was putted in Appendix (II) and well distributed in around the Saw Chaung village, they grow well on the limestone. ***Spathoglottis pubescent*** regard as rare species, ***Pleione pracox*** is endangered, ***Dendrobium longicornu*** is threatens and ***Cymbidium elegans*** regard as very rare species according to (IUCN) and unindentified species of genus ***Habenaria*** was record in study area. ***Anthogonium graci*** was found in Mon, Mandalay and Thanintheri (Kress *et al*., 2003) but in recent study also found in Chin State. ***Cymbidium elegant*** was found in Kachin State (Kress *et al*.,2003) but also found in Chin State.

In conclusion orchids habitat and locations are very important for survive and reproduction. Today wild orchids are gradually disappear by human activity and some species are dangerous to survive and reproduction. The flora and fauna are very diverse in Myanmar but very weak in maintenance and survey record. Botanical collection is still needed to cover the whole floristic diversity of Myanmar, because botanical exploration has sharply decreased in Myanmar 1950. (Kress *at el*,2003) Compared with neighboring countries with intensive orchids studies. Myanmar orchids flora have lagged behind being well documented and studied. So the orchidologist will have to find out continuously and also should attend to all international orchids forum to get update current wild orchids information and report to government for protection of our living jewels. We have known deeply in current study that need to conservation for sustainable rich orchids biodiversity and ecotourism to invite international expert.

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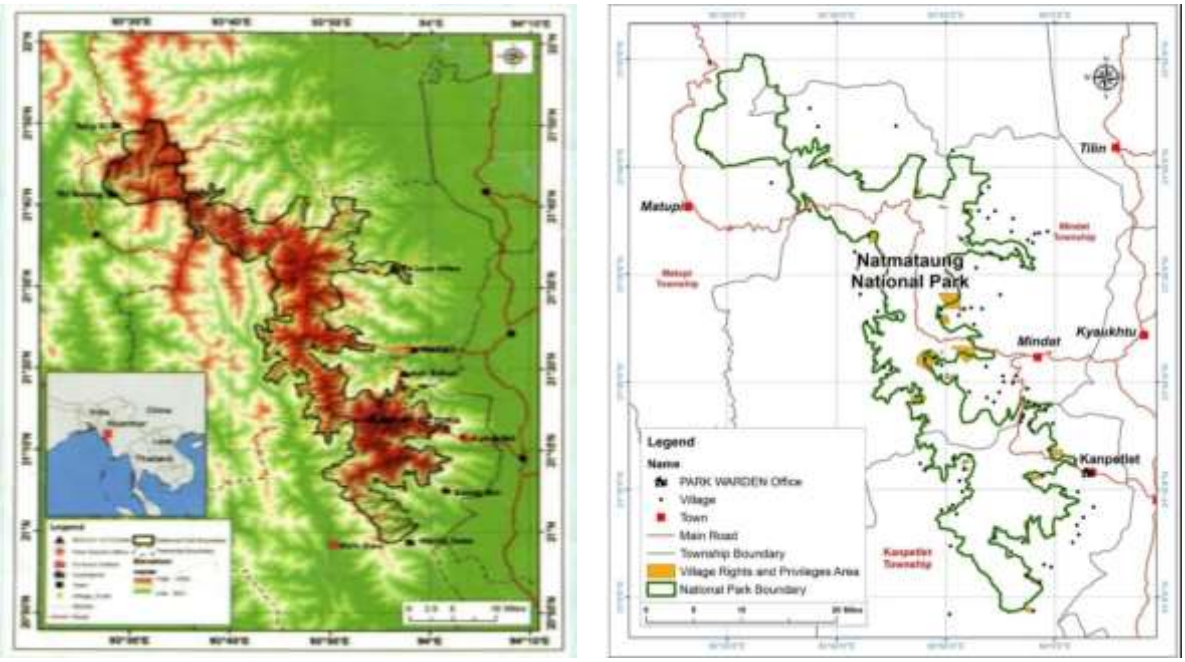
The Study Area



INVESTIGATION ON CULTIVATION OF FOUR PERENNIAL RICE CULTIVARS IN FIELD UNDER MANDALAY CLIMATE

Su Su Hlaing[1], Soe Soe Aung[2], Hlaing Nwe Thynn[3]

Abstract

This study deals with the climatic adaptation of four perennial rice (PR) cultivars (PR 23, PR 25, PR 101, PR 107) for five growing seasons using Single Trial Design, and their growth and yield characters were studied in the Mandalay area, Patheingyi Township. The cultivation was carried out in the field conditions at Let Thit Village, Patheingyi Township, Mandalay Region from 2021 to 2022. The seeds were provided by the Research Center of Perennial Rice Engineering and Technology in Yunnan, School of Agriculture (SOA), Yunnan University (YNU) in China. Plant height and number of tillers were collected every 15-days after transplanting [DAT (Day After Transplanting)] until harvesting. At the harvesting times, number of panicles, panicles lengths, numbers of spikelets, filled-grains weight, filled-grains percentages and 1000 grains weight were collected and measured from ten sampling plants of each plot, and the yield data were collected from the 6.6 x 6.6 ft^2 of two sampling plots. In the growth characters, PR 101 resulted in the tallest plant height during the five growing seasons. The maximum numbers of tillers were found in PR 23. In the yield characters, the maximum numbers of panicles, filled-grains weight, filled-grains percentages and highest yields were found in PR23. The longest panicle lengths were found in PR 107, maximum numbers of spikelets in PR 101, maximum straw dry weight in PR 25 and highest 1000 grains weight in PR107 were obtained respectively. In according of these findings indicated the capability of perennial rice cultivation, well-adaptable growth characters and high yields of perennial rice cultivars over their successive regrowth seasons starting from the first growing seasons until the fifth growing seasons, short duration of life span, low cost of labor and cultural management system in field preparation during rice cultivation. Therefore, the cultivation of PR cultivars would provide many benefits for the efficient food supply and security, and also for the socio-economic development of Mandalay Region.

Keywords: climatic adaptation, perennial rice (PR) cultivar, growth characters, yields

Introduction

Rice (*Oryza sativa* L.) is the primary stable food for more than two billions of people in Asia and about hundred millions of people in Africa and Latin America. Rice is one of the most important cereal crops cultivated in the world. It provides foods for more than half of the world population. Asia is the leader in rice production and accounts for more than 90% of world rice production (Matsnoto *et al.* 2005). Agriculture in Myanmar is also dominated by paddy rice cultivation, which generates a direct or indirect economic livelihood for over 75% of the population. In Myanmar, rice is not only the stable food but also the major export product in the past and future.

With the global population increasing, pressure on the resource base and the impact of climate change, even the marginal lands, which currently support 50% of world population are at risk of degradation under annual cropping, and must be farmed sustainably in future to meet the increasing demand for food and livelihood (Shilai *et al.* 2017). Conversion of annual fields into perennial fields offers many biodiversity friendly benefits. One of those benefits is reduced soil erosion. Annual farming leaves fields fallow at the intervals of growing seasons and offers less root mass through the growth cycle. Perennial plants develop much greater root mass and

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protect the soil year-round. Perennial farming can reduce erosion rates by up to 50% (Pimentel and Huang,1997).

Perennial plants also conserve fresh water better than annual plants. Annual crops lose up to five times more water than perennials (Glover and Regauold, 2010). This means that annual fields require more irrigation which threatens fresh water sources and consequently biodiversity in certain ecosystems. Perennial grain crops hold the promise of stabilizing fragile lands, while contributing grain and grazing in mixed farming systems. Farmers were keen to grow it because of reduced costs and especially savings in labor (Huang *et al*. 2018).

Perennial rice is currently the most advanced of the perennial cereal species, as some cultivated rice strains are already able to have regrowth after harvesting, especially in humid tropical areas. In fact, humid tropical areas could be the first areas to adopt new perennial rice types. In temperate areas, the most important limitations for perennial rice may be drought, cold resistance and longevity (Alessandro 2018).

The aim and objectives of this study was to study the effects of growth characters on four perennial rice cultivars, under field conditions for five growing seasons. The objectives were to study the growth characters of four perennial rice (PR) cultivars for five growing seasons under field conditions and to monitor the yield characters of those PR cultivars for five growing seasons after the cultivation in field.

Materials and Methods

The field experiments were conducted in field located at Let Thit village, Patheingyi Township, Mandalay Region from July 2021 to December 2022.

The seeds of four perennial rice cultivars, namely PR 23, PR 25, PR 101 and PR 107 were used. All the seeds were provided by the Research Center of Perennial Rice Engineering and Technology in Yunnan, School of Agriculture (SOA), Yunnan University (YNU) from China (Figure 1).

Soil Samples Collection and Soil Analysis

The soil samples were randomly collected from the experimental field at the depth of 20 cm. The collected soil samples were analyzed before cultivation at Laboratory of Land Use Department, Department of Agriculture (DOA), Chan Mya Thar Si Township in Mandalay.

Meteorological Determination

During the rice cultivation, the meteorological data of monthly temperature, rainfall and relative humidity (RH) were obtained from the Department of Meteorology and Hydrology Station, Patheingyi Township in Mandalay.

Preparation of Seed Beds

Seeds were soaked into the water for 24 hours and germinated with wrapping wet clothes for 36 hours. The germinated seeds were soaked in water again for 10-15 minutes before sowing to the nursery seed beds. Some water was sprayed 2 times per week during germinating.

Each nursery seed bed was laid out 120 cm long, 90 cm wide and 15 cm height. The 165 g of seeds weight for each cultivar was sown in each seed bed. Then, the 28-day old-seedlings were transplanted into the paddy fields.

Figure 1. Seeds of four perennial rice (PR) cultivars

A. PR 23 B. PR 25 C. PR 101 D. PR 107

Field Preparation and Cultivation

For the field preparation, one stroke of ploughing and two strokes of harrowing, irrigation and soil preparation were carried out for two weeks before transplanting. Transplanting was carried out after three or four leaves were emerged from the seedlings at 28 days after sowing (DAS). The healthy seedlings were selected and transplanted into the designated plots in field conditions.

Irrigation was 2 - 5 times per month according to the available water contents of soil. The compound fertilizers, N: P: K (15: 15:15) were treated three times. The compound fertilizer, 100 kg ha^{-1}, was applied at the transplanting time. For the second time, the combination of compound fertilizer 100 kg ha^{-1} and urea 25 kg ha^{-1} was applied on the paddy fields during the growing stage of tilling. For the third time, the combination of compound fertilizer 100 kg ha^{-1} and urea 50 kg ha^{-1} was applied at the time of flowering (anthesis). Those methods for the fertilizer treatments were recommended by Department of Agriculture. There are two times fertilizer applications for every growing season. First time applications were made within 5 days after the harvesting times and the second time application were made during the initial heading stage. 15 cm length of stem cutting was left after harvest to be regrowth of stems for the next growing season.

Experimental Layout and Design

Field experiments were carried out in field from first to fifth growing seasons. The single trial experimental design was laid out for each cultivar. The plot area for each cultivar was 510 cm × 3750 cm. Each plot included 15 rows and each of which contained 125 plants. The row-to-row spacing and plant-to-plant spacing were 30 cm each other (Figure 3.4).

Data Collection

Data were collected at every 15-days after transplanting (DAT) until harvesting. Ten plants from each plot were randomly collected and measured for the growth, yield components and yields parameters. The 6.6 × 6.6 ft^2 of two plots data were randomly collected for the plot yields at harvest. Filled-grains weight per panicle, filled-grains percentages, 1000 grains weight, grain yields and plot yields were recorded at the harvesting times.

Statistical Analysis

The data were analyzed by using the CROPSTAT, Version 7.0. The treatment means were compared by LSD (Least Significant Differences) at 5% level of significance (IRRI 1997).

Harvesting and Threshing

Harvesting was carried out at maturity stage when 85% of grains were changed into golden color. All plants were harvested and threshed manually. The grains of each plot were allowed separately for sun drying until the well-dried.

Determination of Growth and Yield Characters

The data of plant height, numbers of tillers per plant, numbers of panicles plant^{-1} and numbers of spikelets per panicle were collected from ten sample plants of each plot at every 15-days after transplanting (DAT) and from the 6.6 × 6.6 ft^2 size of two sampling plots at harvest.

Yield per plant (g) was calculated with the following formula (IRRI 2013).

$$\text{Yield plant}^{-1}\text{ (g)} = \frac{1000 \text{ grain weight} \times \text{Filled grain \%} \times \text{Effective tillers} \times \text{Total Spikeletes}}{1000 \times 100}$$

Results and Discussion

Plant Height

The plant heights of all PR cultivars were measured through first to fifth growing seasons. PR 101 showed the tallest plant height in all growing seasons. The plant heights of all PR cultivars were shorter the second growing season than the first growing season. The plant height was gradually tallest in third growing season, and it was found that the plant height of all rice cultivars was gradually shortest from third to fifth growing seasons. In those findings, the PR 101 showed the rapid their growth in all growing seasons, however, it did not obtain the highest yields. (Table 1 and Figure 2-7).

Table 1. Means plant height of four PR cultivars from first to fifth growing seasons

PR Cultivars	Plant height (cm plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	86.90	77.40	104.50	93.10	80.00
PR 25	92.40	79.00	102.00	93.30	81.70
PR 101	139.40	92.00	112.20	100.00	95.70
PR 107	101.80	65.30	82.10	88.50	84.70

Number of Tillers

The number of tillers was collected at the time of harvesting time for all growing seasons. The maximum number of tillers was found in the second growing season and the minimum number in the first growing season. Among all PR cultivars, PR 23 showed the maximum numbers of tillers and the minimum number of tillers was observed in PR 101 (Table 2).

Table 2. Means numbers of tillers of four PR cultivars from first to fifth growing seasons

PR Cultivars	Number of Tillers (plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	18.20	40.50	27.20	35.50	27.60
PR 25	18.00	37.20	26.20	34.00	24.20
PR 101	15.60	36.10	25.10	23.50	30.50
PR 107	19.20	31.10	28.50	20.40	26.80

Number of Panicles

The number of panicles per plant was collected at the harvesting time for all growing seasons. The maximum number of panicles was found in PR 23 and minimum number in PR 107. It was found that if the number of tillers was increase, the number of panicles was also increase, and if the number of tillers was decreased, the number of panicles also decreased, which demonstrated the positive correlation between number of tillers and number of panicles. Therefore, these findings were agreed with Hossain *et al.* (2008), they reported that the number of panicles depends on the number of tillers and proportion of effective tillers (Table 3).

Table 3. Means numbers of panicles of four PR cultivars from first to fifth growing seasons

PR Cultivars	Number of Panicles (plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	15.70	14.20	12.90	14.20	20.80
PR 25	17.60	16.50	14.40	16.50	17.00
PR 101	13.20	13.10	16.80	16.00	15.80
PR 107	14.20	13.60	13.90	13.70	12.50

Panicle Length

The panicle length (cm) was measured at the time of harvesting. The maximum panicle length was found in PR 107 in the first growing season and the minimum length was found in PR 23 in the fifth growing season (Table 4).

Table 4. Means panicle lengths of four PR cultivars from first to fifth growing seasons

PR Cultivars	Panicle Length (cm plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	25.51	23.20	25.10	23.10	16.90
PR 25	25.20	21.80	23.40	25.70	25.70
PR 101	31.40	28.00	29.80	24.40	20.80
PR 107	31.50	27.40	26.50	25.20	20.60

Number of Spikelets

The number of spiklelets per panicle was measured at the time of harvesting. The maximum number of spikelets was found in PR 101 (Table 5).

Table 5. Means numbers of spikelets of four PR cultivars from first to fifth growing seasons

PR Cultivars	Number of Spikelets (panicle^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	8.09	7.97	6.90	8.08	8.37
PR 25	7.52	7.43	6.85	7.76	7.03
PR 101	9.31	8.06	6.65	8.59	7.25
PR 107	6.29	6.52	6.34	6.54	6.26

Filled-Grains Weight

Filled grain weights of all PR cultivars were measured after harvesting. The maximum numbers of filled-grains weight were found in the first growing seasons. However, the numbers of filled-grains weight were gradually reduced from second to fifth growing seasons (Table 6).

Table 6. Means filled-grains weight of four PR cultivars from first to fifth growing seasons

PR Cultivars	Filled-grains weight (g plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	28.88	3.57	4.62	1.41	4.52
PR 25	28.35	4.25	5.39	2.31	4.24
PR 101	27.87	8.07	9.82	9.95	6.35
PR 107	22.43	4.74	6.63	7.52	3.63

Filled-Grain Percentages

The maximum filled-grain percentage was found in the first growing seasons for all PR cultivars. Filled-grains percentages were gradually reduced from second to fifth growing season (Table 7).

Table 7. Means filled-grains percentages for four PR cultivars from first to fifth growing seasons

PR Cultivars	Filled grain percentage (% plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	94.61	73.81	69.93	47.27	78.86
PR 25	95.53	78.51	71.36	65.10	75.64
PR 101	96.19	87.92	89.91	88.47	83.00
PR 107	84.88	77.03	81.94	84.61	75.61

1000 Grains Weight

The maximum 1000 grains weights of all PR cultivars were found in the first growing seasons and gradually lower in season by season and the lowest grains weights were observed in the fifth growing seasons for all PR cultivars (Table 8).

Table 8. Means 1000 grains weights of four PR cultivars from first to fifth growing seasons

PR Cultivars	1000 grains weight (g plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	27.26	20.64	20.65	19.47	19.51
PR 25	27.86	20.55	22.39	19.50	19.59
PR 101	26.36	18.14	17.94	18.05	18.17
PR 107	33.26	22.50	25.96	20.90	18.53

Straw Dry Weights

The straw dry weights of all PR cultivars were also measured after harvesting. The lowest straw dry weight was observed in PR 23 and PR 25 in their first growing seasons, and PR 101 and PR 107 in their fourth growing seasons (Table 9).

Table 9. Means straw dry weight for four PR lines from first to fifth growing seasons

PR Cultivars	Straw dry weight (g plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	27.60	36.44	36.06	28.80	30.88
PR 25	29.10	38.15	41.65	29.76	32.23
PR 101	54.72	40.58	40.19	33.00	34.43
PR 107	38.88	19.22	16.81	13.21	15.69

Yields

The maximum yield was obtained in the first growing season for all PR cultivars. The yield of all PR cultivars was decreased in their fifth growing season compared with their first growing seasons. The highest yield was found in PR 25 in the first growing season (Table 10).

Table 10. Means yield for four PR lines from first to fifth growing seasons

PR Cultivars	Yield (g plant^{-1})				
	First growing season	Second growing season	Third growing season	Fourth growing season	Fifth growing season
PR 23	12.60	1.72	1.28	1.05	2.72
PR 25	12.42	1.97	1.55	1.64	1.76
PR 101	10.27	1.58	1.77	2.30	1.70
PR 107	11.12	1.54	1.81	1.57	1.15

Physical and Chemical Properties of Cultivated Soil

Composite soil sample was collected from the experimental sites before starting the experiment and was analyzed for various physiochemical properties at Laboratory of Land Use Laboratory, Department of Agriculture (DOA), Chan Mya Thar Si Township, Mandalay. Physicochemical properties of the soil were measured by the standard methods of soil chemical analysis. The analysis for respective years of experimentation revealed that the soil had 1.44% organic carbon, 2.49% humus, 0.27% available nitrogen, 9.00 ppm available phosphorus, 30.42% available potassium and Na, K, Ca and Mg 1.10, 0.64, 28.29 and 5.47 mg kg/soil respectively, 1.36% moisture content of the soil, 0.14 mg/kg EC soil with pH 8.3 (Table 11).

According to the result of soil analysis data, it was found that the indigenous nitrogen content was low in the experimental soil. Therefore, the compound fertilizers containing nitrogen were treated to all PR cultivars. Remarkably, it was found that all PR cultivars were growth in all growing season.

The soil type of experimental area was clay soil and Fugen *et al.* (2016) reported that clay soil was suitable for cultivation of rice and the grain yield in clay soil was 46% higher than in sandy soil. Clay soil has more fine particles that can hold water and nutrients, thus it can retain more water and nutrients needed by water-loving rice plant.

The soil samples of experimental site were analyzed and these result show that pH of the sample soil was 7.85 and it is moderately alkaline. The suitable soil pH for rice cultivation is at pH 6 (Harrell & Saichuk 2011) and most of the plants can absorb nutrients well between pH 6.0 - 7.5 (Yoshida 1972). According to the recommendation of Land Use Laboratory, Department of Agriculture, rice prefers the pH 5.5 - 7.5, therefore PR can grow very well in these designated field plots.

Table 11. Physical and chemical properties of cultivated soil of PR cultivars

Physical and Chemical Properties	Results				
		Na^+	K^+	Ca^{++}	Mg^{++}
Total N (%)	0.27	-	-	-	-
Available P_2O_5 (ppm)	9.00	-	-	-	-
Available K_2O (mg/ 100 g)	30.42	-	-	-	-
Exchangeable Cations	-	1.10	0.64	28.29	5.47
Moisture (%)	1.36	-	-	-	-
pH (1: 2.5)	8.3	-	-	-	-
EC ms / cm	0.14	-	-	-	-
Organic Carbon (%)	1.44	-	-	-	-
Humus (%)	2.49	-	-	-	-

Source: Laboratory of Land Use Laboratory, Department of Agriculture (DOA), Chan Mya Thar Si Township, Mandalay.

Meteorological Data during PR Cultivation

The meteorological report was obtained from Department of Meteorology and Hydrology Station, Patheingyi Township, Mandalay (Table 12).

Monthly meteorological data were also recorded and concerning with the meteorological data, the mean temperature for cultivated land was 29.07°C. This temperature was not agreed with Yoshida (1981).

Table 12. Meteorological data during the cultivation of PR cultivars (2021-2022)

Month	Rainfall (mm)		Temperature (°C)		Mean Relative Humidity (%)	
	2021	2022	2021	2022	2021	2022
January	0.00	0.00	23.91	22.46	65.44	68.71
February	0.00	0.00	25.74	23.01	49.00	57.13
March	0.00	0.03	30.14	30.64	40.18	46.58
April	0.07	0.11	32.28	32.01	50.92	55.77
May	0.17	0.22	32.93	30.17	57.34	70.45
June	0.02	0.02	32.35	31.42	59.00	63.90
July	0.26	0.12	31.19	35.08	68.03	66.18
August	0.50	0.17	28.86	34.69	73.17	70.27
September	0.27	0.14	29.69	31.11	79.14	70.55
October	0.21	0.23	30.11	30.17	74.79	72.42
November	0.06	0.00	27.10	27.23	75.45	65.08
December	0.00	0.00	23.91	25.22	71.92	67.40
Mean	0.13	0.09	29.02	29.48	63.70	64.54

Source: Department of Meteorology and Hydrology Station, Patheingyi Township, Mandalay.

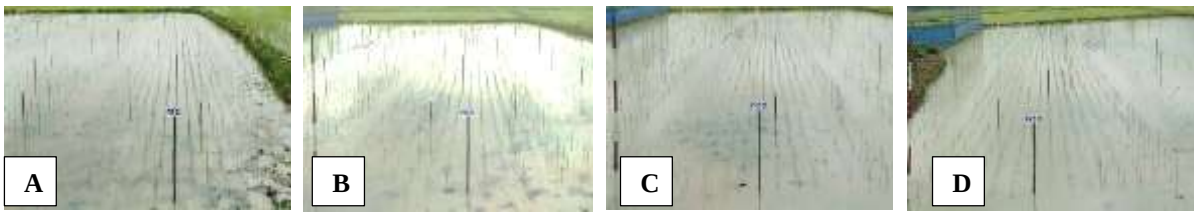

Figure 2. First growing seasons of perennial rice (PR) cultivars in field
A. PR 23 B. PR 25 C. PR 101 D. PR 107

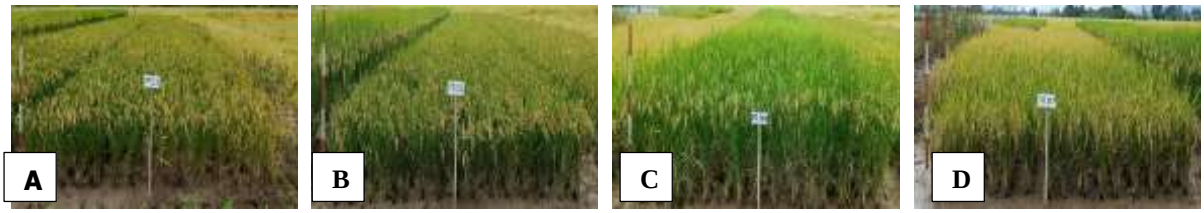

Figure 3. First growing seasons of perennial rice (PR) cultivars in field
A. PR 23 B. PR 25 C. PR 101 D. PR 107

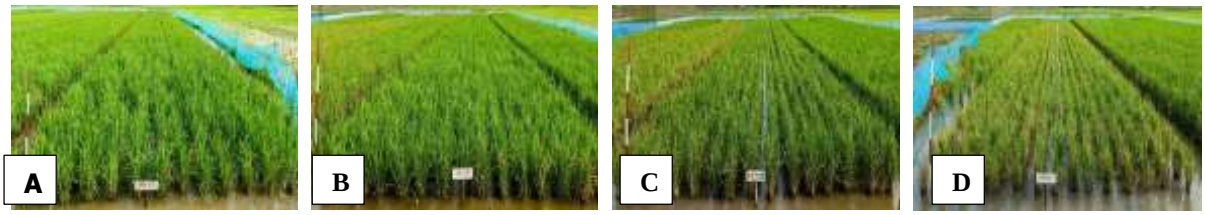

Figure 4. Second regrowth seasons of perennial rice (PR) cultivars in field
A. PR 23 B. PR 25 C. PR 101 D. PR 107

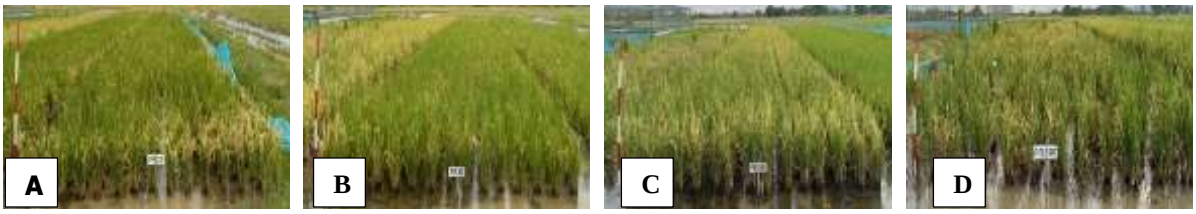

Figure 5. Third regrowth seasons of perennial rice (PR) cultivars in field
A. PR 23 B. PR 25 C. PR 101 D. PR 107

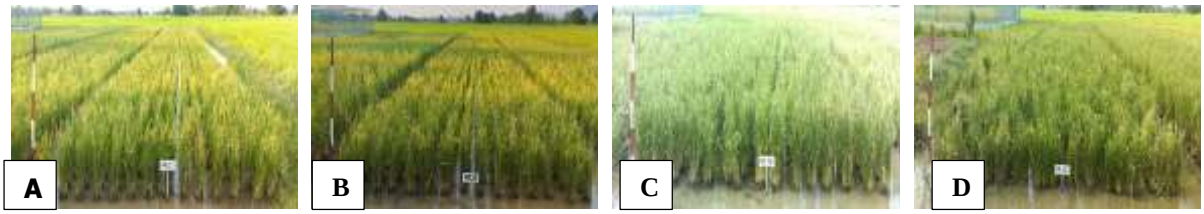

Figure 6. Fourth regrowth seasons of perennial rice (PR) cultivars in field
A. PR 23 B. PR 25 C. PR 101 D. PR 107

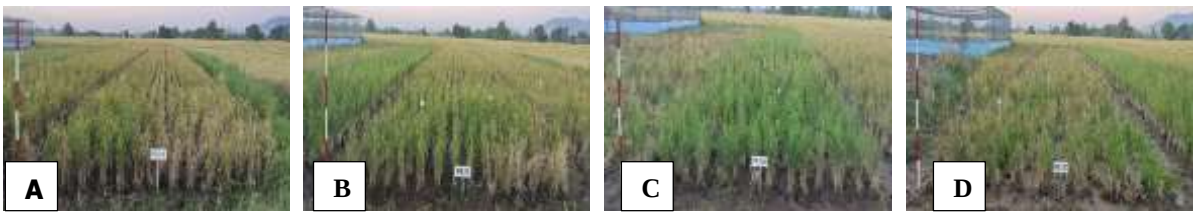

Figure 7. Fifth regrowth seasons of perennial rice (PR) cultivars in field
A. PR 23 B. PR 25 C. PR 101 D. PR 107

Conclusions

This study demonstrated the well-adaptable growth characters of four PR cultivars in the experimental field in Mandalay Region, particularly in Patheingyi Township and the high production yields in four PR cultivars over all of five growing seasons. Besides, the PR cultivars obviously performed early flowering, early harvesting maturities, and continuously growing. Moreover, the time and costs of field preparation, cultural management system would be reduced significantly during their cultivation and also found as the well adaptability under the Mandalay climate. Therefore, it was believed that the cultivation of PR cultivars would provide many benefits for the efficient food security and socio-economic development, especially in Mandalay Region.

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TAXONOMIC STUDY ON TEN WILD MUSHROOMS FROM NGALAIK RESERVED FOREST IN NAYPYITAW UNION TERRITORY

Su Hlaing Htwe[1], Aye Nann Han[2]

Abstract

The taxonomic study on wild mushrooms was undertaken from Ngalaik Reserved Forest in Naypyitaw Union Territory. The study areas are situated between North latitude 19° 59.974' and East longitude 96°16. 795'. Ten species were collected during the year from July to October in 2022. The 10 species belonging to 6 genera, 4 families and 2 order were collected, preserved, classified and identified. The collected species were identified as *Agaricus arvensis* Schaeff., *Agaricus bitorquis* (Quel.) Sacc., *Lepiota castaneidisca* Murrill., *Termitomyces bulborhizus* T.Z.Wei, *Termitomyces clypeatus* R.Heim., *Termitomyces eurhizus* (Berk.) R.heim, *Termitomyces heimii* Natarajan., *Podaxis pistillaris* (L.)Fr., these 8 species are growing on the soil *Podoscypha petalodes* (Berk.) Boidin.and *Ganoderma lucidum (*Curtis) P. Krst., are growing on decaying wood and root. In the present study, the edible, inedible, and medicinal wild mushrooms species were also found. Among them 7 species were edible 2 species were inedible and 1 species were usable for medicinal mushroom. The edible mushrooms were found under genus of *Agaricus arvensis* Schaeff., *Agaricus bitorquis (*Quel.), *Podaxis pistillaris* (L.), *Termitomyces bulborhizus* T. ZWei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan. Inedible 2 species are *Lepiota castaneidisca* Murrill., *Podoscypha petalodes* (Berk.) Boidin. *Ganoderma lucidum* (Curtis) P.Karst. was usable for the medicinal purpose. An artificial key to the studied species was constructed and presented.

Keywords: Taxonomic of ten wild mushrooms, Ngalaik Reserved Forest, Naypyitaw Union Territory

Introduction

Fungi are important organisms that serve many vital functions in forest ecosystem including decomposition, nutrient cycling, symbiotic relationships with trees and other plants, biological control of other fungi, and as the causal agents of diseases in plants and animals. Mushrooms are sources of food for wildlife and fungi that cause decay in living trees are beneficial to many species of birds and mammals. Macrofungi are distinguished from other fungi by their fruiting structure that we know as mushrooms (Ostry *et.al* 2010).

Mushrooms are familiar to everyone. Edible as well as medicinal properties of mushrooms were known to many of the ancient civilization. There are about 45,000 known species of fungi and about 2000 of them are considered edible (Nair 1990). Mushrooms are one of the most important sources of nutritious food and of great economic importance. They also have rich nutritional value with high contents of proteins, vitamins, minerals, fibers, trace elements and low no calories and cholesterol (Smith *et al*. 2002).

Mushroom, even today, are still not widely accepted as useful medicinal. More than 1,000 kinds of wild mushrooms are sold as edible mushrooms around the world. More than 400 kinds are medicinal and are being used by people in almost every country (Lincoff 2010). In this research, the study area, Ngalaik Reserved Forest is situated between North latitude 19° 59.974' and East longitude 96°16. 795'. In this research, the 10 wild mushrooms were collected in Ngalaik Reserved Forest.

In 2015, Aye Aye Maw presented taxonomic studies on wild mushrooms from Monywa District. The taxonomic studies on the wild mushrooms from Southern Shan State were presented

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by Ohnmar Htwe (2017). Meiktila, Popa and Yinmarbin areas in Mandalay Region were studied by Nilar Cho (2018), Katha District, Sagaing Region were revealed by Chaw Thiri Mon (2020). Loikaw District in Kayah State were studied by Au Au Khaing (2021). Although previous researchers had done to identify and classify the mushrooms in Myanmar, the taxonomic studies on wild mushrooms from Naypyitaw Union Territory are still lacking. Therefore, it is needed to carry out this research. The present research works were focused on those areas for fulfillment the information on the wild mushroom flora of Naypyitaw Union Territory.

The aim and objectives of this study were to identify and classify the morphological characteristics of the wild mushrooms from Ngalaik Reserved Forest, Ottarathiri Township in Naypyitaw Union Territory, to record their taxonomic characteristics and distribution, and to fulfill for providing the scientific information and the compilation of the wild mushroom flora in Myanmar.

Materials and Methods

The study areas of on wild mushrooms was undertaken from Ngalaik Reserved Forest in Naypyitaw Union Territory were situated between North latitude 19° 59.974' and East longitude 96°16. 795. The fresh specimen of wild mushrooms were from Ngalaik Reserved Forest from July to October in 2022.

All the fresh specimens were recorded with digital camera to get their actual habitat and noted their fruiting characteristics. The collection, preservation and the spores print technique were followed by Thomas (1948), Krieger and Schaffer (1967) and Schaffer and Pacionic (1981). The fleshy mature specimens were selected. The stipe was removed by cutting it off as close as possible to the point of attachment of cap. It is obtained by placing a cap with the hymenium facing down on a sheet of white, black paper or a piece of glass-slide. A blow can serve as a cover after a few hours, a layer of the spores was deposited. The real color of the spores was determined by spore prints.

The collected specimens were preserved in Formalin-Acetic acid - Alcohol (FAA) by the ratio of 5: 5: 90. Some of the dried specimens were placed in plastic bags and plastic bottles. The classification and identification of collected specimens were done by comparing the literature: Thomas (1948), Alexopoulos (1962), Krieger (1967), Pacioni (1981), Keizer (1998). An artificial key to the studied species was also constructed and presented. The herbarium specimens will numbered and deposited at the herbarium room of Department of Botany, University of Mandalay for the references and other scientific studies.

Result

Ten species belonging to 6 genera, 4 families and 2 order were collected, from Ngalaik Reserved Forest in Naypyitaw Union Territory. These species are *Agaricus arvensis* Schaeff., *Agaricus bitorquis* (Quel), *Lepiota castaneidisca* Murrill, *Podaxis pistillaris* (L.) Fr. *Termitomyces bulborhizus* T.Z.Wei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan, *Ganoderma lucidum* (Curtis) P.Karst. *Podoscypha petalodes* (Berk.) The morphological and spores characters of those species were classified and identified. The lists of collected species and their comparable morphological characteristics were shown in Table 1-2 and Figure 1-10.

Table1. List of collected wild mushroom species from Ngalaik Reserved Forest

Territory Sub-Class	Order	Family	No.	Scientific Name
Homobasid-omycetidae	Agaricales	Agaricaceae	1.	*Agaricus arvensis* Schaeff
			2.	*Agaricus bitorquis* (Quel) Sacc.
			3.	*Lepiota castaneidisca* Murrill.
			4.	*Podaxis pistillaris* (L.) Fr.
		Lyophyllaceae	5.	*Termitomyces bulborhizus* T.Z.Wei
				Termitomyces clypeatus R. Heim
			7	*Termitomyces eurhizus* (Berk.) R.heim
			8	*Termitomyces heimii* Natarajan
	Polyporales	Ganodermataceae	9	*Ganoderma lucidum* (Curtis) P.Krst.
		Meruliaceae	10	*Podoscypha petalodes* (Berk.) Boidin.

Table 2. Comparable morphological characteristics of wild mushrooms form Ngalaik Reserved Forest in Naypyitaw Union Territory

No	Scientific name	Growing habitat	Edible/ Inedible	Caps			Gills/Pores	
				Colour	Shape	Umbonate	Colour	Attachment
1	*Agaricus arvensis* Schaeff.	soil	edible	white	expanded	absent	chocolate - brown	free
2	*Agaricus bitorquis* (Quel.) Sacc.	soil	edible	white	expanded	absent	dark-brown	free
3	*Lepiota castaneidisca* Murrill.	soil	inedible	white with reddish bown	expanded	present	white	free
4	*Podaxis pistillaris* (L.) Fr.	sandy soil	edible	white	cylindrical	absent	white	-
5	*Termitomyces bulborhizus* T.Z.Wei	soil	edible	greyish brown	expanded	present	pale pink	
6	*Termitomyces clypeatus* R.Heim.	soil	edible	pale brown	expanded	present	pale pink	free
7	*Termitomyces eurhizus* (Berk.) R.heim	soil	edible	yellowish brown	expanded	present	pale pink	free
8	*Termitomyces heimii* Natarajan.	soil	edible	white	expanded	present	white	free
9	*Gandoderma lucidum (*Curtis) P.Krst.	decaying	inedible	reddish-brown	kidney-shaped	absent	cream colored	free
10	*Podoscypha petalodes* (Berk.) Boidin.	wood	inedible	pale brown	funnel	absent	cream colored	-

Table 2 Continued

No.	Scientific name	Stipes				Spores			
		Shape	Colour	Hollow/Solid	Rings	Colour	Shape	Texture	Size
1.	*Agaricus arvensis* Schaeff.	equal	white	hollow	present	brown	ellipsoid	smooth	10.0-15.5× 7.5-10.5µm
2	*Agaricus bitorquis* (Quel) Sacc	equal	white	solid	present	brown	ellipsoid	smooth	10.0-15.0× 7.5-12.5µm

No.	Scientific name	Stipes				Spores			
		Shape	**Colour**	**Hollow/Solid**	**Rings**	**Colour**	**Shape**	Texture	Size
3.	*Lepiota castaneidisca* Murrill.	bulbous	white	hollow	present	white	ellipsoid	smooth	10.0-12.5× 7.5-10.5µm
4.	*Podaxis pistillaris* (L.) Fr.	equal	white	solid	absent	white	ellipsoid	smooth	10.5-20.0× 6.5-7.5µm
5.	*Termitomyces bulborhizus* T.Z.Wei	equal	white	solid	absent	pink	ellipsoid	smooth	12.5-15.0× 10.0-12.5µm
6.	*Termitomyces clypeatus* R.Heim.	equal	yellowish brown	solid	absent	pink	ellipsoid	smooth	10.5- 12.5× 7.5-10.0µm
7.	*Termitomyces eurhizus* (Berk.) R.heim	equal	pale brown	solid	absent	pink	ellipsoid	smooth	12.5-17.5× 7.5-12.5µm
8.	*Termitomyces heimii* Natarajan	equal	white	solid	present	white	ellipsoid	smooth	12.5-20.0× 7.5-10.0µm
9	*Ganoderma lucidum* (Curtis) P.Krst.	equal	reddish brown	solid	absent	brown	ellipsoid	smooth	8.5-10.5× 5.5-6.5µm
10	*Podoscypha petalodes* (Berk.) Boidin.	flattened cylindrical	pale yellow	solid	absent	white	ellipsoid	smooth	7.5-10.0× 5-7.5µ

Figure 1. *Agaricus arvensis* Schaeff.
(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 2. *Agaricus bitorquis* (Quel.) Sacc
(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

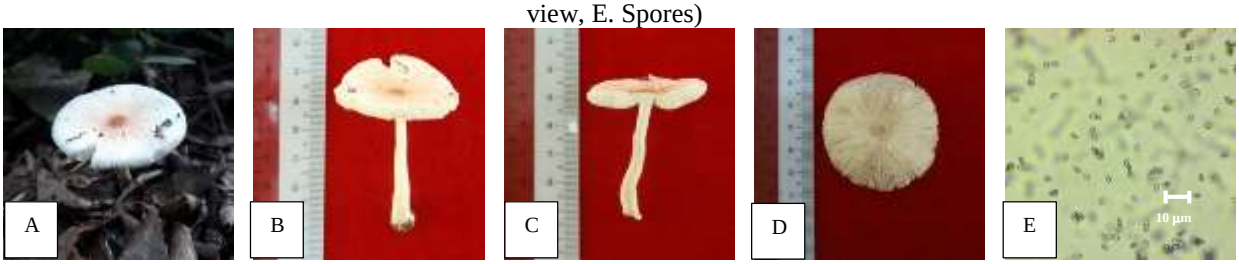

Figure 3. *Lepiota castaneidisca* Murrill.
(A. Growing habitat, B. Fruiting body in lateral view, C. Pileus in lower view, E. Spores)

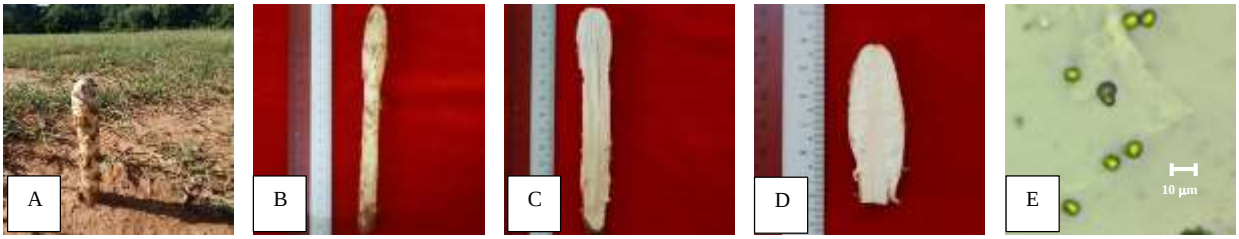

Figure 4. *Podaxis pistillaris* (L.) Fr.
(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 5. *Termeitomyces bulborhizus* T.Z Wei

(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

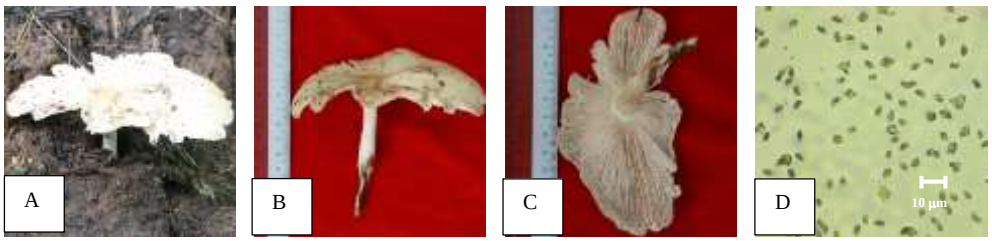

Figure 6. *Termitomyces clypeatus* R. Heim
(A. Growing habitat, B. Fruiting body in lateral view, C. Pileus in lower view, D. Spores)

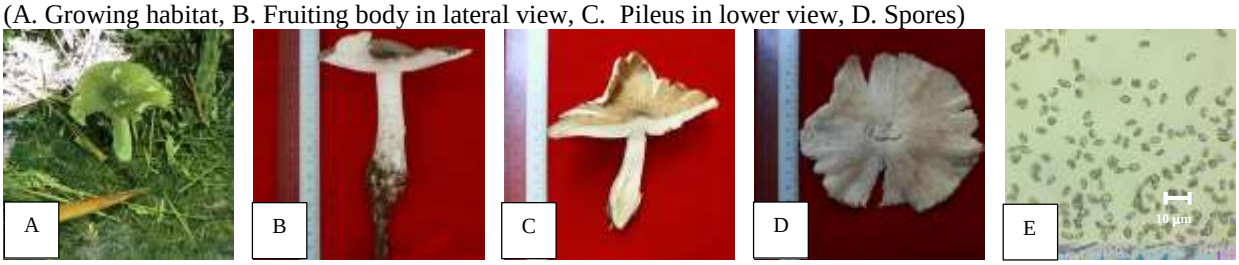

Figure 7. *Termeitomyces eurhizus* (Berk.) R.Heim
(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

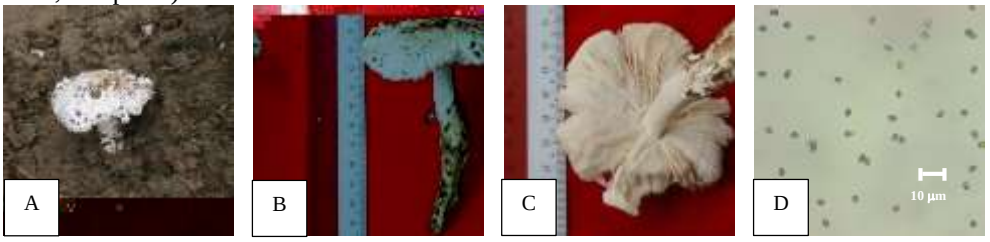

Figure 8. *Termeitomyces heimii* Natarajan
(A. Growing habitat, B. Fruiting body in lateral view, C. Pileus in lower view, D. Spores)

Figure 9. *Ganoderma lucidum* P.Karst
(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

Figure 10. *Podoscypha petalodes* (Berk.) Boidin.

(A. Growing habitat, B. Fruiting body in lateral view, C. Fruiting body in longitudinal section, D. Pileus in lower view, E. Spores)

An Artificial Key to the Studied Species

1. Hymenium pores ---------- 2
1. Hymenium gills ---------- 4
2. Gleba present; cap shaped cylindrical, white ---------- 4. *Podaxis pistillaris*
2. Gleba absent; cap shaped kidney or funnel, reddish or pale- brown ---------- 3
3. Stipe equal, reddish brown; spore brown ---------- 9. *Ganoderma lucidum*
3. Stipe flattened cylindrical, pale yellow; spore white ----------10. *Podoscypha petalodes*
4. Umbo absent ---------- 5
4. Umbo present ---------- 6
5. Stipe hollow; gills color chocolate- brown ----------1. *Agaricus arvensis*
5. Stipe solid; gills color dark- brown ---------- 2. *Agaricus bitorquis*
6. Spore color white ---------- 7
6. Spore color pink ---------- 8
7. Stipe bulbous, hollow; cap color with reddish brown ---------- 3. *Lepiota castaneidisca*
7. Stipe equal, solid; cap color white without reddish brown ----------8. *Termitomyces heimii*
8. Stipe color yellowish brown; spore10.5- 12.5×7.5-10.0µm ----------
----------6. *Termitomyces clypeatus*
8. Stipe color white or pale brown; spore12.5-15.0×10.0-12.5µm ---------- 9
9. Cap color greyish brown ---------- 5. *Termitomyces bulborhizus*
9. Cap color yellowish brown ---------- 7. *Termitomyces eurhizus*

Discussion and Conclusion

The taxonomic study on wild mushrooms was undertaken from Ngalaik Reserved Forest in Naypyitaw Union Territory. The study areas are situated between North latitude 19° 59.974' and East longitude 96°16. 795'. Ten species were collected during the year from July to October in 2022. The 10 species belonging to 6 genera, 4 families and 2 order were collected, preserved, classified and identified.

Among them 7 species were gill mushrooms type, *Agaricus arvensis* Schaeff., *Agaricus bitorquis (*Quel.), *Lepiota castanneidisca* Murrill, *Termitomyces bulborhizus* T.Z Wei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan. These were gills free type. The 1 species of puffball was *Podaxis pistillaris* (L.) Fr, these 8 species are growing on the soil. The 2 species of porous were, *Ganoderma lucidum* (Curtis) P.Karst. and *Podoscypha petalodes* (Berk.) Boidin., these species are growing on decaying wood and root.

Five species, *Lepiota castanneidisca* Murrill, *Termitomyces bulborhizus* T.Z Wei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan. were present the umbo on the cap. The others, *Agaricus arvensis* Schaeff., *Agaricus bitorquis (*Quel.), *Podaxis pistillaris* (L.) Fr, *Ganoderma lucidum* (Curtis) P.Karst. and *Podoscypha petalodes* (Berk.) Boidin., were absent umbo on the cap. These findings were in agreement with Thomas (1948), Nair (1990).

Two kinds of mushroom species; pore mushrooms and gillsmushrooms were recorded in this study. Among them, *Ganoderma lucidum* (Curtis) P.Karst and *Podoscypha petalodes* (Berk.) Boidin was pore mushrooms type.

Various cap shapes were also observed in this study areas. *Agaricus arvensis* Schaeff., *Agaricus bitorquis (*Quel.), *Lepiota castanneidisca* Murrill, *Termitomyces bulborhizus* T.Z Wei, *Termitomyces clypeatus* R.Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan were convex to expanded, *Podaxis pistillaris* (L.) Fr, is cylindrical, *Podoscypha petalodes* (Berk.) Boidin. is funnel shaped, *Ganoderma lucidum* (Curtis) P.Karst is kidney-shaped. These findings were in agreement with Keizer (1998), Phillips (2006).

The stipe shapes of 8 species were equal in *Agaricus arvensis* Schaeff., *Agaricus bitorquis* (Quel), *Podaxis pistillaris* (L.) Fr. *Termitomyces bulborhizus* T.Z.Wei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan, *Ganoderma lucidum* (Curtis) P.Karst. *Podoscypha petalodes* (Berk.) is flattened cylindrical shaped, *Lepiota castaneidisca* Murrill is bulbous. The hollow stipes were observed in *Agaricus arvensis* Schaeff and *Lepiota castanneidisca* Murrill. *Agaricus bitorquis (*Quel.), *Termitomyces bulborhizus* T.Z Wei, *Termitomyces clypeatus* R.Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan ,*Podaxis pistillaris* (L.) Fr, *Podoscypha petalodes* (Berk.) Boidin., *Ganoderma lucidum* (Curtis) P.Karst were solid stipes. These findings were in agreement with Keizer (1998), Thomas (1948).

The spores colour were brown in *Agaricus arvensis* Schaeff., *Agaricus bitorquis* (Quel), *Ganoderma lucidum* (Curtis) P.Krst., the spores colour were white in *Lepiota castaneidisca* Murrill., *Podaxis pistillaris* (L.) Fr., *Termitomyces heimii* Natarajan *Podoscypha petalodes* (Berk.), the spores colour were pink in *Termitomyces bulborhizus* T.Z.Wei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim. Nair (1990). Among them 7 species

were edible, 2species were inedible and 1 species were usable for medicinal mushroom. The edible mushrooms were found under genus of *Agaricus arvensis* Schaeff., *Agaricus bitorquis (*Quel.), *Podaxis pistillaris* (L.), *Termitomyces bulborhizus* T.Z Wei, *Termitomyces clypeatus* R. Heim, *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan *Ganoderma lucidum* (Curtis) P.Karst. The inedible mushrooms were found under genus *Lepiota castaneidisca* Murrill and *Podoscypha petalodes* (Berk.) Boidin. The medicinal purpose mushrooms were found under genus of *Ganoderma lucidum* (Curtis) P.Karst. These finding were in agreement with Thomas (1948), Phillips (2006), Robert & Joanne (2013), Atkinson, (1901). All the species were identified on the basic of their morphological characters as well as spores shape and texture.

Some wild mushroom species from this Ngalaik Reserved Forest in Naypyitaw Union Territory were also found in Kayah State, Mandalay Region, and Monywa Distinct. These are *Agaricus arvensis* Schaeff., *Agaricus bitorquis (*Quel.). *Ganoderma lucidum* (Curtis) P.Karst.in Monywa Distinct (Aye Aye Maw 2015). *Lepiota castaneidisca* Murrill., *Podaxis pistillaris* (L.) Fr., *Termitomyces eurhizus* (Berk.) R.Heim, *Termitomyces heimii* Natarajan in Mandalay Region (Nilar Cho, 2018), *Termitomyces bulborhizus* T.Z Wei in Kayah State (Au Au Khaing 2021).

Therefore, it would be concluded that the present study was one of the systematic records of wild mushrooms to be used by researchers in various fields of studies. This study will be provided the partial fulfillment of the information on the wild mushrooms distribution in Ngalaik Reserved Forest in Naypyitaw Union Territory and will be beneficial to accomplish the mushroom flora in Myanmar.

Acknowledgements

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MANGROVE VEGETATION DIVERSITY IN TWO VILLAGES OF KYAUKTAN TOWNSHIP, YANGON REGION

Theint Thandar Htet[1]

Abstract

Mangrove forest vegetation analyses were carried out from December 2022 to September 2023 at Kayin Chaung (KC) and Kalartan (Shwe Pyi Thit, SPT) Villages, Kyauktan Township, Yangon Region. A total of 36 quadrats (10 × 10) m^2 were established at two different sites (11 quadrats in Kayin Chaung Village and 25 quadrats in Kalartan Village). A total of 23 species belong to 22 genera of 14 families were recorded in Kayin Chaung Village and 20 species belong to 19 genera of 14 families were recorded in Kalartan Village. Species diversity, species richness and evenness were calculated by Shannon-Wiener and Simpson's indices. The quantitative analysis of species richness showed that the species richness in Kayin Chaung Village (23.83) was higher than that of Kalartan Village (20.92). The results of species diversity showed that Kayin Chaung Village was relatively diverse (H = 2.90, D = 0.93) than Kalartan Village (H = 2.55, D = 0.90). As a result of Shannon-Wiener evenness, the plant species in Kayin Chaung Village (E = 0.92) was more evenly distributed than Kalartan Village (E = 0.85). The Important Value Index (IVI) for tree species was determined by the sum of relative density, relative frequency and relative dominance by using the methods of Curtis. According to the IVI value, the ecologically successful species were *Sonneratia caseolaris* (L.) Engl. (La-mu) and *Sonneratia apetala* Banks (Kan-pa-la) in both of the villages.

Keywords: Diversity, mangrove forest, Kyauktan Township

Introduction

Mangrove forests, also called mangrove swamps, mangrove thickets or mangals, are productive wetlands that occur in coastal intertidal zones (Tue *et al*., 2012; Luo Ling and Gu Ji Dong, 2018). Mangrove forests grow mainly at tropical and subtropical latitudes because mangroves cannot withstand freezing temperatures. There are about 80 different species of mangroves, all of which grow in areas with low-oxygen soil, where slow moving waters allow fine sediments to accumulate (National Ocean Service, NOAA, 2021).

Myanmar is the largest country in mainland Southeast Asia, with a continuous coastline of almost 3,000 km, extending along the Bay of Bengal and Andaman Sea. Yangon is located in the southern part of the country in the east bank of the Yangon or Hlaing River (eastern mouth of the Ayeyarwady River), 40 km (25 mi) north of the Gulf Martaban of the Andaman Sea. Kyauktan Township is situated in the lowermost part of Myanmar, existing in the southern part of Yangon. In this research, Kayin Chaung Village (latitude 16º 34′ 31.980″ N longitude 96º 24′ 27.350″ E) and Kalartan Village (latitude 16º 29′ 29.537″ N longitude 96º 24′ 53.011″ E) are selected which are located in Kyauktan Township, Yangon Region.

In Kayin Chaung and Kalartan Villages, local communities live simply by depending on mangrove forests and their associated resources such as fire wood, coal, medicinal plants, marine products, food and shelter, etc. Therefore, the mangrove forests can assume their rightful importance for social, cultural, economic, and environmental contributions they make to the lives of all who depend on them.

The objective of the present research is to serve the source of information for the management of mangrove vegetation to ensure the conditions and sustainability of the ecosystem.

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Furthermore, mangrove vegetation analysis such as species diversity, species richness, evenness, density, frequency, and abundance can be applied to the monitoring of habitat and conservation management of mangrove forests in Kayin Chaung Village and Kalartan Village, Kyauktan Township, Yangon Region.

Materials and Methods

Study Area

Kyauktan Township is located at the lowermost part of Myanmar, existing in the southern part of Yangon. Kuauktan Township is also included in the Mottama Gulf coastline. In this study, Kayin Chaung Village and Kalartan Village were selected which were included in Kyauktan Township, Yangon region. The household and population of the study area was shown in Table 1 and the location map of the study area was shown in Figure 1.

Methodology

To assess the plant species diversity, a total of 36 quadrats (10 × 10) m^2 with 11 quadrats in Kayin Chaung Village and 25 quadrats in Kalartan Village were established. Inside each quadrat, all trees with at least 5 cm girth at breast height (GBH) were identified and measured the trunk diameter (cm) and total height (m). The location (latitude and longitude) of each study point was recorded by using a Global Positioning System (GPS). Water salinity of the study points were measured by using the hand refractometer (REF 201/211/201 bp). Soil samples were collected from the soil depth of 0 - 30 cm below the soil surface. They were tested in the soil laboratory of the Department of Agriculture (Land Use) Yezin in Nay Pyi Daw, Myanmar for the soil analysis of soil texture, soil pH, organic matter and nutrient contents (N, P, K).

Plant specimens were collected, pressed, dried and identified with the help of available references and Myanmar names were recorded by Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003).

Jackknife estimate of species richness

The number of species in a community is referred to as its species richness. Species richness is widely used in ecology as a measure of species diversity (Baumgärtner, 2005). Jackknife estimate was adopted in order to estimate the species richness per study area. According to the Heltshe and Forrester (1983), the formula for Jackknife estimate of species richness is:

$$\hat{S} = S + \left(\frac{n-1}{n}\right)^{k}$$

Where,

$\hat{S}$ = Jackknife estimate of species richness

S = Observed total number of species in “n” sample plots

n = Total number of plot sample

k = Number of unique species

Measurement of plant species diversity and evenness

Plants species diversity

Species diversity is the number of different species in a particular area (species richness) weighed by some measure of abundance such as number of individuals or biomass. Two commonly used measurements are Shannon’s index and Simpson’s index. Shannon-Wiener diversity index

places more weight on the rare species while Simpson's diversity index emphasizes on the common species (Weidelt, 2000).

Shannon-Wiener Index

$$H = -\sum_{i=1}^{S} (P_i)\,(\log_2 P_i)$$

Where,

H = Shannon-Wiener index of species diversity
S = Number of species
P_i = Proportion of total sample belonging to the i^{th} species

Simpson's Index

$$D = 1 - \sum_{i=1}^{S} (P_i)^2$$

Where,

D = Simpson's index of species diversity
S = Number of species
P_i = Proportion of individual of i^{th} species in the community

Evenness (E)

Another measure of species diversity is the species evenness, which is the relative abundance with which each species is represented in an area. Species evenness is a diversity index, a measure of biodiversity which quantifies how equal the communities are numerically. Shannon-Wiener function (1963) is the most meaningful measure of evenness as follows:

$$E = \frac{H}{H_{max}}$$

$$H_{max} = \log_2 S$$

Where,

E = Evenness (Range 0-1)
H = Index of species diversity
S = Number of species
H_{max} = Species diversity under conditions of maximal equitability

Coefficient of similarity

Coefficient of similarity is generally used as a mean of comparing stands from floristic point of view (Lamprecht, 1989). The study sites were composed by means of similarity coefficient calculated using Sorenson's index of similarity. The index is used as a mean for comparing the floristic similarity between two forests. The formula is as follows:

$$Ks = \frac{2c}{a+b} \times 100$$

Where,

Ks = Coefficient of similarity
a = Number of species in one stand
b = Number of species in the other stand
c = Number of species common to both stand

Evaluation of density, relative density, frequency, relative frequency, mean basal aera and relative dominance

Forest vegetation and methods for determining tree height and cover were quantitatively analyzed for density, relative density, frequency, relative frequency, dominance, relative dominance, and important value index for each tree species that were used for description of vegetation structure are as follows:

$$\text{Density (D)} = \frac{\text{No. of individuals of the species in all the sample plots}}{\text{Total no. of sample plots studied}}$$

$$\text{Relative Density (R. D)} = \frac{\text{No. of individuals of the species}}{\text{No. of individuals of all the species}} \times 100$$

$$\text{Frequency (F)} = \frac{\text{No. of sample plots in which the species occur}}{\text{Total no. of plots sampled}}$$

$$\text{Relative Frequency (R. F)} = \frac{\text{No. of occurrences of the species}}{\text{No. of occurrences of all the species}} \times 100$$

$$\text{Mean Basal Area (MBA)} = \frac{\text{Total basal area}}{\text{Number of trees}}$$

$$\text{Relative Dominance (R. Dm)} = \frac{\text{Total basal area of the species}}{\text{Total basal area of all the species}} \times 100$$

$$\text{IVI} = \text{R. D} + \text{R. F} + \text{R. Dm}$$

The Important Value Index (IVI) of any species in a community ranges between 0 and 300. Values of IVI help in understanding the ecological significance of the species in the respective vegetation type (Lutz, 1928-1930).

Species distribution by frequency class

Each tree species was grouped into five frequency classes based on Raunkiaer's law of frequency. The frequency classes are A, B, C, D, and E according to Raunkiaer's frequency spectrum by Raunkiaer (1934). The law also known as the law of homogeneity and frequency class A represents the species that are rare (r), class B that are seldom present (s), class C that are often

present (o), class D that are mostly present (m), and class E that are constantly present (c). Frequency class A to E were suggested by Raunkiaer's from 0 to 100.

Table 1 Household and population of study area

No.	Village name	Household	Male	Female	Total population
1	Kayin Chaung	247	835	782	1617
2	Kalartan (Shwe Pyi Thit)	167	387	357	744

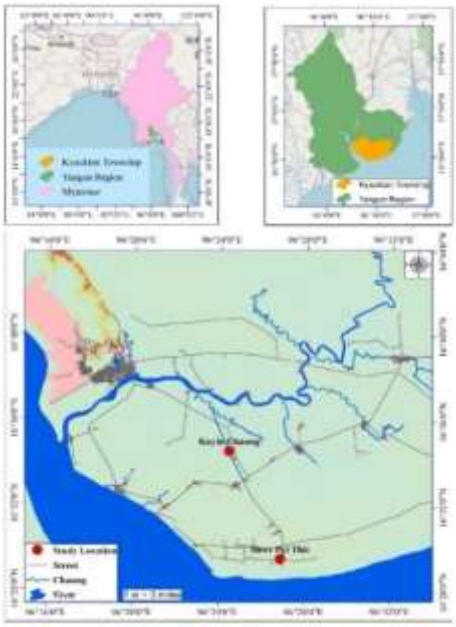

Figure 1. Location map of the study area

Results

The number of family, genera and species of trees which were found in Kayin Chaung Village were 23 species belong to 22 genera and 14 families. There were 20 species belong to 19 genera and 14 families found in Kalartan Village as shown in Table 2.

The species richness is commonly expressed as the number of species per unit area, which is also mentioned as the species density. According to the results, Jackknife estimate of species richness in Kayin Chaung Village and Kalartan Village were 23.83 and 20.92 respectively as shown in Table 3. Among the two study areas, Kayin Chaung Village had higher species richness than Kalartan Village.

Among the different measurement of species diversity indices, Shannon-Wiener Index (H), Simpson's Index (D) and Shannon-Wiener Evenness (E) were used because these indices do not only take taxa richness into account but also depend on relative distribution of individuals. It was observed that the values of Shannon-Wiener Index (H) and Simpson's Index (D) of Kayin Chaung Village were (H = 2.90, D = 0.93) and those that of the values in Kalartan Village were (H = 2.55, D = 0.90). So, it was considered that Kayin Chaung Village was relatively diverse than Kalartan Village.

Shannon-Wiener function is the most widely used index of species diversity because it incorporates both species richness and abundance (E). According to Krebs (1999), 'E' means "Equitability or Evenness" and varies between 0 and 1. A higher value E indicates the presence of many species in approximately equal quantities. As a result of Shannon-Wiener Evenness (E), Kayin Chaung Village was more evenly distributed with the value of 0.92 than that of Kalartan Village with the value of 0.85.

The floristic compositions of two different sites were composed by means of similarity coefficient calculated by using Sorenson's index of similarity. Sorenson's index (1948) is based on the presence or absence of species. If both stands are floristically identical, the coefficient of similarity (Ks) value is 100 and if they are completely different, the value of Ks is zero (0). Sorenson's similarity index (i.e., based on the number of common species) indicated that species composition had 74.42% similarity between Kayin Chaung Village and Kalartan Village, referring that the species composition had moderate floristic similarity.

The results of quantitative analysis of study area, relative density, relative frequency, relative dominance and ranking of ecological significance by Important Value Index (IVI) of tree species in Kayin Chaung Village and Kalartan Village were given in Tables 4 - 5.

The Important Value Index (IVI) of the species was determined by the sum of relative density, relative frequency and relative dominance. Kayin Chaung Village was dominated by *Sonneratia apetala* Banks (IVI = 57.29%), followed by *Sonneratia caseolaris* (L.) Engl. (IVI = 55.85%) and *Ziziphus mauritiana* Lam. (IVI = 16.63%). Kalartan Village was dominated by *Sonneratia caseolaris* (L.) Engl. (IVI = 88.73%), followed by *Sonneratia apetala* Banks (IVI = 39.12%) and *Derris trifoliata* Lour. (IVI = 20.56%).

The horizontal structures of the study area were shown in Tables 6 - 7. In Kayin Chaung Village, *Sonneratia apetala* Banks species were observed to be the biggest tree (≥ 116 cm GBH) which was 1.24% of the total species (Table 6). There were six species in lower class (5 - 15 cm GBH) which were 18.12% of the total species. In Kalartan Village, two species, *Sonneratia apetala* Banks and *Sonneratia caseolaris* (L.) Engl. were to be the biggest trees (≥ 116 cm) which were 1.15% of the total species (Table 7). There were eight species in lower class (5 - 15 cm GBH) which were 60.44% of the total species.

Stratification or vertical structure of the community determines the different growth forms. In Kayin Chaung Village, thirteen species (50.79% of total individuals) were found in the height class of < 2 m. Two species, *Sonneratia apetala* Banks and *Sonneratia caseolaris* (L.) Engl., were found in the height class of ≥ 8 m (4.56% of total individuals) as shown in Table 8. In Kalartan Village, thirteen species (55.40% of total individuals) were found in the height class of < 2 m; two species *Sonneratia apetala* Banks and *Sonneratia caseolaris* (L.) Engl were found in the height class of ≥ 8 m (2.29% of total individuals) as shown in Table 8.

According to the Raunkiaer (1934), five frequency classes of species frequency distribution found in the study area was shown in Table 9. The frequency gives an approximate indication of homogeneity or heterogeneity of a stand. In the present study area, high distribution values were found in higher frequency classes C, D, and E whereas low distribution values were found in lower frequency classes A and B. It indicated that the study area had a high degree of floristic homogeneity.

According to the results of weather data, the study area is influenced by tropical monsoon climate with high temperature and abundant rainfall and characterized by seasons: summer, rainy and winter. Generally, March, April and May of 2020 and 2021; and March and April of 2022 were recorded as having maximum temperatures (between 36.2ºC and 39.1ºC). Also, the study area received maximum rainfall during June (671 mm) in 2020; during June (819 mm) in 2021; and during August (796 mm) in 2022. (Source: Department of Meteorology and Hydrology, Kaba Aye Station, Yangon Region).

The structure and nutrient contents of soil is important, particularly for plants. According to the results of soil analysis, the soil texture of both Kayin Chaung Village and Kalartan Village was clay loam. The nitrogen content of both of the villages was low. The phosphorus content of both of the villages was medium and the potassium content of both Kayin Chaung Village and Kalartan Village was high as shown in Table 10.

Salinity of water is strongly related to the distance from the sea, topography, tidal action and the rain. It is an important factor that affects the rate of growth, survival, height and distribution of mangrove ecosystems (Tri, 1999). The salinity percentage of Kayin Chaung Village (between 1.0% - 2.0%) is lower than that of Kalartan Village (between 8.0% - 9.0%) by using the hand refractometer (REF 201/211/201 bp).

Table 2. Number of families, genera and species of mangrove in study area

Taxonomic Rank	Kayin Chaung Village	Kalartan Village
Family	14	14
Genus	22	19
Species	23	20
Individuals	1337	2666

Table 3. Consolidated details of mangrove species inventory in Kayin Chaung Village and Kalartan Village

Description	**Kayin Chaung Village**	**Kalartan Village**
No. of Sample Plots	11	25
No. of Species	23	20
Individual Species	1337	2666
Unique Species	2	2
Species Richness	23.83	20.92
Shannon-Wiener Diversity Index (H)	2.90	2.55
Simpson's Diversity Index (D)	0.93	0.90
Shannon-Wiener Evenness (E)	0.92	0.85

Table 4. Ranking of Important Value Index (IVI) in Kayin Chaung Village

No.	**Scientific Name**	**R.D (%)**	**R.F (%)**	**R.Dm (%)**	**IVI (%)**
1	*Sonneratia apetala* Banks	3.74	3.17	50.37	57.29
2	*Sonneratia caseolaris* (L.) Engl.	17.28	5.82	32.75	55.85
3	*Ziziphus mauritiana* Lam.	6.36	3.17	7.09	16.63

No.	Scientific Name	R.D (%)	R.F (%)	R.Dm (%)	IVI (%)
4	*Pithecellobium dulce* (Roxb.) Benth.	5.61	4.23	2.83	12.67
5	*Hygrophila phlomoides* Nees	5.91	5.82	0.00	11.73
6	*Hibiscus tiliaceus* L.	6.73	4.76	0.12	11.62
7	*Eupatorium sp.*	5.46	5.82	0.00	11.28
8	*Malachra capitata* (L.) L.	5.09	5.82	0.00	10.91
9	*Derris trifoliata* Lour.	4.86	5.82	0.15	10.83
10	*Mimosa pudica* L.	4.04	5.82	0.00	9.86
11	*Terminalia catappa* L.	3.74	2.12	3.67	9.53
12	*Urena lobata* L.	3.59	5.82	0.00	9.41
13	*Calotropis gigantea* (L.) W.T.Aiton	5.61	3.70	0.08	9.39
14	*Stachytarpheta indica* (L.) Vahl	3.37	5.82	0.00	9.19
15	*Acanthus ilicifolius* L.	3.22	5.82	0.00	9.04
16	*Volkameria inermis* L.	2.99	5.82	0.04	8.85
17	*Ipomoea violacea* L.	3.44	5.29	0.03	8.76
18	*Chromolaena odorata* (L.) R.M. King & H. Robinson	2.92	4.23	0.00	7.15
19	*Hellenia speciosa* (J.Koenig) S.R.Dutta	2.84	4.23	0.00	7.07
20	*Sesbania bispinosa* (Jacq.) W.Wight	1.05	3.17	1.16	5.39
21	*Typha angustifolia* L.	1.72	1.59	0.00	3.31
22	*Samanea saman* (Jacq.) Merr	0.22	1.06	1.27	2.56
23	*Syzygium grande* (Wight) Walp.	0.22	1.06	0.42	1.70
	Total	**100**	**100**	**100**	**300**

R.D = Relative Density; R.F = Relative frequency; R.Dm = Relative Dominance; IVI = Important Value Index

Table 5. Ranking of Important Value Index (IVI) in Kalartan Village

No.	Scientific Name	R.D (%)	R.F(%)	R.Dm(%)	IVI(%)
1	*Sonneratia caseolaris* (L.) Engl.	20.33	9.06	59.34	88.73
2	*Sonneratia apetala* Banks	0.30	1.81	37.01	39.12
3	*Derris trifoliata* Lour.	11.25	8.33	0.97	20.56
4	*Volkameria inermis* L.	10.43	5.80	0.25	16.47
5	*Acanthus ilicifolius* L.	9.75	5.80	0.00	15.55
6	*Ipomoea violacea* L.	6.11	9.06	0.17	15.34
7	*Eupatorium sp.*	7.76	5.80	0.00	13.56
8	*Hygrophila phlomoides* Nees	6.98	4.71	0.00	11.69
9	*Hibiscus tiliaceus* L.	2.18	8.33	1.16	11.67
10	*Urena lobata* L.	5.74	5.80	0.00	11.54
11	*Calotropis gigantea* (L.) W.T.Aiton	2.44	7.25	0.11	9.79
12	*Hyptis brevipes* Poit.	4.28	5.07	0.00	9.35

No.	Scientific Name	R.D (%)	R.F(%)	R.Dm(%)	IVI(%)
13	*Malachra capitata* (L.) L.	3.98	4.71	0.00	8.69
14	*Acrostichum aureum* L.	3.64	4.35	0.00	7.99
15	*Pithecellobium dulce* (Roxb.) Benth.	0.60	3.99	0.43	5.02
16	*Ziziphus mauritiana* Lam.	0.53	3.26	0.34	4.13
17	*Stachytarpheta indica* (L.) Vahl	1.61	2.17	0.00	3.79
18	*Physalis angulata* L.	1.28	2.17	0.00	3.45
19	*Phoenix paludosa* Roxb.	0.53	1.81	0.06	2.40
20	*Terminalia catappa* L.	0.30	0.72	0.16	1.18
	Total	**100**	**100**	**100**	**300**

R.D = Relative Density; R.F = Relative Frequency; R.Dm = Relative Dominance; IVI = Important Value Index

Table 6. Population density of tree species across GBH class interval in Kayin Chaung Village

GBH (cm)	Total no. of individuals	No. of species	% of total species
5 -15 cm	102	6	18.12
16 - 25 cm	128	5	22.74
26 - 35 cm	124	5	22.02
36 - 45 cm	95	4	16.87
46 - 55 cm	47	5	8.35
56 - 65 cm	6	2	1.07
66 - 75 cm	6	2	1.07
76 - 85 cm	5	2	0.89
86 - 95 cm	15	2	2.66
96 - 105 cm	18	1	3.20
106 - 115 cm	10	1	1.78
≥ 116 cm	7	1	1.24
Total	**563**		**100**

Table 7. Population density of tree species across GBH class interval in Kalartan Village

GBH (cm)	Total no. of individual	No. of species	% of total species
5 -15 cm	472	8	60.44
16 - 25 cm	172	3	22.02
26 - 35 cm	73	1	9.35
36 - 45 cm	32	1	4.10
46 - 55 cm	12	1	1.54
56 - 65 cm	4	1	0.51
66 - 75 cm	4	1	0.51
76 - 85 cm	1	1	0.13
86 - 95 cm	-	-	-
96 - 105 cm	1	1	0.13
106 - 115 cm	1	1	0.13
≥ 116 cm	9	2	1.15
Total	**781**		**100**

Table 8. Population of tree species in vertical structure of Kayin Chaung Village and Kalartan Village

Height Class (m)	Kayin Chaung Village			Kalartan Village		
	NS	NI	TI(%)	NS	NI	TI (%)
<2 m	13	679	50.79	13	1477	55.40
2 – 4 m	10	539	40.31	7	887	33.27
4 – 6 m	1	38	2.84	1	141	5.29
6 – 8 m	1	20	1.50	1	100	3.75
≥8 m	2	61	4.56	2	61	2.29
Total		**1337**	**100**		**2666**	**100**

NS = No. of species; NI = No. of individuals; TI = % of total individuals

Table 9. Species distribution by frequency class in Kayin Chaung Village and Kalartan Village

Frequency Class	Frequency Range	Kayin Chaung Village		Kalartan Village	
		NS	TSD (%)	NS	TSD(%)
A	1 – 20 %	2	8.70	3	15
B	21 – 40 %	2	8.70	3	15
C	41 – 60 %	3	13.04	5	25
D	61 – 80 %	4	17.39	5	25
E	81 – 100 %	12	52.17	4	20
Total		**23**	**100**	**20**	**100**

NS = No. of Species; TSD = % of total species distribution

Table 10. Results of soil interpretation

Sr No.	Sample	pH Soil: Water 1:2.5	Texture	Organic Carbon	Total N	Available Nutrients	
						P	K_2O
1	KC	Moderately Alkaline	Clay Loam	Very Low	Low	Medium	High
2	SPT	Slightly Alkaline	Clay Loam	Very Low	Low	Medium	High

KC = Kayin Chaung; SPT = Shwe Pyi Thit

Source: Department of agriculture (land use) Yezin, Naypyidaw

Discussion and Conclusion

In this part of study, species composition and diversity of Kayin Chaung Village and Kalartan Village were investigated. Two areas were chosen with 11 sample plots in Kayin Chaung Village and 25 sample plots in Kalartan Village. In Kayin Chaung Village, 23 species comprising 22 genera and 14 families and 1337 individuals were recorded; 20 species contributing 19 genera and 14 families and 2666 individuals were represented in Kalartan Village.

According to the results of Shannon-Wiener index (H) and Simpson's index (D), the diversity value of Kayin Chaung Village was (H = 2.90, D = 0.93) and that of Kalartan Village was (H = 2.55, D = 0.90) respectively. Both of the villages had high diversity but Kayin Chaung Village was relatively higher than Kalartan Village. The Species Evenness or Species Abundance Distribution (SAD) is important in characterizing ecosystems (Paul *et al*., 2005). According to the results of quantitative analysis, the plant species in Kayin Chaung Village (E = 0.92) were more evenly distributed than Kalartan Village (E = 0.85). Species richness is widely used in ecology as a measure of species diversity (Baumgärtner, 2005). According to the results of Jackknife estimate of species richness, the species richness in Kayin Chaung Village (23.83) was higher than Kalartan Village (20.92). Sorenson's index is used as a mean for comparing the floristic similarity between two forests. The result of coefficient of similarity between Kayin Chaung Village and Kalartan Village had 74.42% of similarity. Therefore, these two villages had moderate floristic similarity.

The important value index is imperative to compare the ecological significance of species (Lamprecht, 1989) It indicates the extent of dominance of a species in a structure of a forest stand (Curtis and McIntosh, 1951). It is stated that species with the greatest important value index are the leading dominants of the forests. The highest IVI and two leading dominant species were *Sonneratia apetala* Banks (Kan-pa-la) (IVI = 57.20%) and *Sonnertaia caseolaris* (L.) Engl. (La-mu) (IVI = 55.85%) in Kayin Chaung Village; and *Sonneratia caseolaris* (L.) Engl. (La-mu) (IVI = 88.73%) and *Sonneratia apetala* Banks (Kan-pa-la) (IVI = 39.12%) in Kalartan Village. Therefore, these could be regarded as the representative and ecological indicator species of the study area.

It is important to examine the species distribution of a stand by their GBH classes. The GBH distribution of individuals in the study area showed that most number of trees were belonged to GBH class (16 - 25 cm) (22.74% of total species) in Kayin Chaung Village and GBH class (5 - 15 cm) (60.44% of total species) in Kalartan Village.

The vertical distribution of the study area was distinguished. The individuals of both of the study areas were concentrated in the height class that were found in < 2 m class: 13 species (50.79% of total individuals) in Kayin Chaung Village; and 13 species (55.40% of total individuals) in Kalartan Village.

According to the results of frequency class distribution by Raunkiaer's frequency classes, high distribution values were found in the higher frequency classes C, D and E and low distribution values were found in lower frequency classes A and B, indicating that both of the study areas had high degree of floristic homogeneity.

Mangroves provide a number of valuable ecosystem services that contribute to human well-being. In the present study area, mangroves play a vital role in supporting local and regional communities with ecosystem services, and thus enhancing the livelihoods of communities. Provisioning (e.g., timber, fuel wood and food resources), regulating (e.g., flood, storm and erosion control), habitat (e.g., breeding, spawning and nursery habitat for fish and prawn species), and cultural (e.g., recreation, tourism and cultural heritage) are among these locally and regionally important ecosystem services.

In conclusion, mangrove species diversity of both Kayin Chaung Village and Kalartan Village was carried out. In addition, mangrove vegetation analysis such as species diversity, species richness, evenness, density, frequency and abundance can be applied to the monitoring of

habitat and conservation management of mangrove forests and can also be a source of information to ensure the condition and sustainability of the ecosystem in Kayin Chaung Village and Kalartan Village.

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IN VITRO SEED GERMINATION AND SEEDLING DEVELOPMENT OF THE ORCHID *PHOLIDOTA ARTICULATA* LINDL.

San Win[1], Nilar[2]

Abstract

In this experiment, the plantlets were initiated from seeds of *Pholidota articulata* Lindl. (Kwyetmeee pan myokywe). The experiment was carried out at Plant Tissue Culture Laboratory of the Htone Bo Agricultural Research Farm, Taunggyi, Department of Agricultural Research from October, 2022 to March, 2023. This study was focusing on evaluating the effect of natural additive as plant growth regulators (PGR). The additive used in this study was 100 ml of coconut water (CW) that supplemented in Murashige and Skoog (MS) medium. Four treatments such as T_1 (MS only), T_2 (half MS), T_3 (Full MS and CW) and T_4 (half MS and CW). Each treatments of 6 replications and experimental design was set up using Completely Randomized Design (CRD). Murashige and Skoog (MS) and Half MS media were used to study their effects in seed germination and protocorm development. Maximum percentage of seed germination was observed in Half MS and coconut water (T_4) medium and minimum percentage of seed germination was in MS only medium (T_1). The best protocorms for plantlet development i.e MS media was supplemented with various combinations of Cytokinins (BAP) and Auxin (NAA). Therefore, *in vitro* developed protocorms were sub-cultured on the Murashige and Skoog (MS) medium, supplemented with coconut water and different concentrations of 6-benzylaminopurine (BAP) and α-naphtalene acetic acid (NAA). The highest number of shoot and number of leaves was observed in MS medium supplemented with 3mg.l^{-1} BAP.

Keywords: *Pholidota articulata*, coconut water, BAP, NAA

Introduction

Orchidaceae is a large family with 25,000-30,000 species and 600-800 general known from the world. (Backer and Ben Den Brink, 1968). The genus *Pholidota* are epiphytic herbs generally grown on rocks and trees. Most plants of the genus *Pholidota* found in India grow as epiphytes. Some are also found growing on moist, mass covered rock structures on large, hilly slopes. Sexual reproduction of orchids in nature is being very slow as only 2-5% of their seeds can germinate after symbiosis with a special mycorrhizal fungus (Pant *et al.*,2017). So, plant tissue culture technique is one of the alternative ways to propagate the plants through seeds without fungal association those cannot be reproduced sexually in nature easily (Pant, 2013). In vitro propagation protocol by plant tissue culture has not been developed yet in this orchid. In vitro propagation of this orchid could be an alternative to fulfill its demand on horticulture and traditional medicine as well as for its conservation.

The aims and objectives are to obtain the *in vitro* plants of *Pholidota articulata* Lindl. From seed culture, to obtain the plantlet production from PLBs potentially *in vitro*, to identify the best hormonal effect for in vitro regeneration of orchid and to establish an effective protocol for *in vitro* seed germination, protocorm and plantlet development of *Pholidota articulata* Lindl.

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Materials and Methods

Experimental Site

The research was carried out at the laboratory of Plant Tissue Culture of the Htone Bo Agricultural Research Farm, Taunggyi, Department of Agricultural Research during October, 2022 to March, 2023.

Collection and Identification of *Pholidota articulata* Lindl.

The specimens were collected from Maung Myanmar Orchid Garden, Taunggyi area. The collected plant specimens were studied by under using dissecting microscope in laboratory, Department of Botany, Taunggyi University. The morphological and taxonomical studies were made from the collected specimen by using available literature such as Backer, 1968 and Shi xiantaoshu, 2010.

Method of Media Preparation

According to the Murashige and Skoog (1962), method of 1000ml medium preparation applied in the present study is as follows;

1. 300ml distilled water was poured into 3000ml beaker.
2. Proper amount of stock solution were added.
3. Sugar 30g was added and stirred.
4. Agar powder 6 g was slowly added and stirred.
5. Before the addition of agar, the pH value was adjusted to be 5.7 with 0.1N NaOH and 0.1N HCl
6. The volume of solution was made up to 1000ml and the level was marked.
7. The solution was gently heated until it starts to boil.
8. Then medium was heated and stirred until agar was completely dissolved and becomes amber-colored.
9. The medium was dispensed into culture bottles.
10. The culture bottles were covered and autoclaved.
11. The media were sterilized in an autoclave at 120-121°C and 1.2 kg/cm^2 for 30 minutes.
12. Then the bottles were cooled and used.

Experiment 1 (Initial Seeds Culture)

Source of Plant Material

The green healthy capsule of *Pholidota articulata* Lindl. which is a native species of Myanmar was obtained from Maung Myanmar Orchid Garden, Taunggyi area.

Culture Bottles

All flasks were washed with soap detergent, then rinsed throughly with hot distilled water and dried.

All flasks with the capacity of 100 ml each containing 20 ml of nutrient medium were used.

Culture Medium

MS media used in this investigation were shown in Table 1. MS media consist of coconut, sugar (2%) and agar (1%).The pH value was adjusted to be 5.5 ± 0.1 with 0.1 N NaOH and 0.1 N

HCl. Sterilization was made in autoclave at 120°C-121°C and 1.0 – 1.5 kg/cm2 (260°F and 15 lb/in2) for 20 minutes.

Decontamination

The green capsules was washed with 10% Clorox solution by a soft brush, then it was dipped into 70% ethyl alcohol and flame rapidly.

Culture Condition

All culture were maintained at 25 ± 2°C under continuous illumination of about 120 foot candles from 4- feet white fluorescent tube and 25%- 35% relative humidity.

Figure 1. Procedures of Seeds Culture

Experiment 2 (Multiplication of shoots on the different nutrient medium)

Source of Plant Material

The protocorm like bodies (PLBs) of *Pholidota articulata* Lindl. were obtained from previous culture of seed germination. Protocorm explants were obtained through *in vitro* cultures, no sterilization was required.

Elongation and Multiplication of Shoots

Protocorms with first initiated leaf developed from the germinating seeds were used for shoots multiplication. MS medium with different treatments and different concentration of BAP and NAA (1 and 3 mg/l) were used for the elongation and multiplication of shoots from the seed derived-protocorms.

Experimental Design

Six treatments such as T_1 control (MS Only), T_2 (MS + Coconut water 200ml/liter), T_3 (M.S + 1 mg/l BAP), T_4 (M.S + 3 mg/l BAP), T_5 (M.S + 1mg.l^{-1}mg/l NAA) and T_6 (M.S + 3 mg/l NAA) with six replications. Each was set up using completely randomized design (CRD).

Culture Condition

All culture bottles were maintained in a culture room where light was supplied by 4 feet fluorescent tubes (1000-1200 lux) light intensity for 8/16 hours (light/dark). Throughout the culture period temperature ranged from 23°C to 25°C. Their experiment were carried out at the tissue culture laboratory.

Data Collection and Statistical Analysis

In this experiment, 6 treatments which six replications each were used for plantlets initiation and number of shoots and number of leaves recorded three months after culture. The treatment means of the plantlets from all replicates were calculated.

The data were analyzed using the IRRISTAT software, version 4, developed by International Rice Research Institute (IRRI), Philippines. The mean separation was calculated by Least Significant Different (LSD) (Gomez and Gomez, 1984).

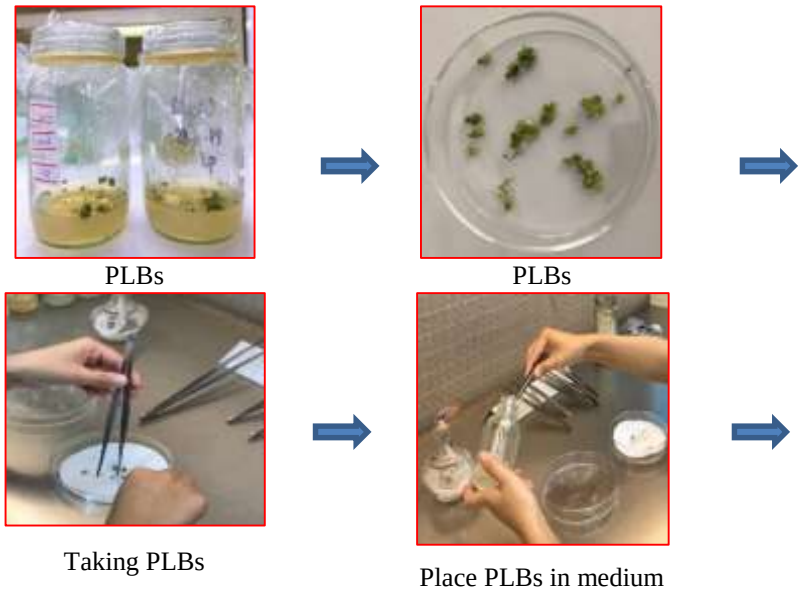


Figure 2. Multiplication of Shoots on the Different Nutrient Medium

Results

Morphological Characters of *Pholidota articulata* Lindl.

Scientific Name	:	*Pholidota articulata* Lindl.
Myanmar name	:	Kwyetmeee pan myokywe
Distribution	:	Kachin State, Kayin state, Mon State, Shan State
Flowering period	:	May – June
Characteristics	:	Flowers white with light
Size	:	0.8 – 1 cm
Number of flowers	:	10 – 20

Epiphytic on tree trunks in forest, lithophytic on shade rocks. Pseudobulbs connected to each other at both ends and stem like, subcylindric, sometimes slightly narrowed, branching or not, sometimes with very short rhizomes between then and producing a few roots. Leaves 2, at apex of new pseudobulb; leaf blade obovate-elliptic, oblong, or narrowly elliptic, veins plicate, subacute or obtuse. Inflorescence at apex of new pseudobulb, floral bracts deciduous during flowering, narrowly ovate-oblong. Flowers greenish white or white and slightly tinged with reddish. Dorsal sepal oblong or elliptic, dorsally carinate, 5-veined; lateral sepals ovate, oblique, slightly wider than dorsal sepal. Petals oblong-lanceolate or suboblanceolate, Column stout, apex winged; rostellum broadly ovate, pollinia 4, waxy. Capsule ellipsoid to obovoid-ellipsoid, slightly 3-ridged Epiphytic on trees in forests, lithophytic on shaded rocks.

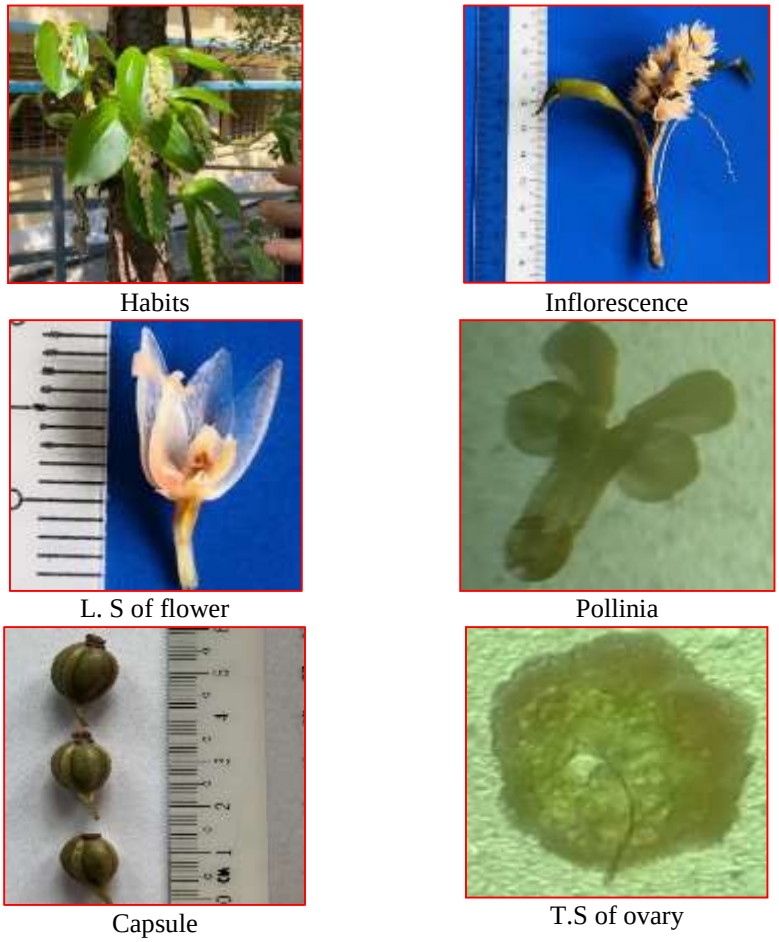


Figure 3. Morphological Characters of *Pholidota articulata* Lindl.

Experiment 1. Germination of Seeds on Different Nutrient Medium

Seeds were inoculated on the full- and half-, of MS medium supplement with coconut water. Sign of seeds germination is by turning them into light yellowish-green on 4th week, they became swollen and elongated spherules in 8th week on all the strengths of ½ MS medium with coconut water.

Protocorms formation was observed in 10th week of seeds culture on ½ MS medium (T_2) and ½ MS medium with coconut water (T_4). They were globular, light yellowish-green and hairy.

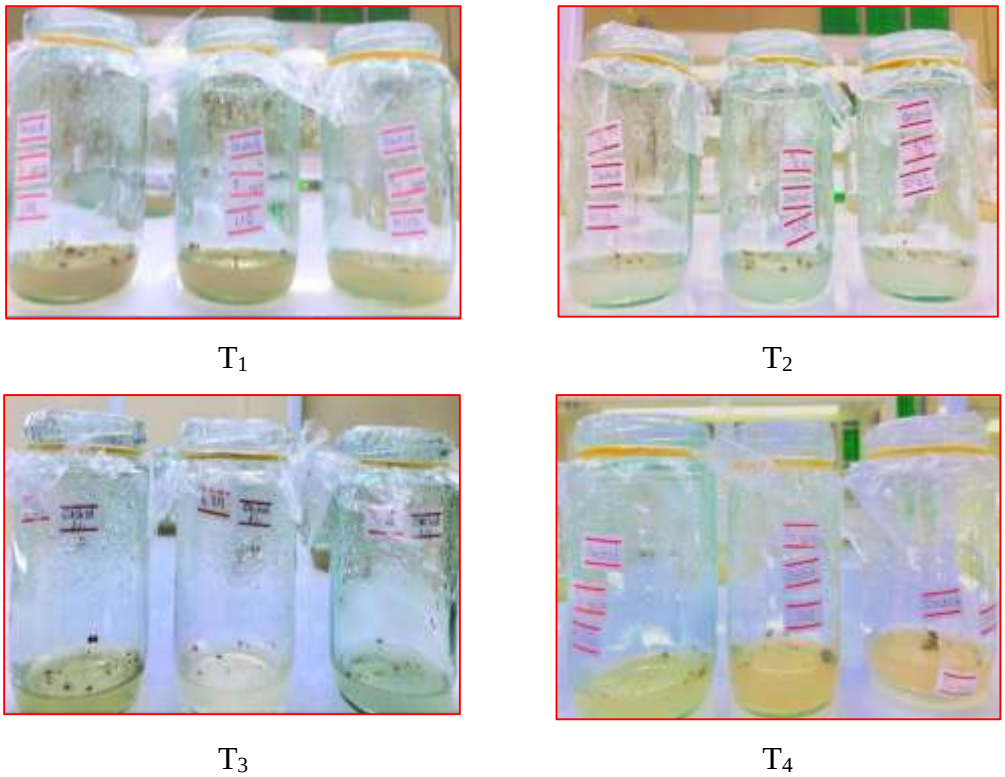
The green, globular hairy protocorms changed into pale yellow over time on these medium. No shoot proliferation or further growth of protocorms was observed in all the concentrations and combinations of the MS medium.

Table 1. *In vitro* Seeds Germination Resulted in Different Treatments of *Pholidota articulata* Lindl.

Treatments	Germination result	Observation
$T_{1\ (control)}$ = MS Only	-	-
T_2 = ½ MS	+	Good germination
T_3 = Full MS+ 100 ml Coconut water	+	Fair germination
T_4 = ½ MS+ 100 ml Coconut water	+	Good germination

- Germination did not occur

\+ Germination was observed



T_1 T_2

T_3 T_4

Figure 4. Development of Protocorms from Seeds on the Full- and Half-strength of MS Medium After12 Week Culture

Experiment 2 (Multiplication of Shoots on the Different Nutrient Medium) Number of Shoots

The new shoots formation were formed three months after culture in all treatments. When compared the various concentrations of treatments, the highest average number of shoots per explants (6.80) were observed at the concentration of (T_4) 3 $mg.l^{-1}$ BAP (Table 2 and Figure 5) showed the multiple shoots of *Pholidota articulata* Lindl.

Table 2. Mean Values of the Number of Shoots on Standard MS Solid Medium Supplemented with Different Treatments

Treatments	Number of Shoots					
	67 days	74 days	81 days	88 days	95 days	102 days
T1	0.00	0.60	1.10	1.70	1.80	2.10
T2	0.00	1.00	1.70	2.20	2.40	2.60
T3	0.00	1.20	1.30	2.10	2.20	4.50
T4	0.00	1.90	3.40	3.90	4.70	6.80
T5	0.00	2.30	3.10	4.80	5.10	5.20
T6	0.00	0.20	0.80	0.90	0.90	1.90
F-test	ns	**	*	**	**	**
5% LSD	0.00	0.69	1.35	1.40	1.40	2.37
CV %	0.0	37.1	50.4	48.4	37.3	40.4

Each value represented the mean from 6 replications. Each replicate consisted of 2 plants. Mean differences in each column was determined by LSD at 5% level of significant. * = Significant at 5%, ** = highly significant , ns = not significant.

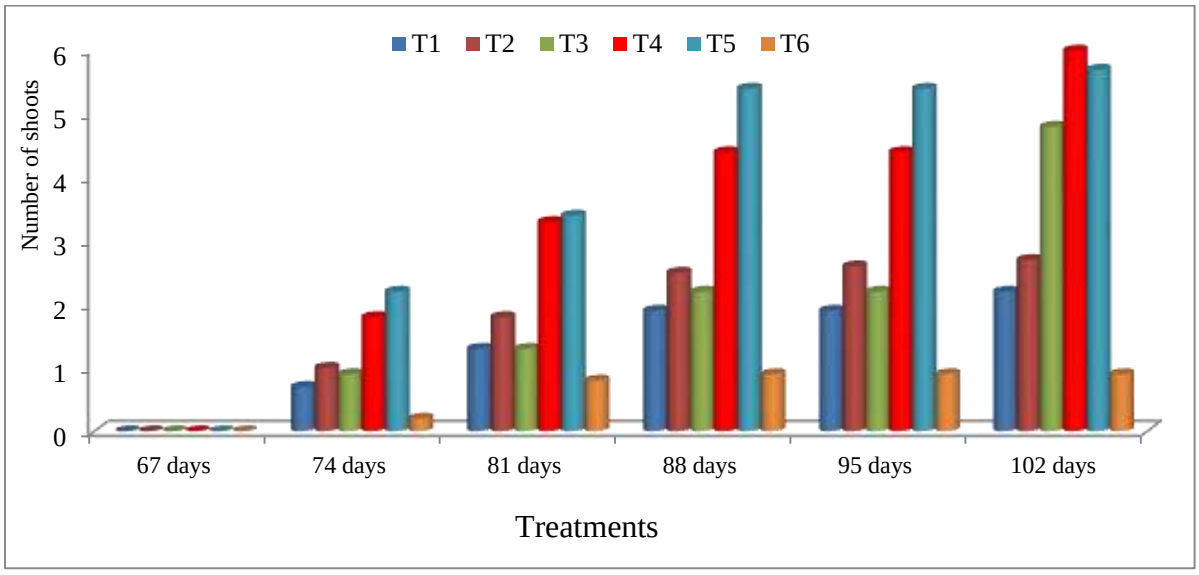


Figure 5. Mean Values of the Number of Shoots on Standard MS Solid Medium Supplemented with Different Treatments

Number of Leaves

The numbers of leaves formation were formed three months after culture in all treatments. When compared the various concentrations of treatments, the highest average number of leaves (9.40) were observed at the concentration of (T_4) 3 mg/l BAP (Table 3 and Figure 6).

Table 3. Mean Values of the Number of Leaves on Standard MS Solid Medium Supplemented with Different Treatments

Treatments	Number of leaves					
	67 days	74 days	81 days	88 days	95 days	102 days
T_1	2.10	4.20	4.40	4.60	4.70	5.20
T_2	3.40	4.00	4.10	4.30	4.40	5.10
T_3	0.65	1.80	2.00	2.10	2.20	4.10
T_4	2.20	3.90	4.90	5.20	5.40	9.40
T_5	2.20	3.40	4.40	4.50	4.70	7.40
T_6	2.40	3.60	4.10	3.90	3.90	4.20
F-test	ns	ns	*	*	*	ns
5% LSD	2.31	1.79	1.95	2.01	2.03	4.42
CV %	71.1	37.5	35.4	35.7	35.4	55.9

Each value represented the mean from 6 replications. Each replicate consisted of 2 plants. Mean differences in each column was determined by LSD at 5% level of significant. * = Significant at 5%, ns = not significant.

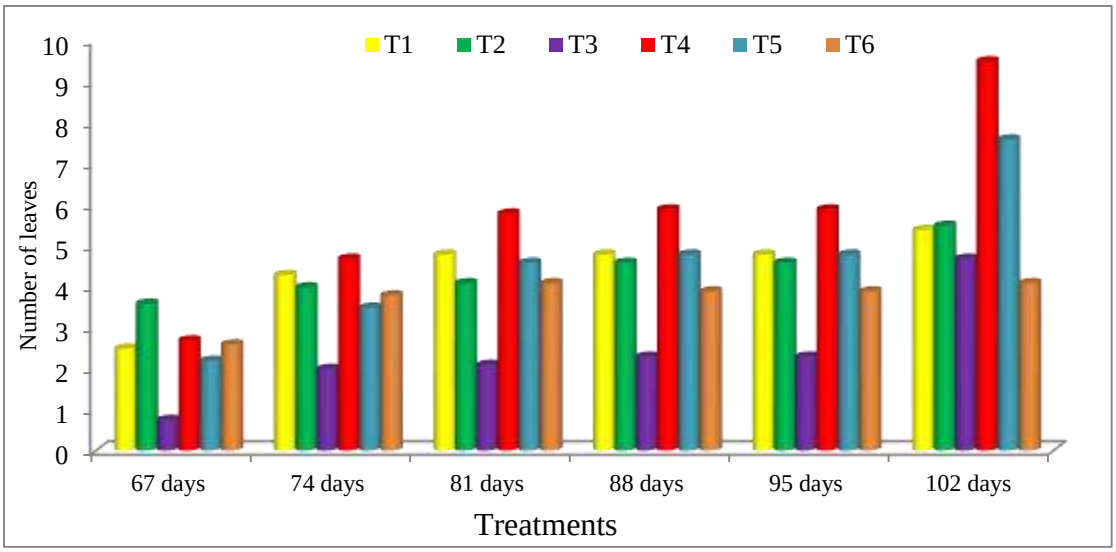


Figure 6. Mean Values of the Number of Leaves on Standard MS Solid Medium Supplemented with Different Treatments

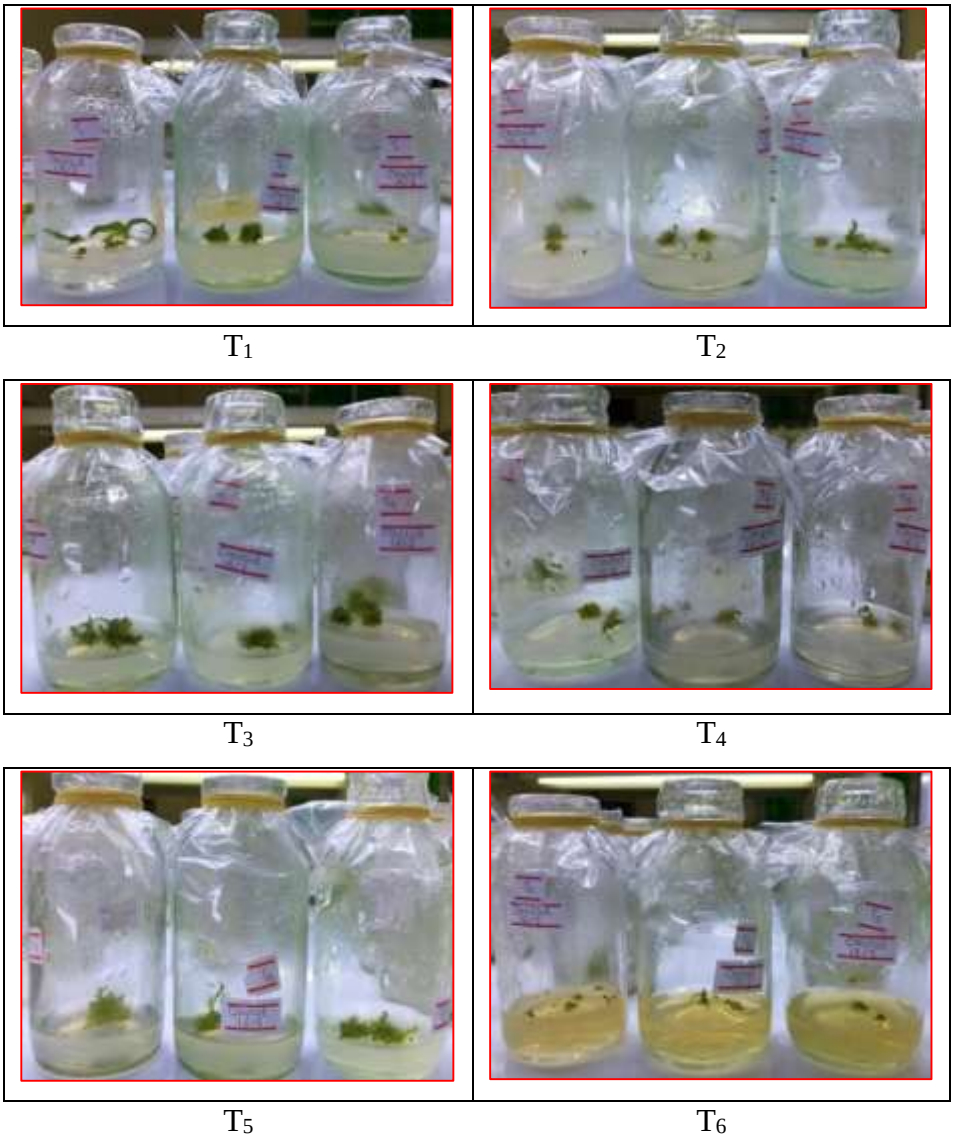


Figure 7. Effects of Different Treatments on Shoot Development of *Pholidota articulata* Lindl. From PLBs Subculture When Placed MS Solid Medium After 102 Days in Culture

Discussion and Conclusion

The morphological characters of *Pholidota articulata* Lindl. are epiphyte, perennial herb, pseudobulb connected to each other at both ends and stem like, subcylindric. Leaves narrowly elliptic, ovate, or oblong. Inflorescence terminal, emerging from apex of pseudobulb, distichous, pendulous, slender, racemose, laxly or densely many flowered. Capsule ellipsoid to ovoide-ellipsoid, slightly 3-ridged and a fruit with numerous minute seeds. This finding was in agreement with Backer and Ban Den Brink, 1968 and Shi xiantaoshu, 2010.

Seeds were germinated in four different treatments full MS and half MS. Seeds germination data were collected following 70 days of culture. Protocorm formation data were recorded at an interval of 7 days up to 70 days. On MS medium protocorm appears after 7 to 8 weeks of germination. Appearance of protocorm was 5 weeks slower in Half MS treatment (T_2) and 4 to 5 weeks in half MS with coconut water (T_4) as compared to MS medium. The process of germination started with the swelling of embryos after 3 weeks of inoculation of the immature yellowish seed. After 5 weeks spherules were observed which were subsequently transformed to protocorm like

bodies after 7 weeks. Addition of 100 ml coconut water to basal half MS medium gave the maximum proliferation. This result was in accord with the study of Peixe *et al*., 2007.

Protocorms were transferred on the MS medium supplemented with Coconut water 200 ml/liter, BAP and NAA (1 and 3 mg/l) for their elongation and multiplication. MS medium supplemented with 3 mg/l BAP was found to be the most effective condition for the multiplication of shoots from protocorms.

The results show that the seeds of *Pholidota articulata* Lindl. can be germinated and seedlings can be developed successfully under *in vitro* conditions.

According to the present study, there were statistically significant differences between nutrition media in terms of germination and seedling development parameters. Different components like macro elements, micro elements, vitamins, and organics showed significant differences for asymbiotic *in vitro* germination. Half-strength of MS medium was found to be the most appropriate for the non-symbiotic seeds germination and protocorms formation of *Pholidota articulata*. MS medium supplemented with 3 mg/l BAP was found to be the most effective condition for the multiplication of shoots from protocorms. Hence, this protocol might be useful for non-symbiotic germination, mass propagation and conservation of *Pholidota articulata* Lindl.

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EFFECTS OF HORMONES AND COCONUT WATER ON CLONAL MULTIPLICATION OF POTATO (*SOLANUM TUBEROSUM* L.)

Myint Myint Khaing [1], Aye Nu Yin[2]

Abstract

Shoots were produced from tissue culture of *Solanum tuberosum* L. Two experiments were carried out at the Plant Tissue Culture Laboratory, Department of Agricultural Research, Htone Bo, Agricultural Research Farm, Taunggyi during March to June, 2022. The shoot tips of potato cultivar Markies were inoculated in Murashige and Skoog (MS) media supplemented with the different concentration of alpha-Naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP) and coconut water. Hormonal effects on plant height, shoots, leaves and roots formation were studied. The maximum plantlet height and the highest number of root formation were observed in potato cultivar Markies on MS + 200 ml coconut water. The highest leaves formation were observed in ½ MS + 200 ml coconut water medium. The highest number of shoot were found in 0.05 mg/L NAA + 1.0 mg/l BAP +200 ml coconut water supplemented with MS medium.

Keywords: shoot tips, MS media, markies.

Introduction

The *Solanum tuberosum* L. (Potato) are members of the Solanaceae family. The Solanaceae, or nightshades, are an economically important family of flowering plants. The family ranges from herbs to trees, and includes a number of important agricultural crops, medicinal plants, spices, weeds, and ornamentals. The Solanaceae, also called nightshades, comprise more than 3000 species many of which evolved in the Amazonian regions of South America. (Jagatheeswari, 2014). Total world area 18.6 million ha^{-1}, 17. 4 t ha^{-1}(FAO 2011). Myanmar, total area 37000 ha^{-1}, yield 15.11 t ha^{-1} (DAP 2021). Potatoes are fat free food containing carbohydrate, protein, vitamins, antioxidants and minerals (FAO 2008)

The *in vitro* and aseptic cultivation of excised plant part on a nutrient medium is termed as plant tissue culture. The plant tissue culture is a branch of biotechnology. This technique is used for plant propagation, plant breeding, preservation and storage, scientific investigation and others. Plant tissue culture or micropropagation is the aseptic culture of cells, pieces of tissue or organs (Kavi Kisher, 1999).

By using micropropagation, the millions of new plants can be derived from a single plant. Micropropagation used for rapid multiplication and getting true-to-type plants on artificial nutrient media under controlled environment. Used in almost all potato seed producing countries in initial stages of seed production (Sein Hla Bo, 1987).

Seed potatoes are tubers that can be planted in a vegetable plot or in containers depending on the variety to produce a crop of harvestable potatoes. A seed potato is a potato that has been grown to be replanted to produce a potato crop (https:// theunconventionalgardener.com).

The purpose of this investigation was to study nutritional effect on clonal propagation of the tested species. To examine suitable culture system for *in vitro* multiplication of selected potato cultivar, production of virus and disease free plantlets. To achieve the aim, shoots of tested species

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were cultured in nutrient media. Various source of nutrition, such as hormones and coconut water were supplied to the media. Response of explants to the media was studied in the present work.

Materials and Methods

Source of Plant Materials and Identification

The explants **of *Solanum tuberosum* L.** (shoots) obtained from initial culture which were well established in Plant Tissue Culture Laboratory of the Htone Bo Agricultural Research Farm, Taunggyi, Department of Agricultural Research. Markies are available from the Department of Agriculture; obtained from Heho Garden.

The morphological and taxonomical studies of the specimen were made at the Department of Botany, Taunggyi University by using available literatures.

Culture Media

The shoot of *Solanum tuberosum* L. were cultured on MS basal medium supplement with 30g sucrose, inositol 0.1g and 6g agar for solid media and various concentrations of hormones: naphathalene acetic acid (NAA), 6-benzylaminopurine (BAP), and coconut water. Finally, the preparation of the medium were inoculated into autoclaved at 121º C for 30 minutes.

Table 1. Different concentrations of NAA, BAP and Coconut water with MS medium

Treatments	Basal Medium	PGR concentration			Remark
		NAA (mg/l)	BAP (mg/l)	Coconut water (ml/l)	
T_1	MS	0	0	0	5 Replication
T_2	MS	0.05	1.0	200	
T_3	MS	0.05	0.5	200	
T_4	MS	0	0	200	

Table 2. Different concentrations of NAA and Coconut water with MS medium

Treatments	Basal Medium	PGR concentration		Remark
		NAA (mg/l)	Coconut water (ml/l)	
T_1	½ MS	0	0	5 Replication
T_2	½ MS	0.01	200	
T_3	½ MS	0.05	200	
T_4	½ MS	0	200	

Media Preparation

According to Murashige and Skoog (1962), method of 1000 ml medium preparation applied in the present study.

Culture of Explants

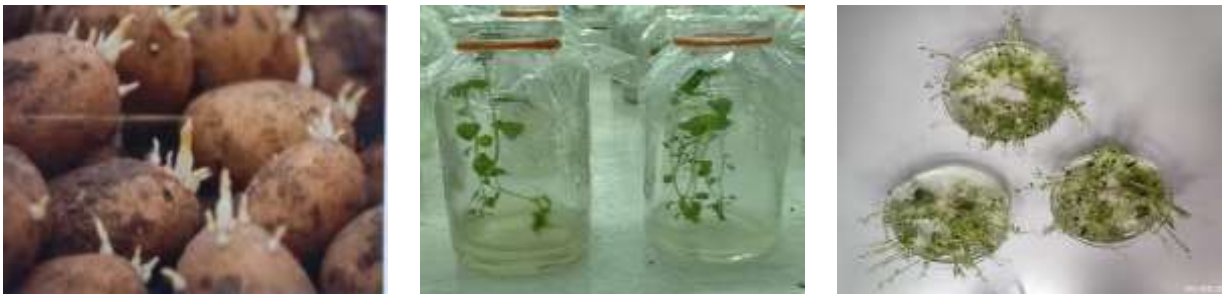
The shoot obtained from initial culture which node cutting of 1 cm in length consisting of one axillary shoot from the middle portion of 6-weeks old plantlets were used as explants source for regeneration. Six weeks old *Solanum tuberosum* L. shoots containing one to two leaves and length of 7.0 – 7.5 cm were selected for each experiment. Two numbers of such shoots were taken and cultured in a prepared medium bottle and each treatment had five replications.

Cultured Conditions

All culture bottles were inoculated in culture room at 20 ± 2˚C with 16/8 hours (light/dark) photoperiod and 30 µmolm^{-1}s^{-1} using white fluorescent lamps.

Measurement and Recording

Growth of shooting stage and rooting stage were measured by recording length of plantlet, number of leaves, the number of shoot and number root. These were recorded at five weeks after culture.



Seed potato | 6-weeks old plantlets in culture | 35- days olded in subculture

Figure 1. *Solanum tuberosum* L. (Potato)

Results

Morphological Characters of *Solanum tuberosum* L.

Scientific Name	:	*Solanum tuberosum* L.
English Name	:	Potato
Myanmar Name	:	A-lu
Family	:	Solanaceae

Annual tufted herbs with tuberous underground stems, cultivated. Stem; ribbed winged, erect or prostrate, about (1-2 ft) in height, pilose. Leaves; alternate, unipinnate, interruptedly imparipinnate compound, petiolate, exstipulate; each pair of large leaflets following pairs of much smaller ones; lowest pair (at base of petiole) small; larger ones ovate-obvate-elliptic; leaflets herbaceous, leaf blade with simple hairs on both surfaces. Inflorescences; terminal or lateral cyme, stout peduncle, pedicels hairy, articulate, 7-20 flowered. Flower; ebracteate, ebracteolate, pedicellate, complete, bisexual, regular, hypogynous. Calyx; sepals 5, synsepalous, lobes long-accuminate, bell-shaped, valvate, pilose, persistent. Corolla; petals 5, synpetalous, campanulate, valvate, white or pale purple, outside hairy; limb slightly lobed. Androecium; stamens 5, apostamenous, antipetalous, epipetalous, the filaments very short, the anthers dithecous, basifixed, introrse, porous dehiscent. Gynoecium; bicarpellary, syncarpous, two locules, axile placentation, many ovules in each locule, the style long, the stigma lobed, ovary superior. Fruits; spherical to ovoid baccate (Figure. 2).

Habit Inflorescence Flower as seem

Figure 2 . Morphological Characters of *Solanum tuberosum* L. (Potato)

Table 3. Survival percentage of potato

Treatments	7 days	14 days	21 days	28 days	35 days
T_1	1	1	1	1	1
T_2	1	1	0.93	1	1
T_3	1	1	1	1	1
T_4	1	1	1	1	1

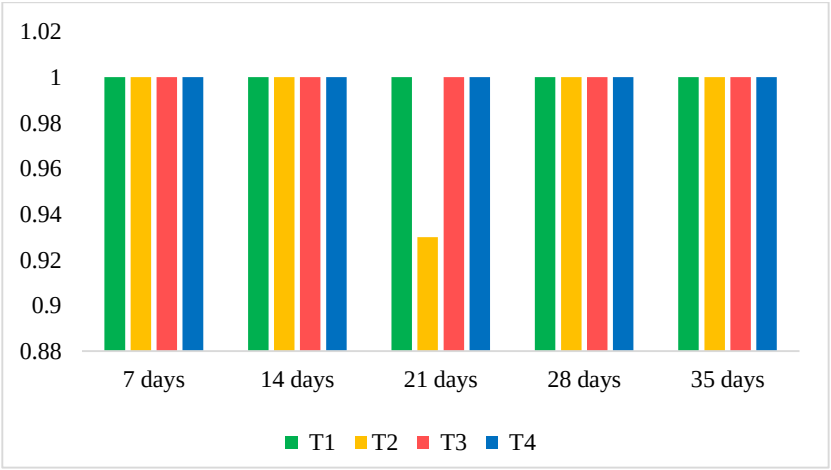


Figure 3 . Survival percentage of potato

Experiment 1: Effects of plant hormone concentration (NAA and BAP) and coconut water in the Shoot Regeneration of potato.

The result obtained in the present investigation using different media, T_1 (control MS only), T_2 (MS + 0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW), T_3 (MS + 0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW), T_4 (MS + 200 ml CW) were as follows:

The result of the plant height showed that T_1 (control) had 4.34, T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) had 4.59, T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 4.2, T_4 (200 ml CW) had 4.74. The maximum plant height were observed in T_4 (200 ml CW).

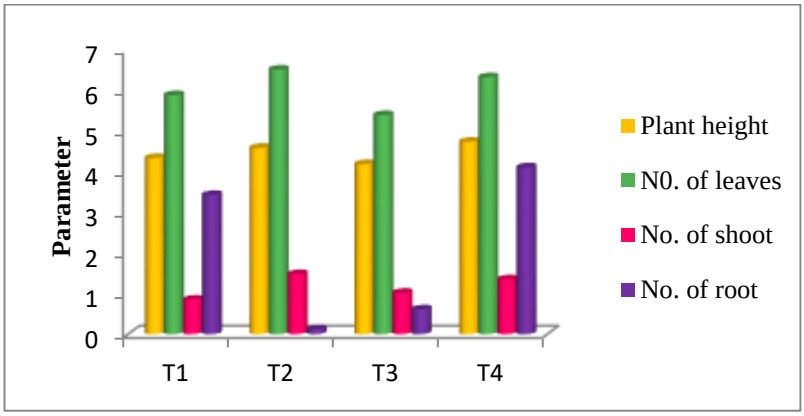
The result of the leaves number T_1 (control) had 5.89, T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) had 6.52, T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 5.4, T_4 (200 ml CW) had 6.32. The highest number of leaves were observed in T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW).

T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) produced more shoot number 1.48 in this experiment. The second best result obtained from T_4 (200 ml CW) had 1.35 and T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 1.02 followed by T_1 (control) had 0.85.

The result of the root number T_1 (control) had 3.43, T_2 (0.05 mg/l NAA + 1.0 mg/l BAP +200 ml CW) had 0.12, T_3 (0.05 mg/l NAA + 0.5 mg/l BAP +200 ml CW) had 0.61, T_4 (200 ml CW) had 4.11. The highest number of root were observed T_4 (200 ml CW) (Table 3 and Figure 3 and 4).

Table 4. Effects of plant hormone (NAA + BAP) and coconut water on the Shoot Regeneration of potato

Treatments	Survival %	Plant Height (cm)	No. of Leaves	No. of shoots	No. of Roots
T_1	100	4.336	5.886	**0.852**	3.434
T_2	100	4.587	**6.52**	**1.48**	**0.118**
T_3	100	**4.196**	**5.4**	1.024	0.612
T_4	100	**4.74**	6.328	1.352	**4.114**



T_1 : Control (MS only)
T_2 : 0.05mg/l NAA+1.0 mg/l BAP +200 ml/l coconut water
T_3 : 0.05 mg/l NAA + 0.5 mg/l BAP + 200 ml/l coconut water
T_4 : 200 ml/l coconut water

Figure 4. Effects of plant hormone (NAA + BAP) and coconut water on the Shoot Regeneration of potato

Maximum length of plantlet and root from T_4 Highest number of leaves and shoot from T2

Figure 5. Effects of (NAA + BAP) and coconut water treatments on the 5-week after the start of the experiment

Experiment 2: Effects of plant hormone concentration NAA and coconut water in the Rooting Stage (Pre-hardening stage) of potato

The result obtained in the present investigation using different media, T_1 (control ½MS only), T_2 (½MS + 0.01 mg/l NAA + 200 ml CW), T_3 (½MS + 0.05 mg/l NAA + 200 ml CW), T_4 (½MS + 200 ml CW) were as follows:

The result of the plant height showed that T_1 (control) had 1.83, T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 2.14, T_3 (½MS + 0.05 mg/l NAA + 200 ml CW) had 2.81, T_4 (½MS + 200 ml CW) had 3.58. The maximum plant height were observed in T_4 ½ MS + 200 ml CW).

The result of the leaves number T_1 (control) had 3.55, T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 4.45, T_3 (½MS + 0.05 mg/l NAA + 200 ml CW) had 5.39, T_4 (½MS + 200 ml CW) had 7.05. The highest number of leaves were observed in T_4 (½MS + 200 ml CW).

T_4 (½MS + 200 ml CW) produced more shoot number 0.65 in this experiment. The second best result obtained from T_3 (½MS + 0.05 mg/l NAA + 200 ml CW) had 0.58 and T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 0.51 followed by T_1 (control) had 0.31.

The result of the root number T_1 (control) had 0.69, T_2 (½MS + 0.01 mg/l NAA + 200 ml CW) had 1.85, T_3 (½MS + 0.05 mg/l NAA + + 200 ml CW) had 3.47, T_4 (½MS + 200 ml CW) had 4.05. The highest number of root were observed in T_4 (½MS + 200 ml CW) (Table 4 and Figure 5 and 6).

Table 5. Effects of plant hormone NAA and coconut water on the Rooting Stage (Pre-hardening stage) of potato

Treatments	Survival %	Plant Height (cm)	No. of Leaves	No. of shoots	No. of Roots
T_1	100	1.830	3.548	0.308	0.692
T_2	100	2.136	4.452	0.508	1.846
T_3	100	2.814	5.392	0.578	3.472
T_4	100	3.576	7.052	0.648	4.048

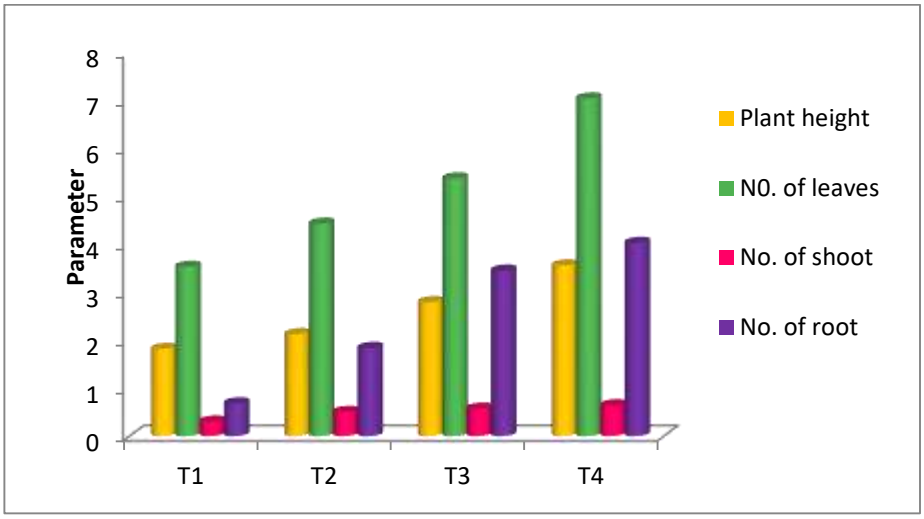


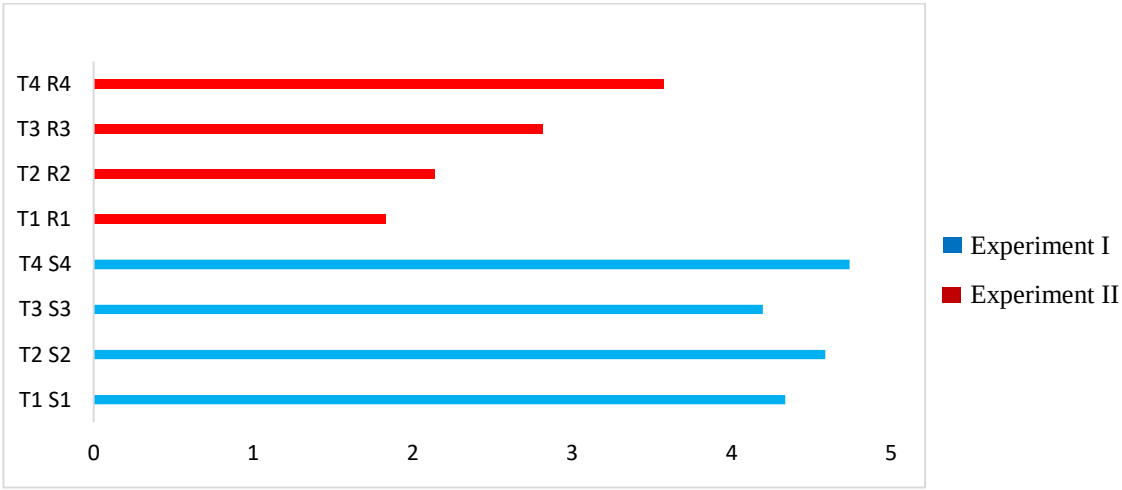
Figure 6. Effects of plant hormone (NAA + BAP) and coconut water on the Shoot Regeneration of potato

Maximum length of plantlet, highest number of leaves, shoot and root from 200 ml/l coconut water

Figure 7. Effects of NAA and coconut water treatments on the 5-week after the start of the experiment

Table 6. Comparison of plant height

$T_1 S_1$	$T_2 S_2$	$T_3 S_3$	$T_4 S_4$	$T_1 R_1$	$T_2 R_2$	$T_3 R_3$	$T_4 R_4$
4.336	4.587	4.196	4.74	1.83	2.136	2.814	3.576



Plant height (cm)

T_1 - Control (MS only)

T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water

T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water

T_4 - MS + 200 ml/l coconut water

T_1 - Control (½ MS only)

T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water

T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water

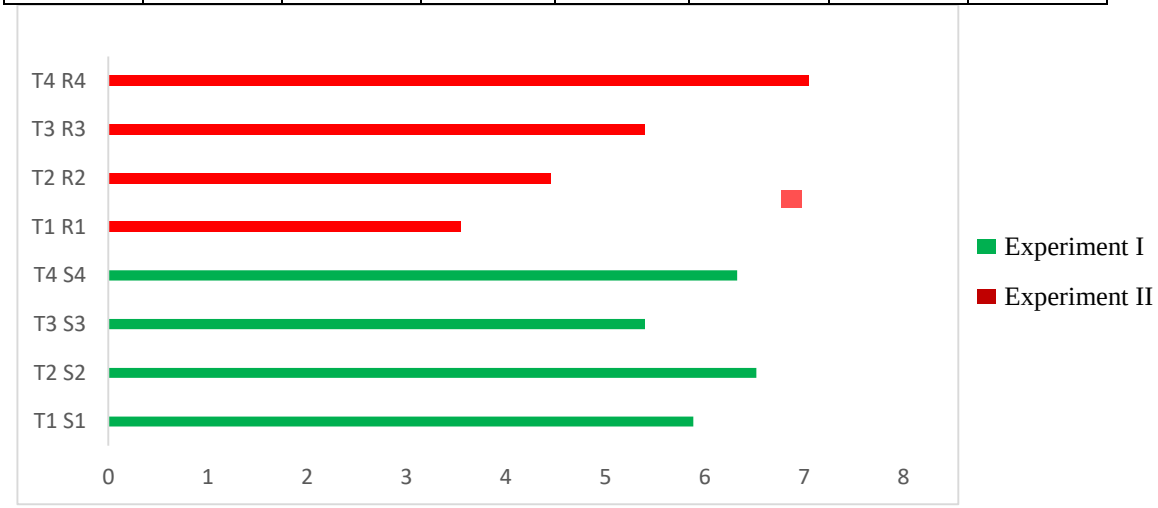
T_4 - ½ MS + 200 ml/l coconut water

S - Experiment I, R- Experiment II

Figure 8. Comparison of plant height (Markies)

Table 7. Comparison on sum of no. of leaves per plant

T_1S_1	T_2S_2	T_3S_3	T_4S_4	T_1R_1	T_2R_2	T_3R_3	T_4R_4
5.886	6.52	5.4	6.328	3.548	4.452	5.392	7.052



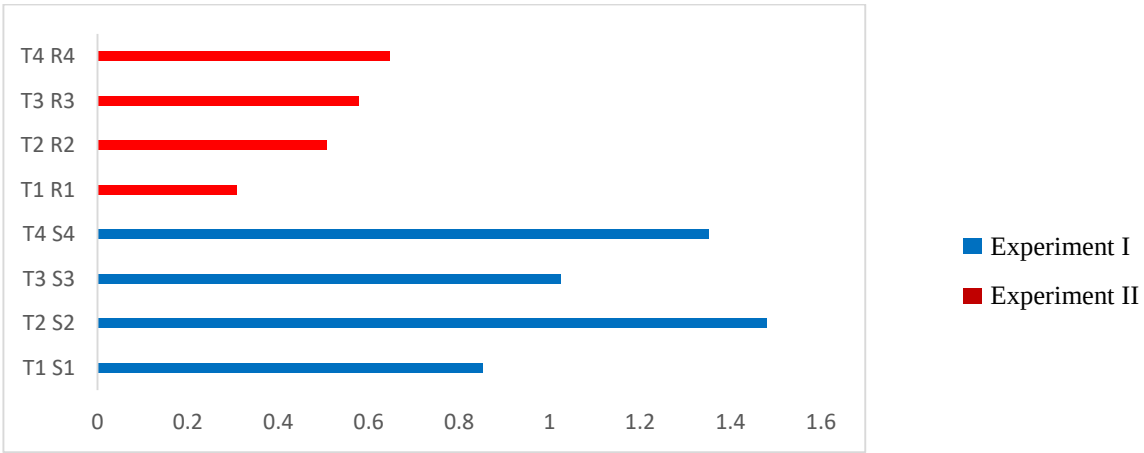
Parameter

T_1 - Control (MS only)
T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water
T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water
T_4 - MS + 200 ml/l coconut water
T_1 - Control (½ MS only)
T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water
T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water
T_4 - ½ MS + 200 ml/l coconut water
S - Experiment I, R- Experiment II

Figure 9. Comparison of no. of leaves per plant

Table 8. Comparison on sum of no. of shoots per plant

T_1S_1	T_2S_2	T_3S_3	T_4S_4	T_1R_1	T_2R_2	T_3R_3	T_4R_4
0.852	**1.48**	1.024	1.352	0.308	0.508	0.578	0.648



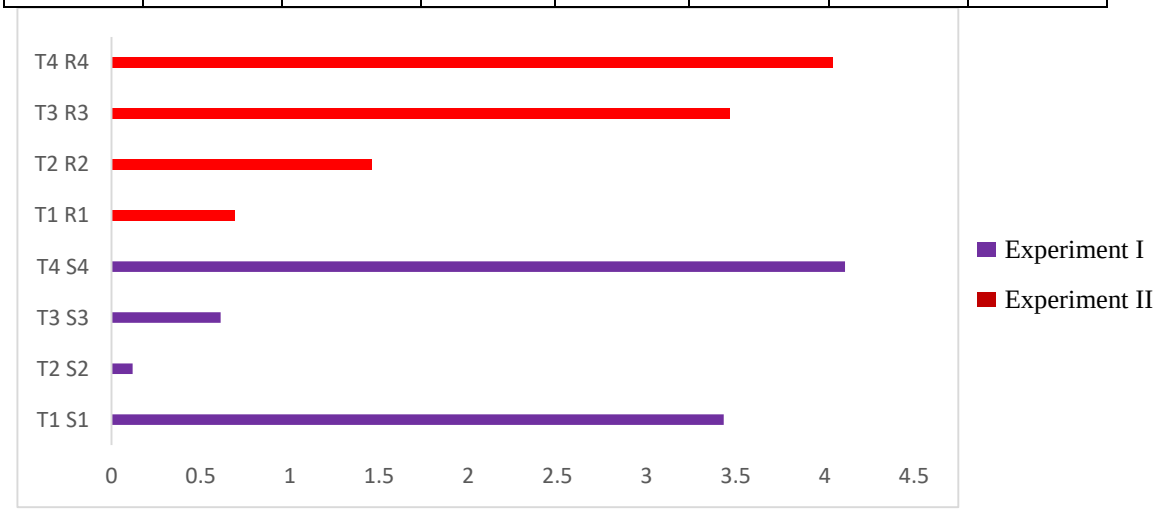
Parameter

T_1 - Control (MS only)
T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water
T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water
T_4 - MS + 200 ml/l coconut water
T_1 - Control (½ MS only)
T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water
T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water
T_4 - ½ MS + 200 ml/l coconut water
S - Experiment I, R- Experiment II

Figure 10. Comparison of no. of shoots per plant

Table 9. Comparison on sum of no. of roots per plant

$T_1 S_1$	$T_2 S_2$	$T_3 S_3$	$T_4 S_4$	$T_1 R_1$	$T_2 R_2$	$T_3 R_3$	$T_4 R_4$
3.434	0.118	0.612	4.114	0.692	1.46	3.472	4.048



Parameter

T_1 - Control (MS only)
T_2 - MS + 0.05mg/l NAA+ 1.0mg/l BAP + 200 ml/l coconut water
T_3 - MS + 0.05mg/l NAA+ 0.5mg/l BAP + 200 ml/l coconut water
T_4 - MS + 200 ml/l coconut water
T_1 - Control (½ MS only)
T_2 - ½ strength MS + 0.01 mg/l NAA + 200 ml/l coconut water
T_3 - ½ MS + 0.05 mg/l NAA + 200 ml/l coconut water
T_4 - ½ MS + 200 ml/l coconut water
S - Experiment I, R- Experiment II

Figure 11. Comparison of no. of roots per plant

Discussion and Conclusion

The plant of *Solanum tuberosum* L. are annual herbs with tuberous underground stems. The stems are erect or prostrate. Leaves interruptedly imparipinnate compound, each pair of large leaflets following pairs of much smaller ones; lowest pair (at base of petiole) small; leaflets herbaceous, with simple hairs on both surfaces. Inflorescence is a terminal or lateral cyme, erect, on a stout peduncle; pedicels hairy, articulate. Flower white or pale purple, anthers at narrowed apex with 2 lateral pores developing into short slits, ovary superior. These characters were in agreement with Backer and Brink (1965).

In this research, potato cultivar Markies were treated with T_1 (Control -MS only), T_2 (0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water), T_3 (0.05 mg/l NAA + 0.5 mg/l BAP + 200 ml/l coconut water) and T_4 (200 ml/l coconut water) for shoot regeneration stage. The cultivar were treated with T_1 (Control - ½ MS only), T_2 (0.01 mg/l NAA + 200 ml/l coconut water), T_3 (0.05 mg/l NAA + 200 ml/l coconut water) and T_4 (200 ml/l coconut water) for rooting stage.

In the present study, among the combination with NAA, BAP and coconut water, 200 ml coconut water supplemented with MS solid medium gave the maximum length of plantlet 4.74 cm. The minimum length of plantlet were observed in (½ MS medium).

As the number of leaves, 200 ml coconut water supplemented with ½ MS solid medium gave the highest leaves number 7.05 in shoot regeneration stage. Control (½ MS medium) gave the smallest number of leaves 3.5 in rooting stage.

As the number of shoot, the highest number of shoot were found in (0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water) in shoot regeneration stage. The lowest of shoot number 0.3 observed in cultivar Markies on control (½ MS medium) in rooting stage.

The result of the root number, 200 ml coconut water supplemented with MS solid medium gave the highest root number 4.11. The lowest of root number 0.11were observed on 0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water medium.

When compared the results of plant growth regulator and coconut water added basal medium, combination of 200 ml coconut water with MS medium showed longest length of plantlets and 200 ml coconut water with ½ MS medium indicated the highest number of leaves in potato.

The proper medium for potato *(Solanum tuberosum* L.) using 200 ml coconut water with MS medium was suitable for longest length of plantlets and the highest number of roots. 200 ml coconut water with ½ MS medium was suitable for highest number of leaves and 0.05 mg/l NAA + 1.0 mg/l BAP + 200 ml/l coconut water medium was suitable for highest number of shoot.

This finding was in agreement with the finding of (Mauney *et al*., 1952) who reported that the plant growth regulators especially auxin and cytokinin present in coconut water.

The cytokinin (BAP) are also used for shoot proliferation by the release of axillary buds from apical dominance (Bhojwani and Razdan, 1983). The investigation of this study were in agreement with the previous finding.

The finding of this study were agreement with (Tulecke and Nickel, 1960) reported that coconut water contains amino acids, inorganic salts and growth promoting substances and the increase in growth is probably due to the presence of these substances.

Coconut water is rich in various minerals and electrolytes like potassium, calcium, manganese, antioxidants amino acids and cytokinins. Coconut water is the best source of potassium (https://pharmeasy.in>blog>11-in...).

It is concluded that the shoots of potato (*Solanum tuberosum* L.) using different combination and concentration of growth regulators and coconut water can reduce the high cost for commercial plant production.

Potatoes are raw, boiled, peeled, or mashed all have medicinal and healing properties. The potatoes are Carbohydrate plant, a fat free food containing carbohydrate, proteins, vitamins, antioxidants and minerals. Moreover, potato is considered as major food crops after maize, rice and wheat. Therefore, potato should be cultivated by using tissue culture method for the production of many plants.

Acknowledgements

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DISTRIBUTION OF BLUE GREEN ALGAE FROM PADDY FIELD IN BO TAUG KONE VILLAGE OF PATHEINGYI TOWNSHIP, MANDALAY REGION

Khin Myo Win[1], Htet Ei Ei Chaw[2], Shoon Lae Yee Htwe[3]

Abstract

Blue Green Algae perform as a bioindicator for paddy field and play the important roles in agricultural soils. The algae specimens and soil samples were collected from paddy field of Bo taug kone village, Patheingyi Township during the months from June 2022 to January 2023. A total of twenty-four species belonging to eleven genera of division Cyanophyta were identified according to their morphological characteristics. The soil pH, electrical conductivity, nitrogen, phosphorus, potassium, moisture and soil texture were measured. Nitrogen fixation blue green algae such as *Anabaena ambigua, A. orientalis, A. variabilis, Cylindrospermum muscicola, Nostoc ellipsosporum, N. microscopicum, N. commune, N. paludosum, N. spongiforme, Gloeotrichia raciborskii* and others blue green algae species were found in the paddy field soil. The present study concludes that the blue green algae enhances soil fertility and is capable of nitrogen fixation for agriculture soil.

Keywords: Bo Taug Kone village, Blue Green Algae, Morphological characteristics

Introduction

Blue green algae are autotrophic which are capable of nitrogen fixation. It has ability to convert nitrogen into nitrate and nitrites, which are easily absorbed by the plant. Thus, blue green algae enhance growth and productivity of the crop plant by providing usable nitrogen through soil and improving the fertility of soil (Jadhav & Talekar, 2019). Blue green algae are the main components of the microbiota in rice fields and play an important role in the maintenance and build-up of soil fertility, consequently increasing rice production (Thajamanbi *et al*., 2016).

Rice field ecosystem provides favorable environment for the growth of various groups of algae with respect to their requirement of light, water, temperature and nutrient availability (Venkataraman, 1981). Blue green algae are extremely important to fix atmospheric nitrogen in rice fields. They can contribute to the natural fertility of the soils through nitrogen-fixation in their heterocysts.

Kamat (1975) stated that the nitrogen fixing algae not only fix atmospheric nitrogen when they grow in paddy fields but they also enrich the soil with nitrogen and thereby make nitrogen available to the plants growing in these areas. Blue green algae such as *Aulosira, Porphyrosiphon, Scytonema, Lyngbya, Microcoleus, Anabaena, Nostoc, Cylindrospermum* and *Aphanothece* grow extensively in the cultivated soils.

In Myanmar, Khin Mya Mya and Than Tun (1976) examined the nitrogen fixing of blue green algae in Patheingyi of Mandalay area. Khin Nilar Than (1994) investigated that the blue green algae in Patheingyi of Mandalay area. The study of blue green algae is relatively scarce for Myanmar. So, this study emphasized on the blue green algae from paddy field of Bo taug Kone village, Patheingyi Township. The soils in Bo taug kone village are mainly used for rice cultivation. Bo taug kone paddy field is irrigated by the water from the irrigation supply channel, Setawgyi Dam. The aim of the study is to identify the blue green algae from paddy field soils, to investigate physico-chemical features of the soil and to reveal beneficial blue green algae for agriculture.

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Materials and Methods

Study area

Bo taug kone village is one of the 140 villages of Patheingyi Township and it is situated in the North-East of Mandalay City. It lies between the latitude of 21º 51'- 22º 09' N and the longitude of 96º 01'- 96º 22' E. Locationally, Patheingyi is 5 miles far from Mandalay and in the North-East of Mandalay City. This area is characterized by a mean temperature of 29°C. The annual rainfall is 33.77 inches and the mean humidity is 71%. The elevation is 250 m above sea level.

Identification of algae

The algae specimens were collected at random during the months from June 2022 to January 2023. The soil samples were collected on sunny days having temperature 28-35°C, at 10:00 am-11:30 am. The collected specimens were added to the plastic bottles and brought to the laboratory of Botany, Yadanabon University. In culture, 3 g of soil sample was put into 100 ml capacity conical flask containing 50 ml BG 11 medium. These cultures were incubated under sunlight to promote the growth of algae. They were examined for ten days. The morphological characters of algae specimens were analyzed by using Olympus microscope. Then, the cell shapes were measured by using ocular meter. The morphological characteristics were taxonomically identified with the help of literature by Desikachary (1959), Komarek and Anagnostids (1985), Prescott (1962), Shameel (2012). And then, the soil samples were taken randomly from different places and combined together for subsequent analysis. Soil analyses of soil sample were done by the Department of Agriculture (Land use division), Mandalay Region.

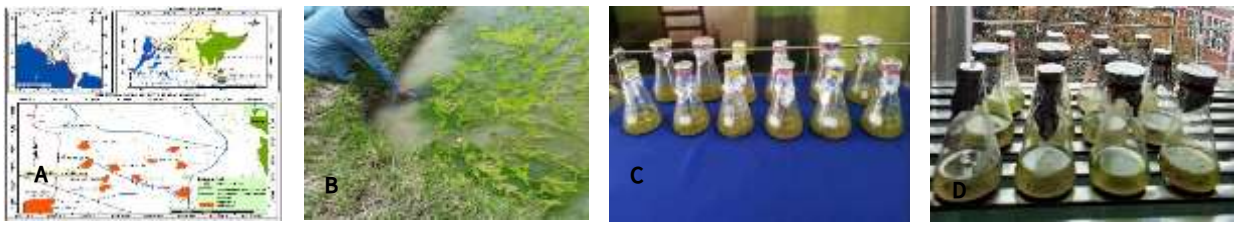


Figure 1. A. Map of study area, B. Algae specimens collect with plastic bottle, C. 3 g of soil inoculated in 50 ml BG 11 Medium, D. Formation of algae after 7 days in BG 11 medium

Results

In the present study, blue green algae 24 species from paddy field of the Bo taug kone village, which belong to 2 classes, 2 order, 4 families and 11 genera were recorded and are described on the following table 1. Cyanophyta (blue green algae) included that unicellular, heterocyst and non- heterocyst filamentous forms. Five different parameters such as pH, EC and concentration of N, P, and K, soil type, moisture and texture were analyzed. The key of the genera and their morphological characteristics of blue green algae are described as follow.

Key to the Genera:

1. Non-filamentous forms ---------- 2
1. Filamentous forms ---------- 4
   2. Sheath present ---------- *Chroococcus*
   2. Sheath absent ---------- 3
3. Cells forming packet-like colonies ---------- *Cyanosarcina*
3. Cells without forming packet-like colonies ---------- *Aphanocapsa*

4. Heterocysts present ---------- 5
4. Heterocysts absent ---------- 8
5. Heterocysts terminal ---------- 6
5. Heterocysts intercalary ---------- 7
6. Trichomes hair present ---------- *Gloeotrichia*
6. Trichomes hair absent ---------- *Cylindrospermum*
7. Trichomes solitary or in a tangled ---------- *Anabaena*
7. Trichomes embedded in obvious and extended firm mucilage ---------- *Nostoc*
8. Trichomes sheath present ---------- *Lyngbya*
8. Trichomes sheath absent ---------- 9
9. Trichomes straight ---------- *Oscillatoria*
9. Trichomes spirally coiled ---------- 10
10. Multicellular, cross wall distinct ---------- *Arthrospira*
10. Unicellular, cross wall indistinct ---------- *Spirulina*

Table 1. Species of Blue Green Algae occurred from paddy field of Bo Taug Kone village.

Phylum	Class	Order	Family	Genus	Species
Cyanophyta	Chroocophyceae	Chroococcales	Chroococcaceae	1. *Aphanocapsa*	*intertexta*
				2. *Chroococcus*	*turgidus* var. *maximus*
				3. *Chroococcus*	*minutus*
				4. *Cyanosarcina*	*burmensis*
	Nostocophyceae	Nostocales	Oscillatoriaceae	5. *Arthrospira*	*khannae*
				6. *Spirulina*	*princeps*
				7. *Spirulina*	*subsalsa*
				8. *Oscillatoria*	*chlorina*
				9. *Oscillatoria*	*rubescens*
				10. *Oscillatoria*	*proboscidea*
				11. *Oscillatoria*	*subbrevis*
				12. *Oscillatoria*	*vizagapatensis*
				13. *Lyngbya*	*martensiana*
				14. *Lyngbya*	*truncicola*
			Nostocaceae	15.*Nostoc*	*ellipsosporum*
				16.*Nostoc*	*microscopicum*
				17.*Nostoc*	*commune*
				18.*Nostoc*	*paludosum*
				19.*Nostoc*	*spongiforme*
				20.*Anabaena*	*ambigua*
				21.*Anabaena*	*variabilis*

Phylum	Class	Order	Family	Genus	Species
			Rivulariaceae	22. *Cylindrospermum*	*muscicola*
				23. *Gloeotrichia*	*raciborskii*
				24. *Gloeotrichia*	*raciborskii* var. *conica*

1. *Aphanocapsa intertexta* Gardner

Colony with no definite, cells agglomerations with up to 50 cells, surrounded by a thin mucilage, cells sub-spherical, light green, with small granules; cells 7.5µm in diameter.

2. *Chroococcus turgidus* var. *maximus* Nygaard

Cells hemispherical, sheath colorless; much lamellate in the inner portion, two-celled colonies, cells 42.5µm in diameter and long, colony 45µm in diameter, 62.5µm in long.

3. *Chroococcus minutus* (Kuetzing) Naegeli

Cells spherical, groups of 4, covered mucilaginous sheath; granule present; cells 25µm in diameter and long, colony 50µm in diameter, 75µm in long.

4. *Cyanosarcina burmensis* (Skuja) Kovacik

Cells irregular, cells join to mucilaginous colonies, cells divide successively in three or more different places, forming small packet-like colonies; cells 8.5µm in diameter, 15.5µm long.

5. *Arthrospira khannae* Drouet et Strickland

Trichome planktonic, multicellular, forming loose spirals, not constricted at the cross walls, ends slightly attenuated, cross walls granulated; cells 5.0µm in diameter, spiral about 20.0µm distant.

6. *Spirulina princeps* W.et G. S West

Trichome loosely spiralled, unicellular, regularly coiled, blue green; distance between spiral 12.5µm in diameter.

7. *Spirulina subsalsa* Oersted ex Gomont

Trichome tightly spiralled, blue green, regularly coiled, tightly coiled, no space between cells; cells 2.5µm in diameter.

8. *Oscillatoria chlorina* Gomont

Trichome straight, not constricted at the cross walls, granules present, apical cell broadly rounded, not capitate, yellow; cells 12.0µm in diameter, 7.5µm in long.

9. *Oscillatoria rubescens* (Kutz.) Gomont

Trichome straight, not constricted at the cross walls, apical cell conical, granule present, ends gradually attenuated, calyptra present, blue green, forming black spot; cells 7.5µm in diameter, 2.5µm long.

10. *Oscillatoria proboscidea* Gomont

Trichome straight, not constricted at the cross walls, apical cells capitate, granule absent at the septa, at the end gradually attenuated and bent, blue green; cells 12.5µm in diameter, 7.5µm in long.

11. ***Oscillatoria subbrevis*** Schmidle

Trichome straight, slightly constricted at the cross walls, granule absent at the septa, slightly curved at the apex, apical cells rounded, yellowish green; cells 7.5µm in diameter, 2.5µm in long.

12. ***Oscillatoria vizagapatensis*** Rao, C.B

Trichome straight, not constricted at the cross wall, apical cell broadly rounded forming a cap with a slightly thickened outer membrane, granules present, blue green; cells 8.0µm in diameter, 9.5µm long.

13. ***Lyngbya martensiana*** Menegh ex Gomont

Filaments caespitose, filament more or less flexible, sheath thin, not constricted at the cross-walls, end cell round, blue green; cells 10.0µm in diameter, 3.0µm in long.

14. ***Lyngbya truncicola*** Ghose

Filaments straight, sheath thin, not constricted at the cross-walls, cells short, apical cell rotund, not attenuated, blue green; cells 10.0µm in diameter, 3.0µm in long.

15. ***Nostoc ellipsosporum*** (Desm.) Rabenhorst

Thallus colonial, gelatinous, irregularly loosely entangled, brownish green; cells cylindrical; 6.5µm in diameter, 14.0µm long; heterocyst sub-spherical, 7.5µm in diameter, 14.0µm long; gonidia ellipsoid; 7.5µm in diameter; 17.5µm long.

16. ***Nostoc microscopicum*** Carmichael

Thallus loosely entangled in mucilage colonial, cells barrel-shaped, 7.5□m in diameter, heterocyst subspherical, 7.5µm in diameter; gonidia barrel-shaped.

17. ***Nostoc commune*** Vaucher ex Bornet & Flahault

Thallus gelatinous mass, colonial, filaments very coiled, densely entangled and interwined, cells sub-spherical, 6.25µm in diameter and 7.5µm long, heterocyst globose, intercalary.

18. ***Nostoc paludosum*** Kutzing ex Born et Flah.

Thallus microscopic, loosely or tightly coiled in gelatinous sheath, yellowish-green; cells barrel-shaped, 5.5µm in diameter, 6.5µm long, heterocyst ovate, 5.0µm in diameter and 7.5µm long; akinetes ovate.

19. ***Nostoc spongiforme*** C.A Agardh

Colony globular of loosely entangled trichome, expanded; cells cylindrical; 5.0µm in diameter; 7.5µm long, the colour changing blue-green to brownish green; heterocysts oblong or ovate, 6.5µm in diameter, 7.5µm long.

20. ***Anabaena ambigua*** Rao, C.B.

Trichome straight or slightly bent, dense clusters, cells barrel-shaped, with deep constriction at the joint, apical cell rounded, granular present; cells 7.5µm in diameter, 5.0µm long, heterocyst spherical, 7.5µm in diameter, 10.5µm long, akinetes ellipsoid, continguous to heterocysts, 12.5µm in diameter, 15µm long, attaching at one both side of the heterocysts.

21. ***Anabaena variabilis*** Kutzing ex Born. et Flah

Trichomes single, flexuous, cells spherical, end cell rounded, heterocyst intercalary, spherical, blue green; cells 6.5µm in diameter, heterocysts 6.5µm in diameter, akinetes not continguous with the heterocysts.

22. *Cylindrospermum muscicola* Kutzing ex Born. et Flah

Thallus mucilagenous, trichome broad, constricted at the cross walls, light blue green; cells quadrate, 4.5- 5.0µm in diameter, heterocysts oblong; 5µm in diameter and 7.5µm long.

23. *Gloeotrichia raciborskii* Woloszynska

Filaments free floating; trichomes heteropolar, ending in a long hair, sheath at the base lamellated, dull brown; cells at the base of the trichome shorter than broad, higher up as long as broad, pale blue green; basal heterocyst spherical, 7.5µm in diameter, cells 2.5µm in diameter and 55µm long.

24. *Gloeotrichia raciborskii* var. *conica* Woloszynska

Filaments free floating; taper towards the end, with a basal heterocyst, with a sheath which often covers the basal heterocyst, sheath thinning out from the base to apex, giving a more or less conical shape, trichomes 7.5µm in diameter, heterocyst 7.5µm long and diameter, cells 55µm long.

Table 2. Soil analytical data of Paddy field in Bo Taug Kone Village

No.	pH (1:25)	Electro Conductivity (mS/cm)	Total Nitrogen (%)	Available Phosphorus (ppm)	Available Potassium (mg/100g)	Soil type	Moisture (%)	Soil texture
1.	7.56	0.17	0.22	5.40	19.47	moderately alkaline	4.40	Sandy loam

Discussion

In this present study, the distribution of blue green algae in the paddy fields from Bo taug kone village in Patheingyi Township, Mandalay region has been studied from June 2022 to January 2023. A total 24 species of blue green algae were identified from paddy field soil. 1 species of *Aphanocapsa intertexta, Arthrospira khannae, Cyanosarcina burmensis* and *Cylindrospermum muscicola*; 2 species of *Anabaena ambigua, A. variabilis, Chroococcus turgidus* var. *maximus, C. minutus, Gloeotrichia raciborskii, G. raciborskii* var. *conica, Lyngbya martensiana, L. truncicola* and *Spirulina princeps, S. subsalsa* and 5 species of *Nostoc* and *Oscillatoria* were recorded. Heterocystous containing cell such as *Anabaena, Cylindrospermum, Gloeotrichia* and *Nostoc* were found in paddy field. *Anabaena* and *Nostoc* species were abundant because akinetes cells are resting spores to withstand adverse environmental conditions in the paddy field. *Cylindrospermum* and *Gloeotrichia* play an important role in the soil nutrients cycling, improvement of plant growth, and development. Singh (1950) reported that *Nostoc* and *Anabaena* can be used in the reclamation of the 'usar' lands. Nitrogen fixation blue green algae are present on paddy field soil to increases the soil fertility and nitrogen fixation will be improved.

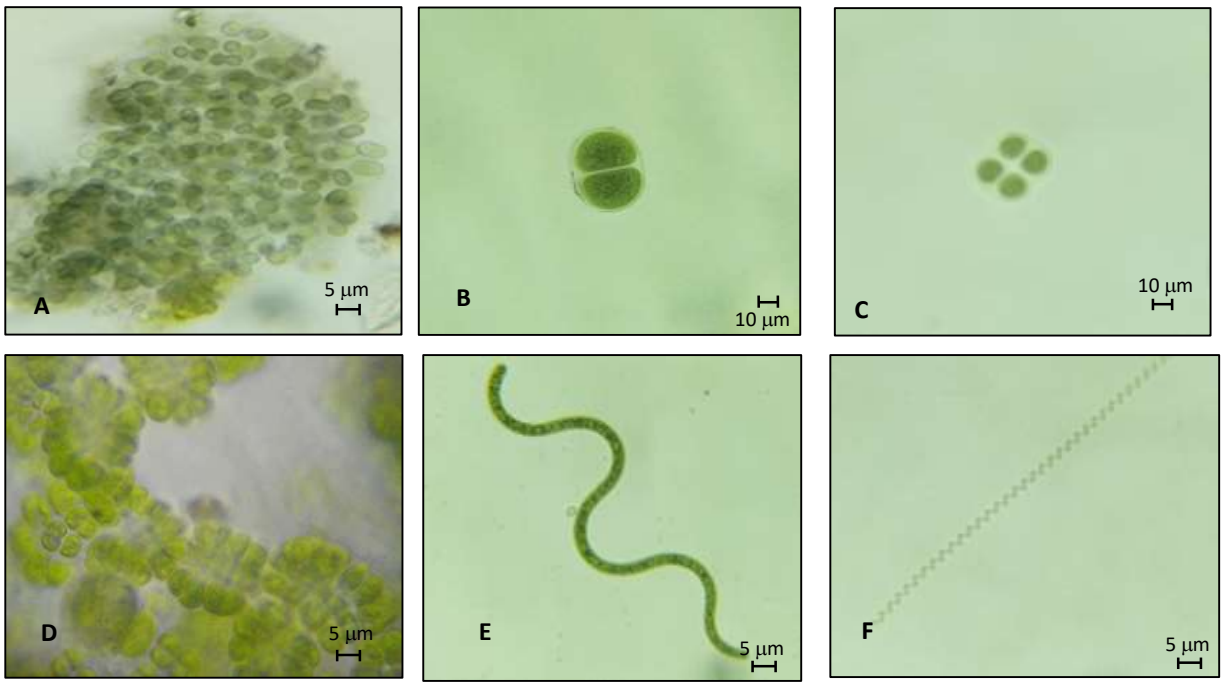
Non heterocystous cells such as *Aphanocapsa intertexta, Chroococcus turgidus var. maximus, C. minutus, Cyanosarcina burmensis, Lyngbya martensiana, L. truncicola Oscillatoria chlorina, O. rubescens, C. proboscidea, O. subbrevis, O. vizagapatensis, Arthrospira khannae, Spirulina princeps* and *S. subsalsa* were observed in this field. The genus of *Anabaena, Nostoc, Oscillatoria, Cylindrospermum* and other genus were commonly found in culture. The genus of *Cyanosarcina* was only found in culture and this genus was not observed on the surface of paddy field. *Oscillatoria* species is not only a source of nitrogen, but also used as organic matter and growth promoting substances for rice cultivation. It can reduce ecological and biochemical imbalance in a rice field. *Spirulina* species is associated with symbiotic bacteria that fix nitrogen which is needed for the growth of plants. Other algae species are able to increase nitrogen in soils.

In this results, the soil of Bo taug kone village was found moderate alkaline and having the pH of 7.56. The alkaline soil is responsible for the growing population of Cyanophyceae. Soil pH affects nutrient solubility in the soil. (Kumari *et al*., 2011) reported that soil pH level of near 7 is optimal for overall nutrient availability, crop tolerance and soil microorganism activity. Blue green algae prefer a slightly alkaline pH 7.5. So, this results agreed with Kumari *et al*., 2011. pH is the most important factor in determining the algal flora composition. Ghadage and Karande (2019) observed an abundant growth of blue green algae in fields with pH ranges from 6.5 to 7.5. In Cultures, optimal pH for growth of cyanobacteria ranges from 7.5 to 10. This result was agreed with Ghadage and Karande. The concentration of total nitrogen, available phosphorus and available potassium have 0.22%, 5.40 ppm and 19.47 in this field. Banakar *et al*., (2020) reported that influences of nutrients especially nitrate and phosphate have in the regulation of Cyanophyceae growth.

The content of the nitrogen, phosphorus and potassium are important factor determining the growth of algae. The moisture of soil and electrical conductivity have 4.40 and 0.17. The Bo taug kone soil texture is sandy loam. Soil texture is also important in agriculture. It was agreed with that of Kamaromy and Padisak (1999) who stated that sandy loam soil has rich algal flora and high algal density. (Jadhav & Talekar., 2019) represented pH and electrical conductivity had a direct influence with the development of algal forms. Rani and Narasimha (2021) stated that a correlation between the composition of the algal flora and soil characters. Blue green algae are economically important both directly and indirectly. The growth of blue green algae depends not only the nutrition in the soil but also their physico-chemical parameters and its environments.

Conclusion

It was concluded that the blue green algae were richly observed from Bo taug kone paddy fields. The blue green algae are beneficial in various ways and its potential application for valuable benefits of human beings as well as other organisms. The blue green algae are present in paddy fields and are helping to maintain soil fertility and to improve the crop production.



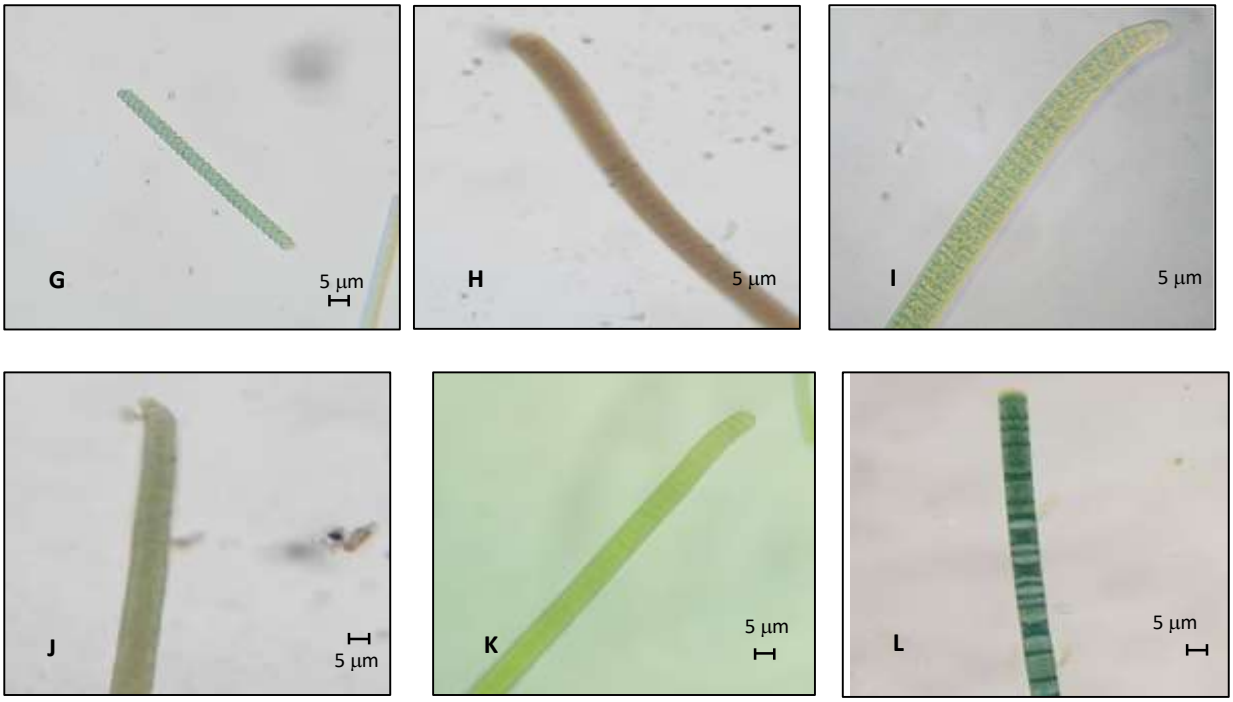


Figure 2. A. *Aphanocapsa intertexta* Gardner

B. *Chroococcus turgidus* var. *maximus* Nygaard

C. *Chroococcus minutus* (Kuetzing) Naegeli

D. *Cyanosarcina burmensis* (Skuja) Kovacik

E. *Arthrospira khannae* Drouet et Strickland,

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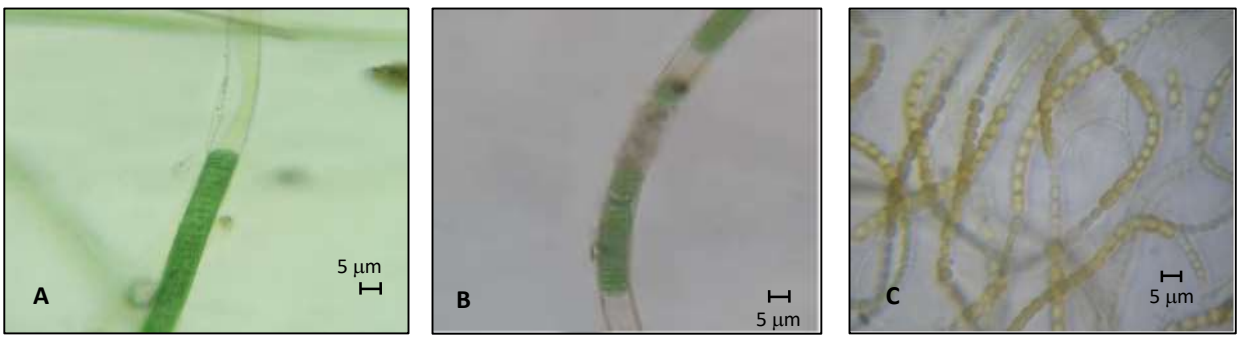
J. *Oscillatoria proboscidea* Gomont

K. *Oscillatoria subbrevis* Schmidle

L. *Oscillatoria vizagapatensis* Rao. C. B



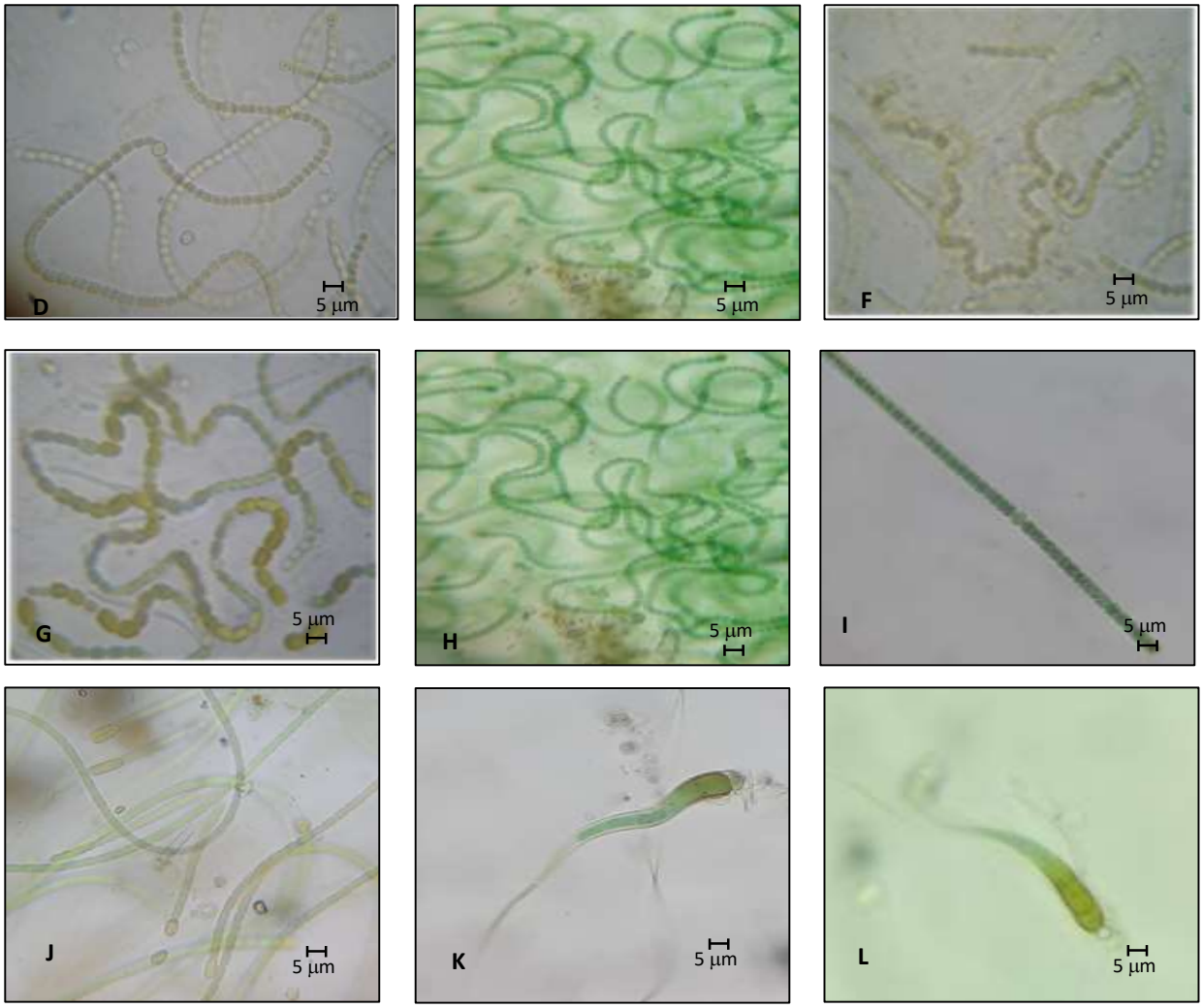


Figure 3. A. *Lyngbya martensiana* Menegh ex Gomont

B. *Lyngbya truncicola* Ghose

C. *Nostoc ellipsosporum* (Desm.) Rabenhorst

D. *Nostoc microsporium* Carmichael

E. *Nostoc commune* Vaucher ex Bornet & Flahault

F. *Nostoc paludosum* Kutzing ex Born.et Flah.

G. *Nostoc spongiforme* C.A Agardh

H. *Anabaena ambigua* Rao, C. B.

I. *Anabaena variabilis* Kutzing ex Born et Flah

J. *Cylindrospermum muscicola* Kutzing ex Born.et Flah

K. *Gloeotrichia raciborskii* Woloszynska

L. *Gloeotrichia raciborskii* var. *conica* Woloszynska

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A STUDY ON POLLEN MORPHOLOGY OF SAPINDALES AND BRASSICALES FROM MANDALAY AND BAGO REGIONS

Hnin Yu Maw[1], Nwe Nwe Yi[2], Swe Swe Linn[3]

Abstract

The pollen morphology of 10 species belonging to 10 genera of 5 families in Sapindales and Brassicales were studied. The specimens were collected from Mandalay and Bago Regions from May 2018 to December 2020. The collected species were 6 species of Sapindales and 4 species of Brassicales. The morphological characters of pollen grains of each species were also studied. The pollen grains of all species examined in this study are generally monads. The types of pollen grains were colpate and colporate. One, nine species were colpate and colporate respectively. The shapes of pollen were oblate spheroidal, spheroidal, suboblate and subprolate. The smallest pollen (9.0 – 14.0 µm) was found in *Limonia acidissima* L. and the largest pollen (30.0 – 32.6 µm) was observed in *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm. The sculpture patterns of pollen grains were psilate in 1 species, reticulate in 4 species and obscurely reticulate in 5 species. The pollen photomicrographs of each species were observed by polar view and equatorial view. The pollen morphology provides for identification and future systematic research work of orders Sapindales and Brassicales.

Keywords: Sapindales and Brassicales, Colpate and colporate, Palynology

Introduction

Pollen is Latin and means “fine dust” or “flour”. Its first use as a scientific word to describe the male sperm carrying units of flowering plants is credited to Carl Linnaeus in *Sponsalia Plantarum* published in 1747. Pollen is the dust of vegetable, which will burst when moistened with the appropriate liquid and propulsively explode a substance which is not discernable by the naked senses (Kesseler 2009).

Sapindales is an order of flowering plants, division Magnoliophyta (Angiospermae) in the subclass Rosidae of the class Magnoliopsida. The order Sapindales consists of 15 families and approximately 6200 species. The largest families are Rutaceae (about 1500 species), Sapindaceae (about 1500), Meliaceae (about 550 species), Anacardiaceae (about 600) species and Burseraceae (about 600 species). Most members of the Sapindales are woody plants with compound or lobed leaves and polypetalous (Arthur 2014).

The Brassicales are an order of flowering plants, belonging to the eurosids II group of dicotyledons. One characters common to many members of the order is the production of glucosinolate (mustard oil) compounds. The only families included were the Brassicaceae and Capparaceae as separate the Tropaeolaceae, Resedaceae and Moringaceae. The families Capparaceae and Brassicaceae are closely related (Hai 2015).

The aim and objectives of this research work were to identify and classify the morphological variation in pollen of Sapindales and Brassicales, to study and record the collected species systematically from the palynological point of view and to provide the different pollen characters which may be used in phylogenetic inferences.

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Materials and Methods

Collection of Plants Materials and Pollen Samples

The specimens were kept under observation from Mandalay and Bago Regions from 2018 to 2020. The collected species were recorded individually by photographs during flowering period. Identification of specimens were carried out by referring to Hooker (1881-1887), Backer et al (1965-1968), Dassanayake (1980-2001). Myanmar name were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003) in Myanmar.

Acetolysis of Pollen Grains

The pollen samples were acetolysed by the standard method of Erdtman (1960). The acetolysis solution was mixed using a measuring cylinder: 9 parts of glacial acetic acid, and 1 part of concentrated sulphuric acid was added. The acid was dropped gently down the side of the tube. The pollen samples from the glass vial were put into a test-tube and then acetolysis mixture (1cc) was poured into the test-tube containing the pollen samples and stirred with a glass rod. The test-tube was heated in a water-bath at 75°C about 20-30 minutes. After centrifuging and decanting, a few drops of dilute glycerine solution was added to the residue, then transferred and stored in air-tight glass vial.

A drop of sample was taken with a glass rod and placed on a slide, then covered with a cover slip. The terminology was used in accordance with Erdtman (1952), Moore & Webb (1978) and Hesse *et al.* (2009).

Results

1.Family – Anacardiaceae

Bouea oppositifolia (Roxb.) Meisn., Pl. Vasc. Gen. 2:55. 1837. (Figure 1 A)

Myanmar name : Taw mayan

Outstanding characters

Perennial trees, 5 – 25 m high; branches spreading crown. Leaves simple, opposite and decussate, exstipulate, glabrous. Inflorescences axillary paniculate, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous.

Pollen morphology (Figure 1 B, C)

Tricolporate, oblate spheroidal, small, 10.8 – 18.0 × 12.0 – 15.6 µm in length and breadth; amb triangular; colpi longicolpate, 9.6 – 15.6 × 2.4 – 6.0 µm in length and breadth; pori lolongate, 4.8 – 7.2 × 2.4 – 6.0 µm in length and breadth; exine about 1.2 µm thick, sexine thicker than nexine; sculpturing psilate.

2. Familly – Rutaceae

Casimiroa edulis La Llave, Nov. Veg. Descr. 2:2. 1825. (Figure 1 D)

Myanmar name : Thagya thi

Outstanding characters

Perennial, evergreen trees, up to 6 m high; stems and branches terete, glabrous. Leaves palmately compound, alternate, exstipulate, oil gland on both surfaces. Inflorescences terminal or

axially, panicle, many-flowered, pubescent. Flowers bisexual, actinomorphic, pentamerous, hypogynous, greenish white, pubescent.

Pollen morphology (Figure 1 E, F)

Tricolporate, subprolate, small, 13.2 – 16.8 × 10.8 – 13.2 µm in length and breadth; amb triangular; colpi ¾ way up to the pole, 12.0 – 14.4 × 3.6 – 5.4 µm in length and breadth; pori circular, 2.4 – 7.2 µm in length and breadth; exine about 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, lumina heterobrochate, 0.6 – 2.4 µm width; muri simplibaculate, about 1.2 µm wide.

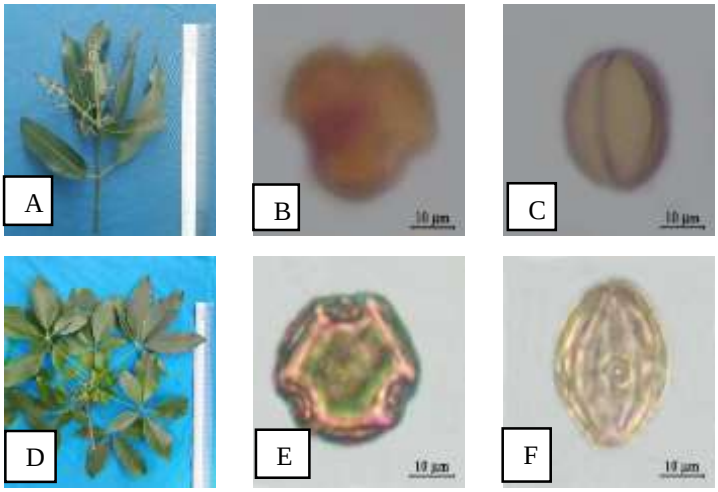

Figure 1. A. Inflorescences of *Bouea oppositifolia* (Roxb.) Meisn., B. Pollen in polar view of *B. oppositifolia* (Roxb.) Meisn., C. Pollen in equatorial view of *B. oppsitifolia* (Roxb.) Meisn., D. Inflorescences of *Casimiroa edulis* La Llav, E. Pollen in polar view of *C. edulis* La Llave, F. Pollen in equatorial view of *C. edulis* La Llave

3. *Limonia acidissima* L., Sp. Pl. (ed. 2) 554. 1763. (Figure 2 A)

Myanmar name : Thi, Mak pyen sum

Outstanding characters

Perennial, deciduous, armed trees with dark brown bark, up to 8 m high. Leaves unipinnately compound, imparipinnate, alternate, exstipulate, shining, glabrous on both surfaces. Inflorescences axillary or terminal, racemes. Flowers bisexual, actinomorphic, hypogynous, pentamerous, dark red, turning to yellowish-green.

Pollen morphology (Figure 2 B, C)

Tetracolporate, suboblate, small, 9.6 – 15.6 × 14.6 – 20.4 µm in length and breadth; amb quadrangular; colpi longicolpate, 8.4 – 14.4 × 2.4 – 4.8 µm in length and breadth; pori lalongate, 2.4 – 4.8 × 3.6 – 6.0 µm in length and breadth; exine 1.2 – 3.6 µm thick, sexine thicker than nexine; sculpturing reticulate, lumina heterobrochate, about 2.4 µm width; muri simplibaculate, about 1.2 µm wide.

4. Family – Meliaceae

Chukrasia tabularis A. Juss., Bull. Sci. Nat. Geol. 23(140): 241. 1830. (Figure 2 D)

Myanmar name : Yinma

Outstanding characters

Perennial, trees, up to 9 m high; stems and branches terete. Leaves unipinnately compound, paripinnate, alternate; exstipulate, pubescent. Inflorescences terminal or axillary, paniculate cyme, many-flowered, pubescent. Flowers bisexual, actinomorphic, pentamerous, hypogynous.

Pollen morphology (Figure 2 E, F)

Tetracolporate, oblate, small, 14.4 – 16.8 × 18.0 – 22.8 µm in length and breadth; amb quadrangular; colpi ¾ way up to the pole, 12.8 – 14.4 × 3.6 – 6.0 µm in length and breadth; pori lalongate, 3.6 – 6.0 × 4.8 – 7.2 µm in length and breadth; annuli present, 1.2 – 3.6 µm in diameter; exine 0.6 – 1.2 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

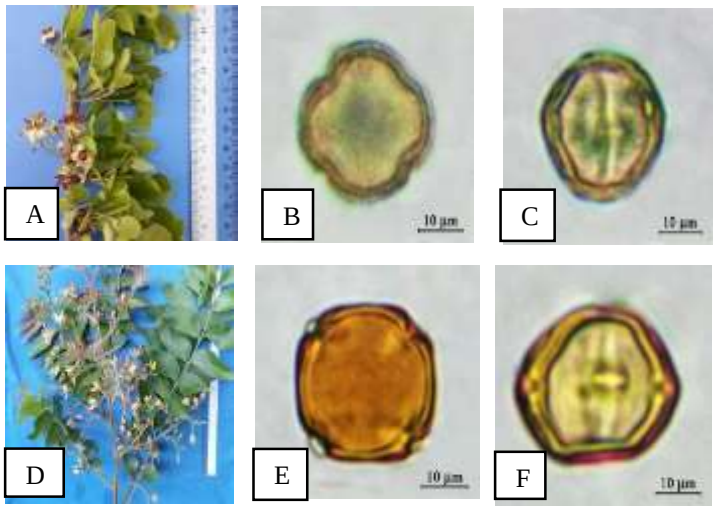

Figure 2. A Inflorescences of *Limonia acidissima* L., B. Pollen in polar view of *L. acidissima* L., C. Pollen in equatorial view of *L. acidissima* L., D. Inflorescences of *Chukrasia tabularis* A. Juss., E. Pollen in polar view *C. tabularis* A. Juss., F. Pollen in equatorial view of *C. tabularis* A. Juss.

5. *Cipadessa baccifera* (Roth.) Miq., Ann. Mus. Bot. Lugduno-Batavi 4:6. 1868. (Figure 3 A)

Myanmar name : Unknown

Outstanding characters

Perennial, erect shrubs, about 3m high. Leaves unipinnately compound, imparipinnate, alternate; exstipulate, pubescent. Inflorescences axillary paniculate cyme, many-flowered. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white.

Pollen morphology (Figure 3 B, C)

Tetracolporate, oblate, small, 12.0 – 14.4 × 18.0 –21.2 µm in length and breadth; amb quadrangular; colpi ¾ way up to the pole, 10.8 – 13.2 × 4.8 – 6.2 µm in length and breadth; pori lolongate, 4.8 – 7.2 × 3.6 – 5.0 µm in length and breadth; exine 2.4 – 3.6 µm thick, sexine thicker than nexine; sculpturing psilate.

6. *Walsura trijuga* (Roxb. ex Sims) Kurz, J. Asiat. Soc, Bengal, Pt. 2, Nat. Hist. 44 (2): 148. 1875.

(Figure 3 D)

Myanmar name : Tagat ta gyi

Outstanding characters

Perennial, tree up to 6 m high; stems and branches terete, glabrous. Leaves unipinnately compound, imparipinnate, alternate; exstipulate. Flowers bisexual, actinomorphic, pentamerous, hypogynous.

Pollen morphology (Figure 3 E, F)

Tetracolporate, oblate spheroidal, medium, 19.2 – 24.0 × 21.6 – 26.4 µm in length and breadth; amb quadrangular; colpi longicopate, 17.2 – 22.8 × 3.5 – 5.7 µm in length and breadth; pori lolongate, 3.8 – 6.0 × 2.5 – 5.0 µm in length and breadth; exine about 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, lumina heterobrochate, about 1.2 µm width; muri simplibaculate, about 0.6 µm wide.

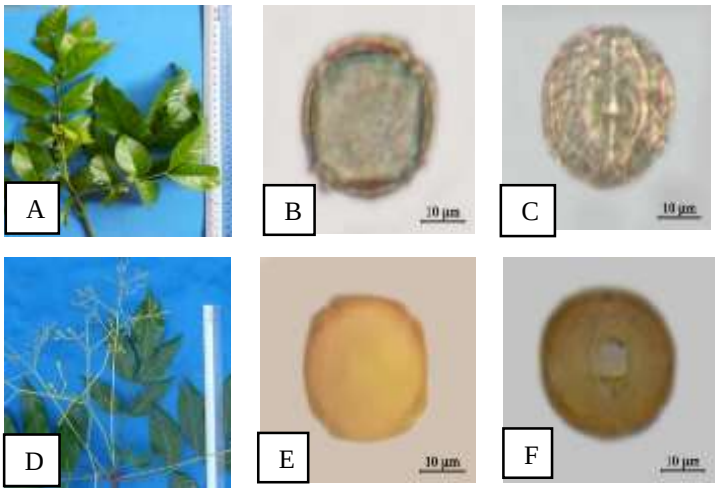

Figure 3. A. Inflorescences of *Cipadessa baccifera* (Roth.) Miq., B. Pollen in polar view of *C. baccifera* (Roth.) Miq., C. Pollen in equatorial view of *C. baccifera* (Roth.) Miq., D. Inflorescences of *Walsura trijuga* **(**Roxb. ex Sims) Kurz, E. Pollen in polar view of *W.trijuga* **(**Roxb. ex Sims) Kurz, F. Pollen in equatorial view of *W. trijuga* **(**Roxb. ex Sims) Kurz

7. Family – Capparaceae

Crateva adansonii DC., Prodr. 1: 243. 1824. (Figure 4 A)

Myanmar name : Kadet

Outstanding characters

Perennial, deciduous, small to medium size tree, glabrous. Leaves palmately trifoliate compound, alternate, glabrous on both surfaces. Inflorescences axillary or terminal corymb, many-flowered, pubescent. Flowers bisexual, zygomorphic, tetramerous, hypogynous, creamy white.

Pollen morphology (Figure 4 B, C)

Tricolpate, subprolate, small, 15.6 – 19.2 × 13.2 – 15.6 µm in length and breadth; amb triangular; colpi ½ way up to the pole, 8.4 – 10.8 × 3.6 – 5.0 µm in length and breadth; exine 1.2 – 2.4 µm thick, sexine thicker than nexine; sculpturing reticulate, lumina heterobrochate, 0.6 – 1.2 µm width; muri simplibaculate, about 1.2 µm wide.

8. *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm., Repert. Spec.Nov. Regni Veg. 41: 128. 1936. (Figure 4 D)

Myanmar name : Thamon

Outstanding characters

Perennial, trees, up to 5 m high; stems and branches terete, glabrous or slightly pubescent. Leaves palmately compound, alternate. Inflorescences axillary or terminal paniculate racemes, many-flowered, pubescent. Flowers bisexual, zygomorphic, tetramerous, hypogynous, pale green.

Pollen morphology (Figure 4 E, F)

Tricolporate, prolate spheroidal, medium, 30.0 – 34.8 × 27.6 – 31.2 µm in length and breadth; amb triangular; colpi longicolpate, 27.6 – 31.2 × 4.8 – 8.4 µm in length and breadth; pori lalongate, 3.6 – 7.2 × 6.0 – 8.4 µm in length and breadth; exine about 3.6 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

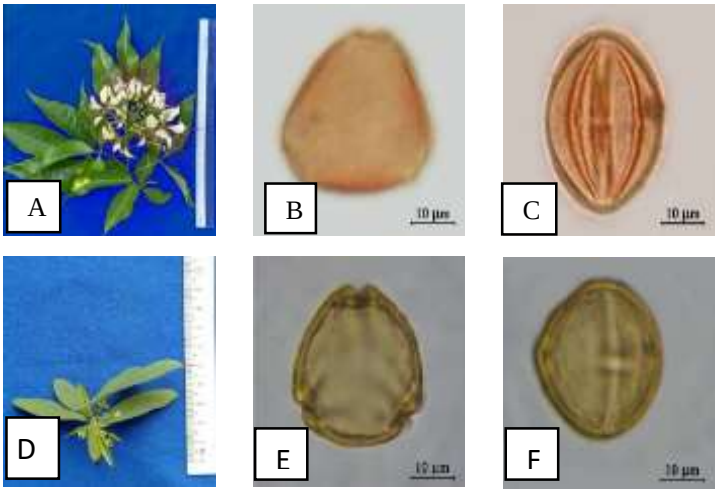

Figure 4. A. Inflorescences of *Crateva adansonii* DC., B. Pollen in polar view of *C. adansonii* DC., C. Pollen in equatorial view of *C. adansonii* DC., D. Inflorescences of *Hypselandra variabilis* (Collett & Hemsl.) Pax & K. Hoffm., E. Pollen in polar view of *H. variabilis* (Collett & Hemsl.) Pax & K. Hoffm., F. Pollen in equatorial view of *H. variabilis* (Collett & Hemsl.) Pax & K. Hoffm.

9. Family – Cleomaceae

Cleome rutidosperma DC., Prodr. 1: 241. 1824. (Figure 5 A)

Myanmar name : Pan hingala

Outstanding characters

Annual, erect or spreading herbs; stems and branches terete. Leaves palmately trifoliolate compound, alternate; exstipulate, both surfaces sparsely glandular. Inflorescences axillary cymes; peduncles terete, glabrous. Flowers bisexual, actinomorphic, tetramerous, hypogynous, pale purple.

Pollen morphology (Figure 5 B, C)

Tricolporate, prolate spheroidal, medium, 24.0 – 28.8 × 22.8 – 25.2 µm in length and breadth; amb triangular; colpi ¾ way up to the pole, 21.6 – 26.4 × 2.4 – 4.8 µm in length and

breadth; pori lalongate, 3.6 – 6.0 × 4.8 – 6.8 µm in length and breadth; exine about 2.4 µm thick, sexine thicker than nexine; sculpturing obscurely reticulate.

10. *Cleome viscosa* L., Sp. Pl. 2:672. 1753. (Figure 5 D)

Myanmar name : Gangala

Outstanding characters

Annual, erect, foetid herbs; stems and branches striated, often pale purplish, viscid glandular. Leaves palmately compound, alternate; exstipulate. Inflorescences terminal racemes, many-flowered. Flowers bisexual, zygomorphic, tetramerous, hypogynous, yellow.

Pollen morphology (Figure 5 E, F)

Tricolporate, prolate, medium, 22.8 – 31.2 × 16.8 – 20.4 µm in length and breadth; amb rounded triangular; colpi longicolpate, 21.6 – 30.0 × 6.0 – 7.6 µm in length and breadth; pori lalongate, 3.6 – 6.0 × 4.8 – 8.4 µm in length and breadth; exine 1.2 – 3.6 µm thick, sexine slightly thinner than nexine; sculpturing reticulate, lumina heterobrochate, 2.4 µm width; muri simplibaculate, about 1.2 µm wide.

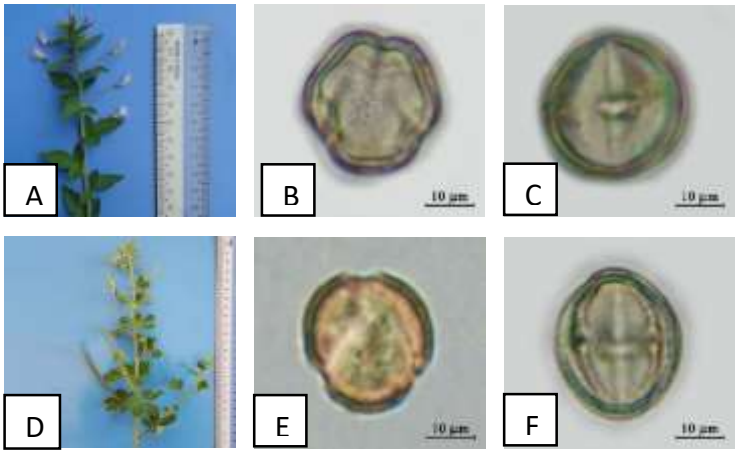

Figure 5. A. Inflorescences of *Cleome rutidosperma* DC., B. Pollen in polar view of *C. rutidosperma* DC., C. Pollen in equatorial view of *C. rutidosperma* DC., D. Inflorescences of *Cleome viscosa* L., E. Pollen in polar view of *C. viscosa* L., F. Pollen in equatorial view of *C. viscosa* L.

Discussion and Conclusion

In the present research, the pollen morphology of 10 species and 10 genera in 5 families belonging to the orders Sapindale and Brassicales has been identified and studied. The specimens were collected in Mandalay and Bago Regions and pollen morphological studies have been carried out.

In the present study, all the collected pollen grains are monad types. The types of pollen grains are occurrs as colpate and colporate. Colporate pollen are found in Sapindales, colpate and colporate pollen grains are observed in Brassicales. These characteristic findings have been similarly stated in earlier researches by Anbari *et al.* (2015), Erdtman (1952) and Mustard (1954).

In equatorial views, the shapes of pollen were oblate spheroidal, spheroidal, suboblate and subprolate. Oblate spheroidal are found in 3 species, subprolate in 3 species, spheroidal in 3 species and suboblate in 1 species.

In polar views, the amb of pollen grains are found as triangular, rounded quadrangular, rounded triangular, and straight quadrangular. Of all families, pollen grains are found to be triangular in 4 species, rounded quadrangular in only one species, straight quadrangular in 4 species and rounded triangular in 2 species. In the same way, these characters are similar to those stated by Khalik *et al.* (2002) and Yates (2005).

In this study, the pollen grains of *Chukrasia tabularis* A. Juss., and *Casimiroa edulis* La Llave were present annuli. They too have the same pollen characters as those described by Hesse *et al.* (2009) and Kesseler and Harley.(2009).

According to result, the different types of pollen characters will highlight not only the interesting pollen features but also the varieties of the pollen morphological data in the study of order Sapindales and Brassicales. These morphological features of pollen will support for identification and classificatioin of flowering plants.

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EFFECT OF CULTURE CONDITION ON PROTEASE PRODUCTION OF PROTEOLYTIC FUNGI FROM BEAN CROP SOIL AND PADDY MILL SOIL

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Abstract

The present study is based on the characterization of protease producing fungi from the bean crop soil in Vegetable and Fruit Research Development Centre (Hlegu Township) and paddy mill soil in Daik-U Township. Screening of fungi was done by employing skim milk agar (SMA) plating technique at room temperature and the zone of proteolysis was noted. The two isolates that gave high positive protease activity results in screening identified as Penicillium sp. (V2) and Cladosporium sp. (D12) by analyzing the macroscopic and microscopic morphological criteria. These fungi were used for protease production via submerged fermentation (SmF). Effects of four different fermentation media (Medium I, Medium II, Medium III and Medium IV), different fermentation period, 1% carbon sources, 1% nitrogen sources, different substrate and casein substrate concentration on protease production were investigated. The highest enzyme activity was obtained with production medium II at four days with 1% casein as best substrate, 1% glucose as carbon source and yeast extract as nitrogen source.

Keyword: Protease activity, Penicillium sp., Cladospordium sp., bean crop soil and paddy mill soil

Introduction

Protein is one of the three major food groups needed for proper nutrition. Proteolytic enzymes or proteinases are the group of enzymes whose catalytic function is to hydrolyze (break down) proteins. Microbial proteases are an important family of proteases and have an advantage over animal and plant proteases, as microbes can be cultured on a large scale in less time, and the growth conditions can easily be optimized in lab conditions. Fungi are an important component of the microbiota typically constituting more of the soil biomass than bacteria depending on soil depth and nutrient condition (Saxena et al., 2017). Carbon sources are very important for microbial growth as they provide carbon skeletons needed for synthesis of new organic molecules (anabolism). The growth of the microbes and enzyme production requires nitrogen sources. Most of the microorganisms require fixed nitrogen source to synthesize proteins, nucleic acids and other cellular components (Suseela et al., 2017).

Proteases are also having extensive applications in the development of environmentally friendly technologies as well as in several bioremediation processes. Several classification systems currently available, provides rich and information about each and every identified protease (Pansuriya and Rekha., 2010). Proteases constitutes large and complex group of enzymes that plays important role in nutrition and various applications in medical and industrial field. A variety of organisms such as bacteria, fungi, yeast, actinomycetes are known to produce these enzymes (Kalpanadevi et al., 2008). Molds of genera Aspergillus, Penicillium and Rhizopus are especially useful for proteases (Sandhya et al., 2005).

Protease is the important industrial enzyme invests an accounting for about 60% of the total enzyme market in the world (Niyonzima and More., 2013). Microorganisms are the most common

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source of commercial enzymes due to their physiological and biochemical properties, cultural conditions and ease of cell manipulation. The growth of industrial enzyme market has expanded to nearly 85 enzymes, which are currently in commercial production. The protease enzyme constitutes two third of total enzyme used in various industries. Furthermore, study was undertaken the assessment of culture conditions on protease production from fungi isolate by submerged fermentation and to evaluate the effect of different media, fermentation period, carbon sources, nitrogen sources, different substrates and substrate concentration on protease activity.

Materials and Methods

Sample collection and isolation of fungi

Soil samples used from different environmental sources like bean crop soil in Vegetable and Fruit Research Development Centre (Hlegu Township) and paddy mill soil in Daik-U Township were collected aseptically from 6 inches depth in sterile zip lock plastic bag. One gram of each soil sample was serially diluted in sterile condition. From diluted suspension 100µL was spread on potato dextrose agar (added 1% casein) medium, incubated at room temperature for three to five days and was monitored every day for the growth of fungal colony. Further these fungi were sub-cultured on potato dextrose agar (added 1% casein) to obtain the pure strain (Sharma et al., 2015). Among them those showing maximum zone of proteolysis were selected for this investigation, named as isolated V (bean crop soil) and D (paddy mill soil).

Primary screening for proteolytic fungi by casein hydrolysis plate assay (Sethis and Gupta., 2015)

Preliminary screening was done by inoculating the fungal isolates on skim milk agar medium containing casein as protein source at room temperature for one to seven days. After incubation, the plates were observed for clear zone of hydrolysis surrounding the colony. The protease was exhibited by a zone of proteolysis, which was exhibited by a zone of proteolysis which was demonstrated by clear area surrounding the fungal growth. The zone diameter was measured in cm and results are recorded (Warcup,1950 and Abe et al.,2015). The enzyme index (EI) expressed as R/r, which R is the degradation zone diameter and r are the colony diameter (Hankin and Anagnostakis,1975, Abe et al.,2015). The species that exhibits maximum clear zone was selected for further identification.

Identification and characterization of protease enzyme

The two fungi were identified according to the Barnett 1960 and Dube 1983.

Fermentation condition (Anson, 1938)

The 100ml of synthetic media containing casein 0.5g, dextrose 0.5g, yeast extract 0.5g, magnesium sulfate 0.02g, dipotassium hydrogen phosphate 0.1g and sodium carbonate 0.01g was taken separately into 300ml one Erlenmeyer flasks and adjusted to pH 8.0. The cotton flasks were then autoclaved at 121°C for 20 mins and allowed to cool to about at room temperature. The contents of the flasks were inoculated with one loop of fungi and incubated at room temperature in a rotary shaker operated at 250 rpm for 4 days. At the end of fermentation period the contents were centrifuge at 10000 rpm (4°C) for 10 mins at room temperature, and the cell free supernatant was used of crude enzyme for enzyme activity assay.

Protease specific activity assay

Protease specific activity assay was performed according to the method of Colowick and Kaplan, 1955. Briefly, 1.3ml of 1% casein dissolved in 50mM potassium phosphate buffer (pH 7) was added to both sample and blank tubes and incubated at room temperature for 30 mins. Subsequently, 0.7ml of crude protease extract was added to only the sample tube and incubated for 30 mins at room temperature. The reaction was stopped with the addition of 3ml of 5% trichloroacetic acid (TCA) solution in both the sample and blank tubes. The mixture was then incubated for 30 mins at room temperature. UV-visible spectrophotometer was used to determine the absorbance value at 275nm. The more tyrosine that is released from casein, the more chromophores are generated and the stronger the activity of the protease. Absorbance values generated by the activity of the protease are compared to a standard curve, which are generated by reacting known quantities of tyrosine with the amount of tyrosine in microgram. From the standard curve the activity of protease samples can be determined in terms of Units, which is the amount in microgram of tyrosine equivalents released from casein per mL minute under assay condition described by Abdilbar et al., 2021.

Effect of different media (Sarker et al., 2013) and fermentation period on protease enzyme activity (Naik et al., 2013)

In this experiment, production of protease by submerged fermentation was study in five different basal medium (medium I, medium II, medium III and medium IV) the composition of fermentation Medium I was casein 10g, yeast extract 10g, glucose 20g, K2HPO4 1g, KH2PO41g, MgSo40.2g, pH 7.0, Medium II was casein 5g, glucose 10g, yeast extract 5g, K2HPO41g, MgSo4.7H2O 0.2g, Na2CO3 0.1g, pH 8.0, Medium III was casein 5g, K2HPO41g, MgSO4.7H2O 2g, NaNO32g, FeSO4.7H2O 0.01g, KCL 2g, pH 7.0 and Medium IV was casein 5g, glucose 25g, yeast extract 0.5g, K2HPO42g, (NH4)2SO41.5g, MgSO4.7H2O 0.3g, CaCl2 0.3g, urea 0.3g, pH 9.0 . The effect of fermentation periods on protease production was determined by incubation for 1 to 7 days. The enzyme activity was measured under assay condition.

Effect of carbon sources on protease enzyme production (Prabhakaran et al., 2015)

The medium was prepared with composition same as the fermentation media but the carbon sources is replaced by different other carbon sources on protease production was investigated. The protease activity was determined as mentioned above.

Effect of nitrogen sources on protease enzyme production (Akcan, 2012)

The influence of different nitrogen sources 1% of beef extract, yeast extract, peptone, KNO3 and NaNO3 were determined for protease production, used incubated the culture at room temperature for the best fermentation period. The cell free filtrate was used to assay for protease activity.

Effect of different substrates on protease enzyme production (Shivakumar Srividya, 2012 and Bijay et al., 2017)

Protease activity was carried out with 1 % various protein substrates including gelatin, casein, wheat bran and soya powder. These substrates present in selected production medium were substituted with 1% of each substrate. After incubation at room temperature for the best fermentation period with each of the substrates separately, enzyme activity was measured under enzyme assay.

Effect of substrate concentration on protease enzyme production (Saxena et al., 2017 and Hariharan et al., 2018)

The effect of casein substrate concentration ranging from (0.5%, 1%, 1.5% and 2%) at room temperature for the best fermentation period using the method described above.

Results

Isolation and screening of fungi

Eleven and twelve soil fungal isolates were isolated on potato dextrose agar (added 1% casein) medium from bean crop soil in Vegetable and Fruit Research Development Centre, Hlegu Township and paddy mill soil in Daik-U Township respectively. These isolated fungi by using serial dilution of pour plate method at room temperature for three to five days.

Preliminary production of proteolytic enzyme on skim milk agar (SMA) medium

The fungal isolates were screened for the ability to produce protease enzyme on skim milk agar (SMA) medium by streak plate method. Among all isolated fungi; two strains were showed clear zones formations around the fungal growth indicating the property of protease. Although only two fungi V2 and D12 showed maximum proteolytic activity with the zone of about 2.5cm for five days and 2cm for six days respectively as shown in figure (1 and 2). Pure colonies were obtained with distinct morphological features.

Morphological characteristics of genus level on prominent fungi

In the present investigations, the selected fungal strains were done by identification of macroscopic and microscopic characters using standard method in table (1). The fungal strain was identified as Penicillium sp. (V2) and Cladosporium sp. (D12) as shown in table (1) and figure (1 and 2).

Table (1) Characters of proteolytic fungi isolated from bean crop soil and paddy mill soil

No	Sources	Macroscopic characters of proteolytic fungi	Microscopic characters of proteolytic fungi	Species
1	Bean crop soil in Vegetable and Fruit Research Development Centre (Hlegu Township)	The mycelium color green color inside, white color peripher and yellow in reverse view.	Conidiospores arising from the mycelium singly, branched near the apex to form a brush-like, conidia-bearing apparatus, ending in a group of phialides, conidia brightly colored in mass, ovoid, in dry basipetal chains.	*Penicillium* sp. (V2)
2	Paddy mill soil (Daik-U Township)	The mycelium color is brown in surface view and black in reverse view.	Conidiophores dark, branched variously near the apex, clustered, conidia dark, lemon-shaped, pigment present.	*Cladosporium* sp. (D12)

Forward view | Reverse view | Proteolytic activity | Micrograph of Penicillium sp. (X400)

Figure. 1. Morphological and microscopical characters of isolated fungi from V2

Forward view | Reverse view | Proteolytic activity | Micrograph of Cladosporium sp. (X400)

Figure. 2. Morphological and microscopical characters of isolated fungi from D12

Optimization parameters of two selected fungi

The present study of four different media, Penicillium sp. and Cladosporium sp. were showed Medium II as shown in figure (4). The protease production by microorganisms is greatly influenced by incubation period. The optimum protease activity was found four days for both species as shown in figure (5). Further increase and decrease in incubation period lead to decline in enzyme activity. The production media was supplement with 1% carbon and nitrogen sources were obtained glucose and yeast extract as shown in figure (6 and 7). The effect of 1% various substrates Penicillium sp. and Cladosporium sp. was exhibited the best activity in casein as shown in figure (8). The range of substrate concentration (0.5% to 2%) which were used in this study to check the optimum protease activity of both species were found 1% concentration as shown in figure (9).

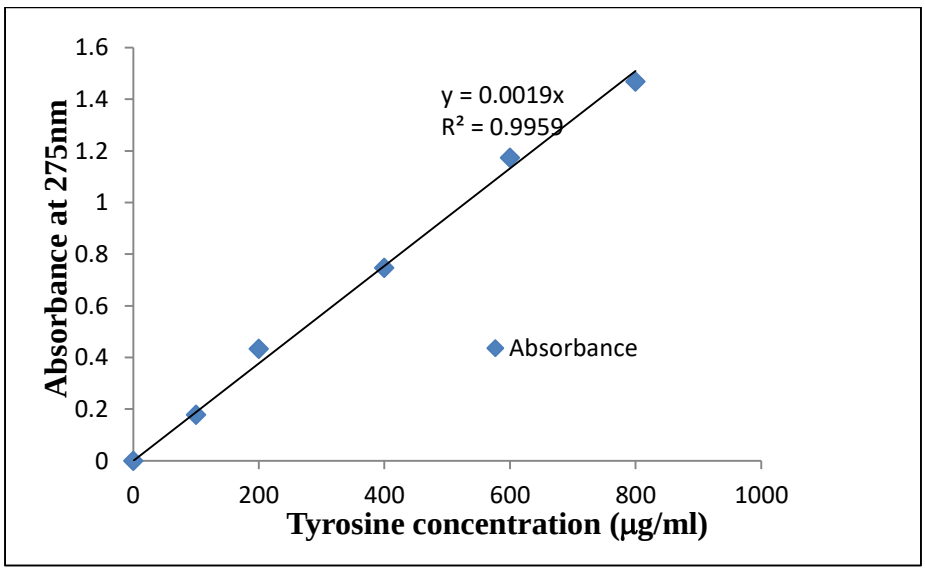

Figure.3. Plot of absorbance as a function of standard tyrosine concentration

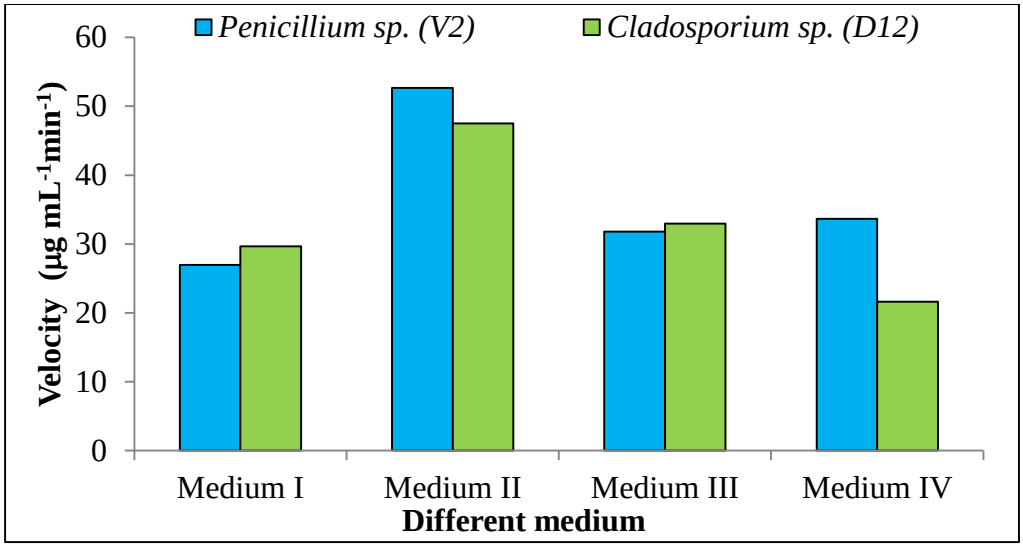

Figure. 4. Plot of velocity as a function of different medium for *Penicillium* sp. and *Cladosporium* sp.

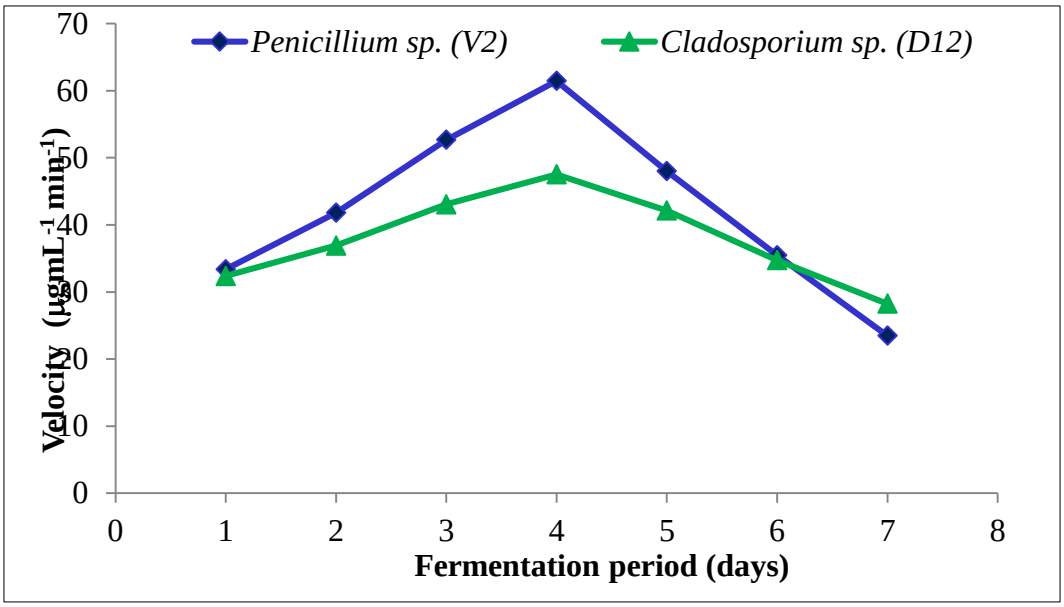

Figure. 5. Plot of velocity as a function of fermentation period for *Penicillium* sp. and *Cladosporium* sp.

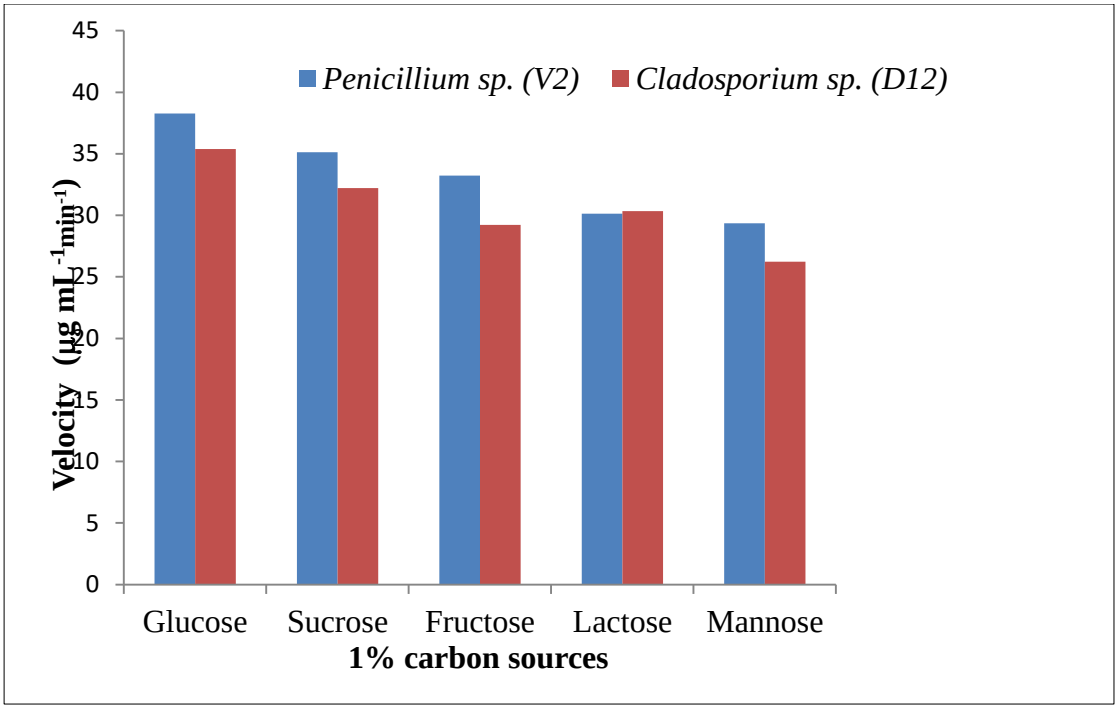

Figure 6. Plot of velocity as a function of carbon sources for *Penicillium* sp. and *Cladosporium* sp.

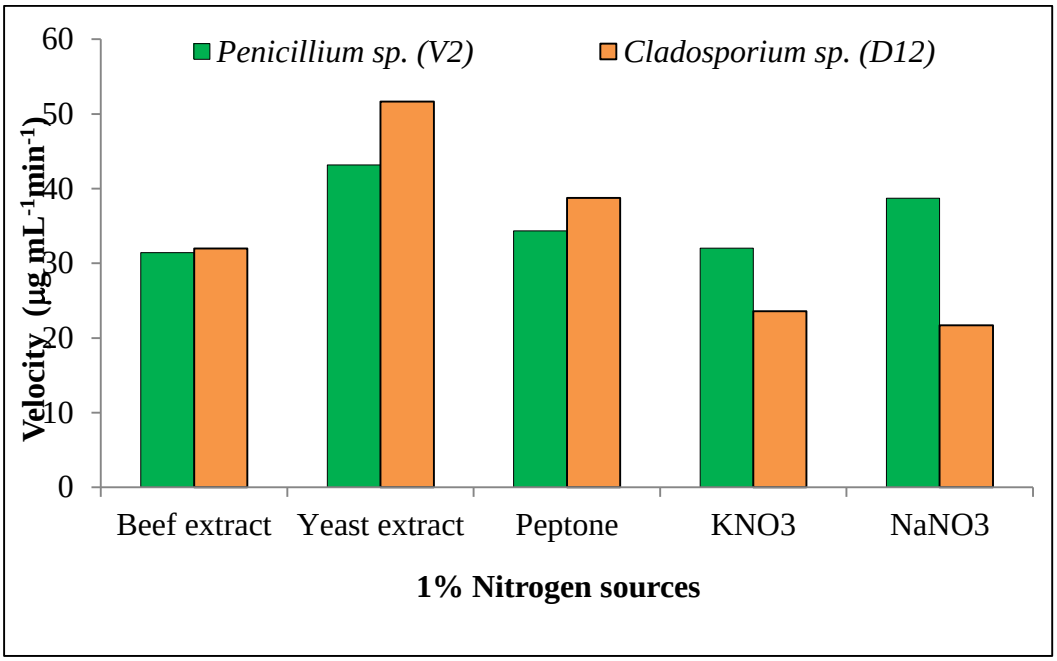

Figure 7. Plot of velocity as a function of nitrogen sources for *Penicillium* sp. and *Cladosporium* sp.

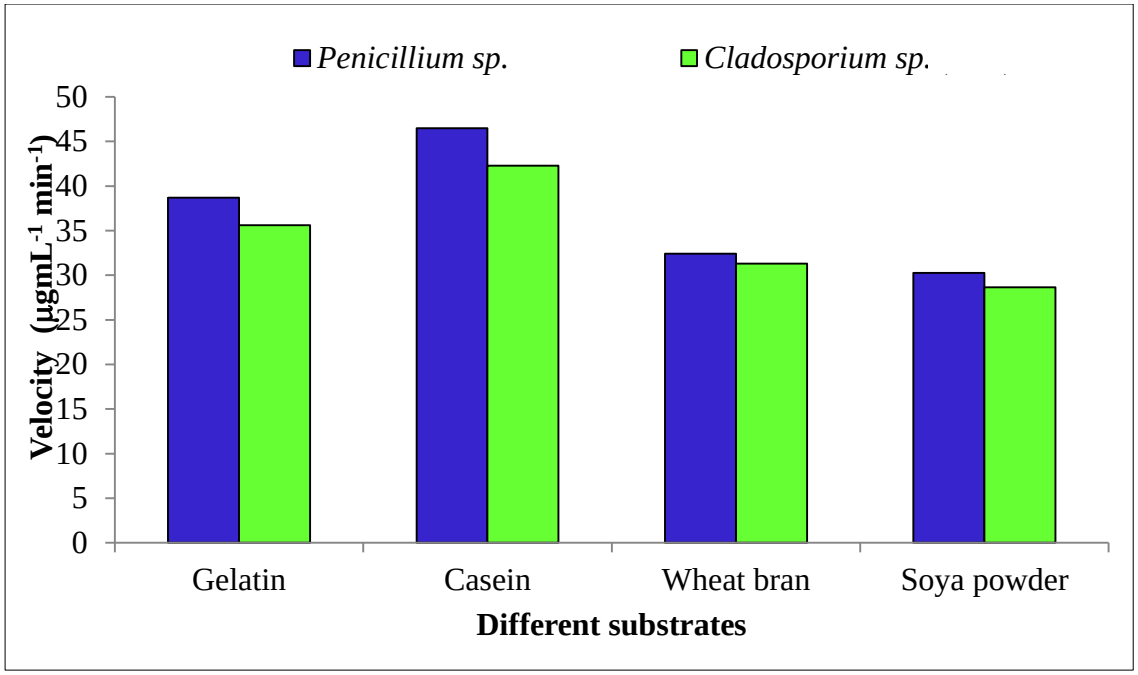

Figure 8. Plot of velocity as a function of different substrates for *Penicillium* sp. and *Cladosporium* sp.

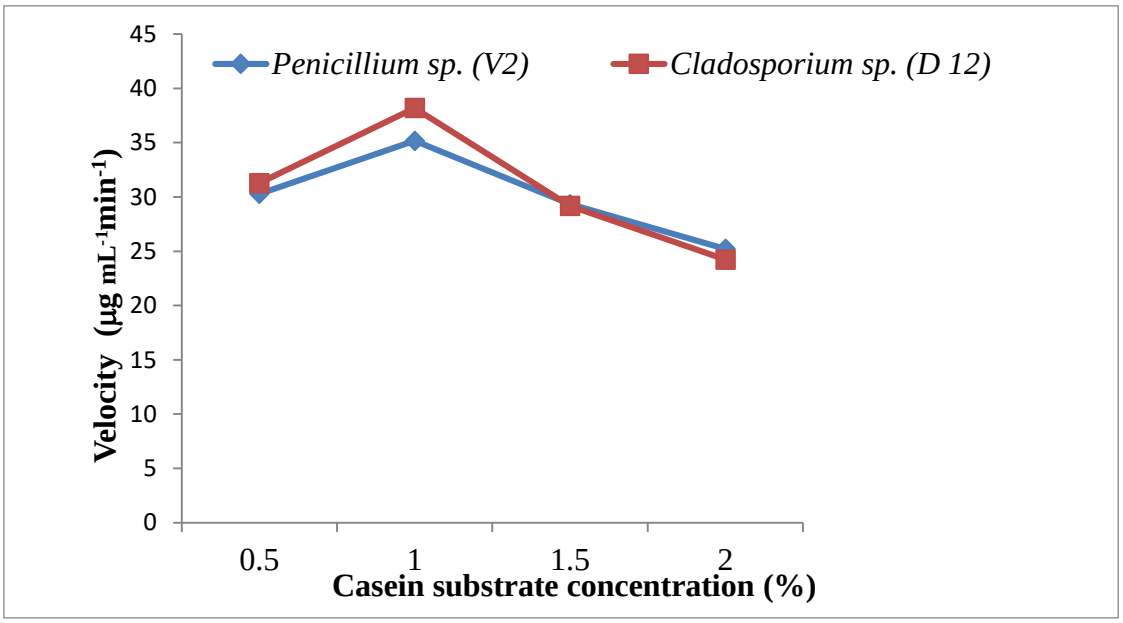

Figure 9. Plot of velocity as a function of casein substrate concentration for *Penicillium* sp. and *Cladosporium* sp.

Discussion and Conclusion

The present study investigated the protease fungi obtained from bean crop soil in Vegetable and Fruit Research Development Centre (Hlegu Township) and paddy mill soil in Daik-U Township. The two strains Penicillium sp. (V2) and Cladosporium sp. (D12) were shown as a maximum clear zone of hydrolysis on skim milk agar (SMA) medium for preliminary study of protease enzyme as shown in figure (1 and 2). Casein is decomposed by the protease to produce

degraded stages of protein products which include tyrosine among them (Haldane, 1965). The enzyme activity of the culture supernatant was determined by measuring the amount of tyrosine released from casein. Therefore, casein was chosen as the basal substrate in the experiment of enzyme-catalyzed reactions in this research works. This result is in accordance with the earlier report of Pelczar and Reid, 1972.

Enzyme properties of some parameter such as different fermentation media, culture period, 1% carbon sources, 1% nitrogen sources, different substrate and casein substrate concentration which were determined using spectroscopic method Wiseman 1975 and Oser 1976. In the present work, the Penicillium sp. and Cladosporium sp. were inoculated into four different submerged fermentation media and different fermentation period at room temperature to obtain the best protease enzyme production. In the present data, the optimum fermentation medium for two strains prominently showed medium II as shown in figure (4). During the fermentation period of Penicillium sp. and Cladosporium sp. prominently showed that 4 days fermentation period provide optimal result as shown in figure (5). In similarly, study was carried out by Gupta et al., 2002. Among the monosaccharides and disaccharides used in suitable 1% carbon sources such as glucose, sucrose, fructose, lactose and mannose, only glucose clearly exhibited in the selected strain as shown in figure (6). Wang and Lee., 1996; EI-Shore et al., 1997; Feroz, 2013 and Benluvankar et al., 2015 also reported that glucose proved to be the best carbon source for improving the productivity of the protease by Conidiobolus coronatus, Aspergillus niger and Penicillium sp. LCJ228. In the case of suitable 1% nitrogen sources (organic and inorganic) such as beef extract, yeast extract, peptone, KNO3 and NaNO3 only yeast extract clearly exhibited in the two selected strains as shown in figure (7). Which is in agreement with the reported of Phadatare et al., 1993, Ashour et al., 1996 and Mohammed, 2015. Different substrates were used for the production of proteolytic enzyme such as gelatin, casein, wheat bran and soya powder. The best substrate for obtaining maximum enzyme activity was found to be casein as shown in figure (8). Similarly result had been reported by Mulimani et al., 2002, Shankar et al., 2011 and Kumar et al., 2012. As casein is a protein, its role in induction of protease synthesis is evident from these results.

The effect of different concentration of casein ranging from 0.5% to 2% were used to determine the activity of protease enzyme. It was found that the higher concentration 1% of casein significantly enhanced maximum protease activity by Penicillium sp. and Cladosporium sp. as showed in figure (9). This result of present value has already been agreed with the literature of Battaglino, 1991; Kamath et al., 2010 and Saxena et al., 2017.

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ISOLATION OF ENDOPHYTIC FUNGI FROM THE LEAVES OF *STEPHANOTIS VOLUBILIS* (L.F.) S. RUESS, LIEDE & MEVE AND THE STUDIES OF ANTIMICROBIAL ACTIVITIES & SOME FERMENTATION PARAMETERS

May Myat Theingi Kyaw[1], Kathy Myint[2]

Abstract

Endophytes are microbes (mostly bacteria and fungi) present in plants asymptomatically. Endophytic fungi are known to produce a wide range of metabolites of pharmaceutical importance. This research paper deals with the endophytic fungi isolated from the leaves of *Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve. It is a well-recognized medicinal plant in traditional medicine. A total of 31 fungal species were isolated from the leaves. The morphology of endophytic fungi was studied and antimicrobial activities of these fungi were carried out by using paper disc diffusion method on six test organisms. All isolated fungi showed antimicrobial activities on *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas* sp., *Staphylococcus aureus*. According to the results, MM-25 showed the best antimicrobial activity against six test organisms. As a result of morphological and microscopical characters, this strain may be *Rhizoctonia* sp. In the study of fermentation parameters, the age of seed culture and the size of inoculum were investigated under two different states such as static and shaking. It was resulted that three-day old cultures provided the highest antimicrobial potentials on six test organisms. Moreover, 25% size of inoculum in fermentation was also observed the best antimicrobial activity on *Staphylococcus aureus* in both static and shaking states.

Keywords: Antimicrobial activity, *Rhizoctonia* sp.

Introduction

Endophytes are ubiquitous that spend their entire or a significant portion of their life cycle within the living tissues of their host plant without causing negative or overt symptoms (Petrini, 1991; Bacon and White, 2000; Kusari and Spiteller, 2012; Meshram *et al*., 2016). During the alliance, neither of the interacting partners is harmed and the benefits obtained are dependent on the interacting partners. Endophytes can provide benefits to their host plants by mediating abiotic and biotic stress tolerance, defending from pests and microbial infections. Thus, endophytes play a significant role in plant symbiosis, protecting their host from pathogens, pests and abiotic stresses (Kusari *et al*., 2014; Meshram *et al*., 2016; Hodkinson and Murphy, 2019).

Endophytes are proving to be a novel source of metabolites for the pharmaceutical and biochemical industries providing biologically active compounds such as antibiotics, antioxidants, anticancer agents, immunosuppressive compounds, insecticides, plant growth-promoting (PG) agents and volatile antimicrobial agents representing a wide range of organic molecules including terpenoids, peptides, carbohydrates, aromatics and hydrocarbons (Strobel, 2018; McNees *et al*., 2019).

The use of herbal medicine for the treatment of diseases and infections is a safe and traditional therapy. Hence, medicinal plants have been receiving great attention worldwide by the researchers because of their safe utility. The plant *Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve is belonging to the family Apocynaceae and is native to North-East Pakistan to South China and West Malesia. It is a climber and grows primarily in the wet tropical biome. It is also found

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to be growing in high rainfall as well as in low rainfall regions. It is a well-recognized medicinal plant in folklore and traditional medicines like Ayurveda, Siddha and Unani (Najafi, 2011). Traditionally, leaves are used as a treatment to boils and abscesses. Different plant parts can be used in cold, eye diseases and snake bites (Nadkarni, 2009).

In the present work, the existence of endophytic fungi in the leaf tissues of *Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve is detected and their potentials of antimicrobial activity were surveyed. Moreover, a strain which showed the highest antimicrobial activity was selected for the fermentation parameters such as age of culture and size of inoculum were studied.

Materials and Methods

Collection of plant sample

Endophytic fungal strains were screened from the fresh leaves of *Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve. The plant samples were collected from the Botanical Garden, Department of Botany, University of Yangon, Kamaryut Township, Yangon Region.

Isolation of endophytic fungi (Suto, 1999)

Isolation of endophytic fungal strains was carried out by the following procedure. The plants were washed in running tap water for about three minutes.

1. The plant part (leaves) was cut into about 1cm pieces.
2. The parts were surface sterilized by soaking in 75% ethanol for 15 seconds.
3. The plant pieces were cut into smaller pieces.
4. These parts were dried on sterilized paper and then placed on agar plates containing nutrient agar medium (NA medium), sucrose yeast extract medium (SY medium), potato glucose agar medium (PGA medium) and glucose yeast extract medium (GY medium and supplemented with chloramphenicol (0.25 mg / 100 ml) to inhibit bacterial growth.
5. These plates were incubated at room temperature for 3-7 days and transferred to pure culture plates.
6. Isolated fungal strains were then transferred into slant culture of each test tube containing respective medium.

Media used to isolate endophytic fungi (Compositions gram per liter)

Nutrient agar medium	
Nutrient agar	28 g
pH	7.4 ± 0.2

Sucrose-Yeast extract (SY) medium (Strobel and Sullivan, 1999)	
Sucrose	10 g
Yeast extract	3.0 g
NaCl	0.5 g
$CaCO_3$	0.1 g
Agar	18.0 g
pH	6.8 ± 0.2

Potato Glucose Agar Medium (PGA) (Atlas,1993)	
Potato	200 g
Peptone	3 g
Glucose	20 g
Agar	20 g
pH	5.6 ± 0.2

Glucose Yeast Extract Medium (GY)	
Glucose	10.0 g
Yeast Extract	3.0 g
NaCl	0.5 g
$CaCO_3$	0.1 g
Agar	18.0 g
pH	6.8 ± 0.2

Chloramphenicol (0.25 mg/ 100 ml) was added after autoclaving.

Antimicrobial activities of isolated fungal strains (Phay, 1997)

The isolated endophytic fungi were grown on different media for 5 days. The fungal isolates were inoculated into seed medium and incubated at room temperature for 3 days. Ten ml of seed culture was transferred into the fermentation medium. The fermentation was carried out for 7 days. The fermented broth was used to check the antimicrobial activity against test organisms by paper disc diffusion assay.

After autoclaving, the conical flasks containing nutrient agar medium were cooled down to 30-35 ºC, 0.3 ml of test organisms were also added into the flasks and shaken and poured into each sterilized Petridishes. After solidification, paper disc impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated for 24-36 hours at room temperature.

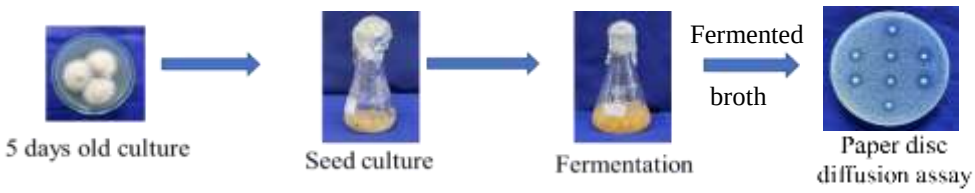


Figure.1. Steps in antimicrobial test of isolated fungi

Morphological and microscopical characters of isolated fungal strains (Barnett, 1998)

Isolated fungi grown on stock culture were transferred on to the plates containing the respective media. Then the plates were incubated at room temperature for 5-7 days. Margins and surface and reverse pigments of isolated fungi were studied for morphological characters and they were identified according to the references of Barnett 1998. The microscopical characters were studied at Microbiology Lab, Department of Botany, University of Yangon.

Fermentation studies

Effects of ages of culture on the fermentation by isolated fungi MM-25

The seed culture of MM-25 was incubated at room temperature. And then about 10 ml of seed culture was separately transferred into fermentation medium at one day intervals. Ages of culture with (1-day, 2-day, 3-day, 4-day, 5-day and 6-day) were utilized for fermentation 6 days. The antimicrobial activity was carried out paper disc diffusion method by using six test organisms (Strobel and Sullivan, 1999). (Figure-2).

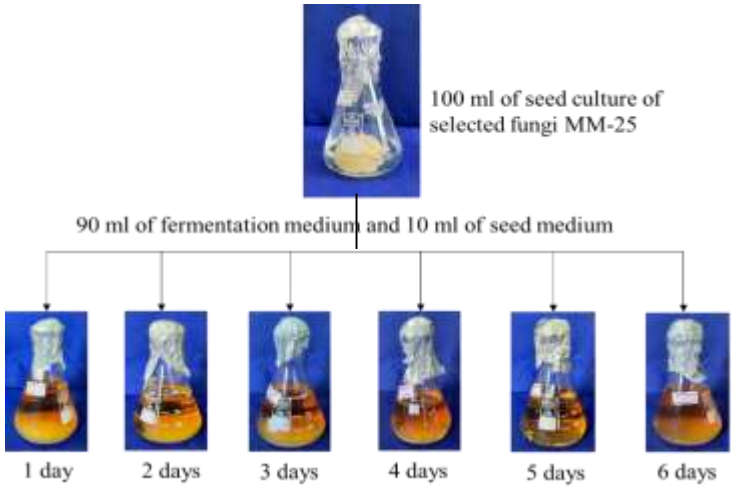


Figure 2. Seed culture and fermentation for age of inoculum

Effect of sizes of inoculum for the fermentation medium of isolated fungi MM-25

The proper cultivation and transfer (size of inoculum) are essential for the production of bioactive metabolites. A piece from fungal plate culture of strain MM-25 was inoculated into 125 ml conical flasks containing 100 ml of Glucose Yeast Extract (GY) medium. The flasks were incubated at room temperature for three days. After three days, the exact culture of the seed cultures was transferred into six conical flasks containing fermentation medium as shown in Figure 5. The fermentation was carried out for six days at room temperature in static state as well as shaking state. After the fermentation period, the fermented broth sample were subjected in the antimicrobial activity test.

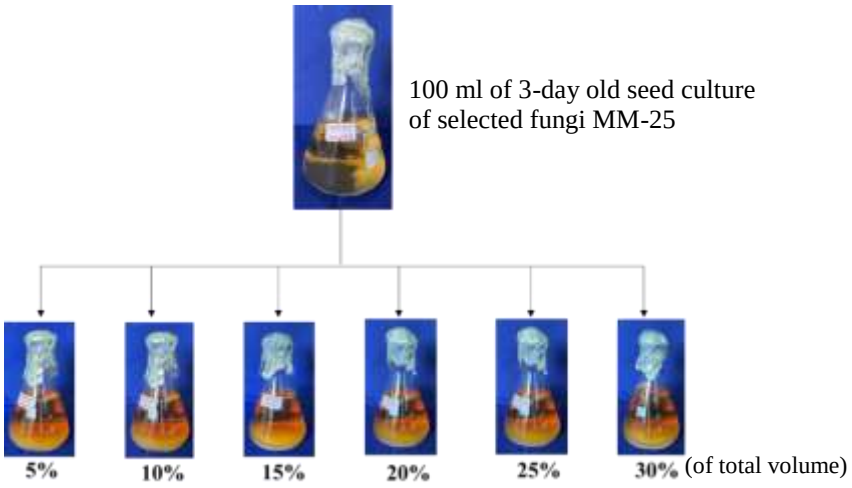


Figure 5. Seed Culture and fermentation for size of inoculum

Conditions of fermentation studies

The seed culture inoculated flask were examined under two fermentation conditions at room temperature. The first is static and the second is shaking condition (reciprocal condition by 180 stroke per minute).

Result

Figure 3. Habit of *Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve

Scientific Name	-*Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve
Synonyms	-*Dregea volubilis* (L.f.) Benth. Ex Hook.f. in Fl.Brit. India 4:46 (1883)
	-*Wattakaka volubilis* (L.f.) Stapf in Bot. Mag. 148: t. 8976 (1923)
Habit	-Climber
Distribution	-Wide
Common Name	-Gwedauk-nwe
Family	-Apocynaceae

Outstanding Characters

Lianas to 12 m. Branches pale gray, lenticellate, branchlets green, smooth. Leaves ovate, sparsely pubescent on the veins; Petiole 2.5-6 cm; leaf blade broadly ovate or suborbicular, 7-18 × 4-17 cm, glabrous or soft pubescent, base shallowly cordate, apex acute or short acuminate; lateral veins ca. 4 pairs. Inflorescences pendent, many flowered; peduncle 2-6 cm, slender, puberulent. Pedicel 2-2.5 cm; flowers green or yellowish green, fragrant. (Figure-8)

Sepals ovate-oblong, 2.5-3 mm, pubescent, ciliate. Corolla glabrous; lobes broadly ovate, 6-12 × 5-12 mm, obtuse, ciliate. Corona yellowish green, 4-4.5 mm in diam. Anther appendages white; pollinia oblong. Ovaries pilose. Follicles narrowly ovoid, 10-15 × 3-4 cm, longitudinally wrinkled-striate or irregularly ribbed. Seeds ovate, ca. 1.2 cm × 6 mm, flattened, marginate.

Flowering period: April-September

Fruiting period: July-December

Isolation of Endophytic Fungi

In the course of investigation of fungi, thirty one fungi were isolated from the leaves of *Stephanotis volubilis* (L.f.) S.Ruess, Liede & Meve (Gwedauk-nwe). These fungi were designated as MM-01 to MM-31. MM-01 to MM-03 were isolated from NA (Nutrient Agar) medium, MM-04 to MM-10 were isolated from PGA (Potato Glucose Agar) medium, MM-11 to MM-17 were isolated from SY (Sucrose/ Yeast Extract) medium and MM-18 to MM-31 were isolated from GY (Glucose/ Yeast extract) medium.

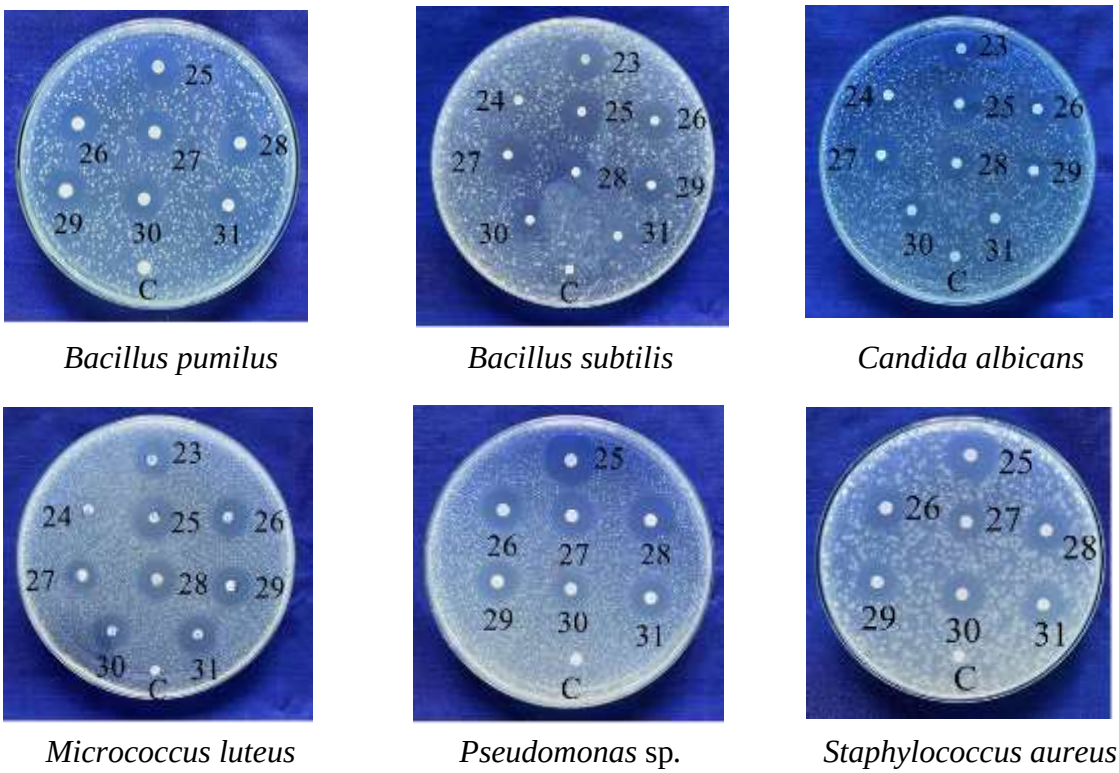
Antimicrobial activity of isolated endophytic fungi

To test the antimicrobial activity, all the endophytic fungi were cultured in respective broth for 2-6 days and their activities were examined against six test organisms. In this study, all strains (MM-01 to MM-31) showed the activities against *Bacillus pumilus, Bacillus subtilis, Candida albicans, Micrococcus luteus, Pseudomonas* sp. and *Staphylococcus aureus.* Among these strains, comparative analysis provided that MM-25 was the most efficient in producing antimicrobial potential as shown in Table 1 and Figure 4.

Table 1. Antimicrobial activities of isolated fungi with 4 days after fermentation by paper disc diffusion method (the numbers indicated the size of clear zones in mm)

	Bacillus pumilus	***Bacillus subtilis***	***Candida albicans***	***Micrococcus luteus***	***Pseudomonas sp.***	***Staphylococcus aureus***
MM-01	20	28	20	22	20	22
MM-02	20	26	21	20	20	21
MM-03	18	21	16	14	19	19
MM-04	19	23	18	18	18	19
MM-05	20	24	19	20	18	23
MM-06	10	22	17	13	15	18
MM-07	20	24	27	15	19	18
MM-08	16	22	15	15	13	14
MM-09	13	15	18	18	17	19
MM-10	12	17	17	12	15	13
MM-11	18	18	15	15	14	12
MM-12	18	21	15	17	14	15
MM-13	20	17	18	13	19	20
MM-14	19	23	20	20	15	20
MM-15	19	22	18	18	17	17
MM-16	17	21	20	20	16	17
MM-17	19	24	18	18	15	19
MM-18	20	18	20	21	19	16
MM-19	15	19	19	15	17	19
MM-20	14	22	19	14	15	18
MM-21	19	23	17	21	13	14
MM-22	17	21	20	20	17	18
MM-23	17	23	19	19	15	15
MM-24	-	-	-	-	-	-
MM-25	22	26	21	24	25	26
MM-26	20	24	14	20	19	21
MM-27	20	25	20	17	19	17
MM-28	20	25	20	21	22	20
MM-29	19	23	15	20	18	16
MM-30	19	25	16	17	15	20
MM-31	17	25	18	21	17	20

Antimicrobial activities of MM-25 with 4 days after fermentation by paper disc diffusion method



Bacillus pumilus *Bacillus subtilis* *Candida albicans*

Micrococcus luteus *Pseudomonas* sp. *Staphylococcus aureus*

Figure. 4. Inhibitory zones of isolated strains against six test organisms

Morphological and microscopical characters of strain MM-25

Colonies of MM-25 were circular to irregular; periphery was whitish filamentous. The inner zone was thicker, raised and white. The center was like a yellow brown doom. The creamy white color can be seen in reverse view of the colony and irregular filamentous edges were prominent. Under the microscope (400X) hyphae were septate, hyaline and multinucleate, branches that are produced at right angle.

Studies on fermentation parameters (age of culture and size of inoculum)

In the studies of fermentation parameters under static and shaking conditions up to 6 days, the observed antimicrobial activities were checked and comparatively shown in Table 2 to 5.

Effects of ages of seed culture on the fermentation by isolated soil fungi MM-25

In the age of inoculum, seed culture one day old, two-day old, three-day old, four-day old, five-day old and six-day old culture were used for the fermentation. According to the result, it was observed that three-day old culture of seed culture was suitable for the fermentation. (Table 2 & 3 and Figure 12).

Table 2. Effect of age of inoculum on antimicrobial activity (inhibitory zone in mm) against six test organisms (Static state)

Test organisms	One-day old culture	Two-day old culture	Three-day old culture	Four-day old culture	Five-day old culture	Six-day old culture
Bacillus pumilus	13	18.5	17.7	17	11	10.4
Bacilus subtilis	13	17.4	16.2	16.2	9.3	7.3
Candida albicans	12.5	15.8	17.4	15.3	14.2	8.8
Micrococcus luteus	9.5	16.5	20.8	19.1	13.7	9.3
Pseudomonas sp.	10	16.1	17.2	15.6	10.7	8.4
Staphylococcus aureus	12.8	18.9	22.9	18	10.2	9.3

Table 3. Effect of age of inoculum on antimicrobial activity (inhibitory zone in mm) against six test organisms (Shaking state)

Test organisms	One-day old culture	Two-day old culture	Three-day old culture	Four-day old culture	Five-day old culture	Six-day old culture
Bacillus pumilus	11.9	19.7	18.5	16.7	12.3	12.1
Bacilus subtilis	13.4	20.7	21.1	17.4	17	15.2
Candida albicans	11	17.1	18.4	17	14	13.2
Micrococcus luteus	10.7	18.6	20.9	16.5	16.4	9.6
Pseudomonas sp.	12.8	18.7	18.5	18.5	14	13.2
Staphylococcus aureus	10.7	19.7	22.1	21.5	14.7	13.9

Static state Shaking state

Figure 12. Inhibitory zones of three-day old seed culture of strain MM-25 against *Staphylococcus aureus*

Effect of sizes of inoculum for the fermentation medium of isolated fungi MM-25

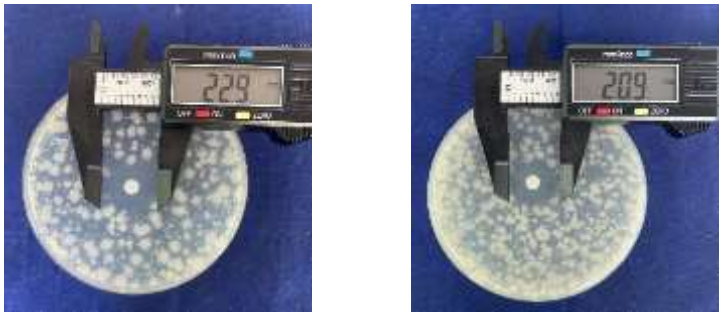
In the study of size of inoculum optimization, different size of inoculum (5%, 10%, 15%, 20%, 25% and 30%) were tested, 25% of the size of inoculum concentration showed the largest clear zone among other concentrations. Therefore, 25% of the seed culture was suitable for the fermentation to produce the bioactive metabolites (Table 4,5 and Figure 13).

Table 4. Effect of size of inoculum on antimicrobial activity (inhibitory zone in mm) against *Staphylococcus aureus* (Static state)

Days / Size of inoculum	1st day	2nd day	3rd day	4th day	5th day	6th day
5%	15.1	9.4	-	-	-	-
10%	18.3	17.2	16.8	16.2	13	-
15%	18	16.9	16.8	16.3	14.9	14.6
20%	22.9	22.3	18	16.9	16.4	14.6
25%	22.9	22.8	19.7	18.1	17	16.2
30%	20.7	19.6	18.7	15	14.7	-

Table 5. Effect of size of inoculum on antimicrobial activity (inhibitory zone in mm) against *Staphylococcus aureus* (Shaking state)

Days / Size of inoculum	1st day	2nd day	3rd day	4th day	5th day	6th day
5%	18	17.1	15.7	13.9	13.5	-
10%	18	17.8	17.6	16.3	16	15.8
15%	20.5	19	17.6	16.7	16.2	15.5
20%	18.1	17.5	17	16.4	15.9	14.9
25%	20.9	20.7	19.9	19.5	18.1	11.3
30%	15.9	15.6	15	14.7	-	-



Static state Shaking state

Figure 13. Inhibitory zones of size of inoculum of strain MM-25 against *Staphylococcus aureus*

Discussion and conclusion

In this present study, thirty one endophytic fungal strains were isolated from the leaves of *Stephanotis volubilis* (L.f.) S.Reuss, Liede & Meve (Gwedauk-nwe). Among thirty-one endophytic fungal strains, MM-01 to MM-03 were isolated from NA (Nutrient Agar) medium, MM-04 to MM-10 were isolated from PGA (Potato Glucose Agar) medium, MM-11 to MM-17 were isolated from SY (Sucrose/ Yeast Extract) medium and MM-18 to MM-31 were isolated from GY (Glucose/ Yeast extract) medium. According to the results of antimicrobial activity, endophytic fungi MM-25 showed the highest activity on different test organisms and it may be *Rhizoctonia* sp.

In Myanmar, Aye Pe (2001), Ni Ni Win (2011), Kyawt Kyawt Aung (2014), Kyi Kyi Khine (2014), Phoo Wint Yee Thaw (2015), Soe Soe Yu Hnin (2018), Hnin Wit Mhon (2018), Kay Thwe Lwin (2021) and Wint Yati (2022) have isolated many endophytic fungal (including *Rhizoctonia* sp.) and bacterial strains from different plant species to isolate the bioactive compounds and had good antimicrobial activity on *Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Malassezia furfur, Micrococcus luteus, Pseudomonas* sp. *Salmonella typhi* and *Staphylococcus aureus.*

In the study of fermentation, seed culture (three-day old) of the age of inoculum was the best for fermentation. In the 1st day, the seed culture didn't show the high activity(< 13.4 mm) on the test organisms. It showed the high activity on *Bacillus pumilus* (18.5 mm) and *Bacillus subtilis* (16.2) on the 2nd day. It also showed the result with highest inhibition rate at the 3rd day on *Candida albicans* (17.4 mm), *Micrococcus luteus* (20.8 mm), *Pseudomonas* sp. (17.2 mm) and *Staphylococcus aureus* (22.9 mm) in the static state. In the shaking state, it showed the high activity on *Bacillus subtilis* and *Pseudomonas* sp. (18.5 mm) at 2nd day. It also showed the highest activity on *Bacillus pumilus* (18.5 mm), *Candida albicans* (18.4 mm), *Micrococcus luteus* (20.9 mm) and *Staphylococcus aureus* (22.1 mm) in 3rd day. In the size of inoculum optimization, fermentation (25%) showed the best antimicrobial activity on the test organism, *Staphylococcus aureus* (22.9 mm in static state and 20.9 mm in shaking condition) at 1st day of fermentation in both static and shaking state.

In conclusion, present study obtained thirty-one endophytic fungi which showed antimicrobial activities and were found to inhibit harmful diseases and infection causing agents such as *Bacillus pumilus, Bacillus subtilis, Candida albicans, Micrococcus luteus, Pseudomonas* sp., *Staphylococcus aureus*. Strain MM-25 may be *Rhizoctonia* sp. according to Barnett (1998) and it indicated the highest antimicrobial activity on tested pathogenic organisms.

In 2021, Sonawane *et al*., had reported that an endophytic fungi *Rhizoctonia* acted as facultative plant pathogen. But it may become a valuable medically important fungi because of the presence of 33 bioactive metabolites in *Rhizoctonia* were found to show active antimicrobial activity on gram positive *Staphylococcus aureus* as well as gram negative *Escherichia coli* bacteria. It is very interested that the extract of fermented broth of *Rhizoctonia* can give anticancer potentials on antileukemic activity, cardioprotective and antifungal activity. Therefore, these fungi may become one of the top targets to do research in the future.

Acknowledgement

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Website

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ISOLATION AND IDENTIFICATION OF SOIL FUNGI FROM PATHEINGYI TOWNSHIP, MANDALAY REGION

Zin Nwe Aye[1], Moe Moe Aye[2], Yee Yee Win[3]

Abstract

This research deals with the isolation and identification of soil fungi from Patheingyi Township in Mandalay Region during July to September, 2018. The soil samples collected from Yay Kyi and Shin Taw Gone Villages. And then, soil samples were undertaken by chemical treatment dilution method. Four fungi were isolated from Yay Kyi and six fungi were isolated from Shin Taw Gone Villages. The morphology of ten isolated fungi the surface view and reverse view are different color. Ten isolated strains were tested by five test organisms for preliminary study of antimicrobial activities. Among them, four fungi showed the antibacterial activity against on *Bacillus subtilis* and *Xanthomonas oryzae,* three fungi showed the antibacterial activity against on *Escherichia coli* and *Pseudomonas fluorescens,* six fungi showed antifungal activities against on *Candida albicans.* ZN-01, ZN-02, ZN-04, ZN-05, ZN-06, ZN-07 and ZN-10 showed the antimicrobial activity against on all test organisms. Moreover, ZN-03, ZN-08 and ZN-09 cannot show the antimicrobial activity against on all test organisms. Especially, ZN-10 showed highest antimicrobial activity of clear zone (28.12 mm) against on *Pseudomonas fluorescens.* Therefore, this strain ZN-10 was selected for further investigation. According to the morphological character, microscopical character and references keys, the fungus ZN-10 is preliminarily identified as *Purpureocillium* sp.

Keywords: soil fungi, antimicrobial activity, identification

Introduction

Patheingyi Township located at 7.5 miles of Mandalay Region. Yay Kyi and Shin Taw Gone Villages located in Patheingyi Township. The villages have kept some of the traditional lifestyles and the main occupations is agriculture. Therefore, the study on isolation of effective fungi from soil samples in Patheingyi Township. Spoonful of soil contains billions of microorganisms. In general, the majority of microbial population is found in the upper six to twelve inches of soil and the number decreases with depth. All soils contain bacteria, fungi and viruses in varying amounts depending on soil conditions (Blackwell, 2011). Fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling as biofertilizers. Soil fungi play an important role as a major decomposer in the soil ecosystem. The soil serves as a reservoir of essential nutrients for many microbial communities, plants and small animal. The microorganisms play major role in soil ecosystem. Microbial composition and functioning changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control (Stefanis *et al*., 2013). Soil fungi are also the major sources of other industrially important compounds like enzyme inhibitors, antitumor agents, insecticides and vitamins (Karthikeyan *et al.,* 2014). *P. fluorescens* encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. *Purpureocillium* sp. has been shown to have potential for biopesticide applications (Singh *et*

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al.,2013). The aim of this study is to isolate soil fungi, their activities and to identify of selected fungus.

Materials and Methods

Collection of Soil Samples

The soil samples were collected from two different places of Patheingyi Township in Mandalay Region, during July to September, 2018 (Table 1). The soil sample were collected from different places (up to 15 cm depth) into sterilized polythene bags after removing the surface soil for the isolation of fungi and brought to the laboratory of Biological Resources and Biotechnology Development Center (BDC) at Pathein University.

Table 1. The collection of soil sample at Patheingyi Township

Soil No.	Collected at		Soil Type	Soil pH	Collected Date
	Places	Location			
S-1	Yay Kyi Village	N 21°57' 11.925, E 96°10'42.324"	Clay Loam	5.6	7.7.2018
S-2	Shin Taw Gone Village	N 21°58' 5.023" E 96°10'5.487"	Clay Loam	6.3	7.7.2018

Chemical Treatment Dilution Method (Hayakawa and Kobayashi, 2005)

Soil samples were air-dried at room temperature for a week. Soil samples were ground and sieved in 2 mm screen. Two g of sieve soil was put into the test tube. Four mL of sterile water was poured into the test tube containing soil and settle for 6 hours to germinate early germinating soil microorganisms. Fourteen mL of 70 % ethanol was added into the test tube containing soil suspension and shaken for 1 minute and dilution with sterile water. The dilution series were cultured on LCA medium. These culture plates were incubated at room temperature 2-5 days. After 2-5 days of isolation, microorganism colonies were appeared. The observed colonies were culture separately on LCA medium. Culture of isolation fungi was carried out 3-5 times with PGA medium until the pure culture was obtained. Then, isolated pure fungi were preserved into slant culture on PGA medium for further experimentation. LCA medium-Glucose:0.2g, Sucrose:0.2 g, K_2HPO_4:0.1 g, KNO_3:0.1 g, $MgSO_4.7H_2O$:0.05 g, KCl:0.05 g, Agar:1.8 g, pH:6.5 and DW: 100 mL. PGA medium - Potato Glucose Agar: 3.9 g, DW: 100 mL, pH: 6.5. LCA = Low Carbon Agar medium, PGA = Potato Glucose Agar medium

Screening of effective soil fungi by paper disc diffusion assay (NITE, 2005)

The isolated fungi were grown for 7 days on PGA medium at room temperature. The isolated fungi were inoculated on seed medium. Seed medium-Glucose:2.5g, Yeast extract: 0.8g, $MgSO_4$:0.02 g, K_2HPO_4:0.01 g, DW:100 mL, pH-6.5 (Nakagawa, 1995) and incubated at room temperature for 3 days. Five mL of seed culture was transferred into the fermentation medium. Fermentation medium-Glucose: 1.5 g, Yeast extract: 0.6 g, Soluble Starch:0.3 g, K_2HPO_4:0.01 g, $MgSO_4$:0.02 g, DW:100 mL, pH-6.5 and incubated at room temperature for 3-7 days. Twenty µL of fermented broth was put on paper disc and placed on assay plate containing test organisms. Assay medium-Glucose:1.0 g, Polypeptone 0.3 g, KNO_3:0.01 g, Agar: 1.8 gm, DW: 100 mL, pH-6.5 (Tomita, 1988).

Test Organisms

This method was carried out at Central Research and Development Center (CRDC) Ministry of industry, lower Myanmar. The test organisms used for this experiment were *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *Escherichia coli* AHU5436, *Pseudomonas fluorescens* IFO94307, *Xanthomonas oryzae* NITE 09582.

Results

Isolation of Soil Fungi

In the course of investigation of fungi, ten fungi were isolated from two different soil samples by using chemical treatment dilution method which collected from Patheingyi Township, Mandaldy Region. Four fungi were isolated from Yay Kyi Village and six fungi were isolated from Shin Taw Gone Village respectively as show in (Table 2 and Figure 1).

Table 2. Isolated Fungi from Two Different Soil Sample

Soil No.	Collected Place	Soil pH	Isolation Method	Isolated Fungi
			Chemical Treatment Dilution	
S-1	Yay Kyi Village	5.6	4	ZN- 01, 02, 03, 04
S-2	Shin Taw Gone Village	6.3	6	ZN- 05, 06, 07, 08, 09, 10
Total isolated soil fungi			**10**	

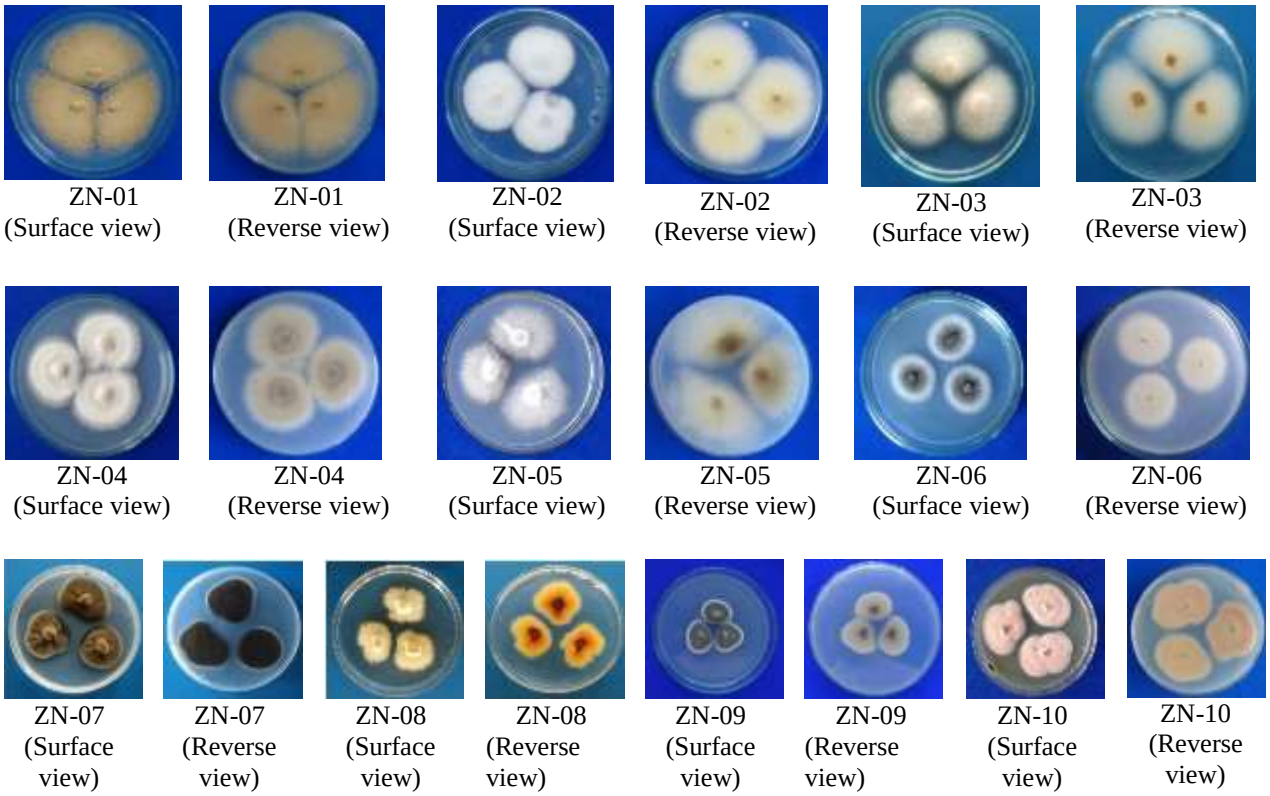


Figure 1. Morphological character of isolated fungi ZN-01 and ZN-10 on PGA medium

Antimicrobial activities of isolated soil fungi

All fungal strains were tested by five test organisms for preliminary study of antimicrobial activities. Among them, ZN-10 exhibited the highest activities as shown in (Table 3 and Figure 2)

Table 3. Antimicrobial Activities of Isolated Soil Fungi (ZN-01 to ZN-10) at 5 days period

Isolated Fungi	Antimicrobial Activity (mm)				
	Antibacterial				Antifungal
	B. subtilis	*E. coli*	*P. fluorescens*	*X. oryzae*	*C. albicans*
ZN-01	17.27	22.58	14.21	20.34	12.33
ZN-02	-	-	-	11.53	13.35
ZN-03	-	-	-	-	-
ZN-04	14.10	-	15.12	-	17.10
ZN-05	-	-	-	14.38	17.35
ZN-06	14.37	-	-	-	16.39
ZN-07	-	23.10	-	24.12	-
ZN-08	-	-	-	-	-
ZN-09	-	-	-	-	-
ZN-10	17.00	24.11	**28.12**	24.60	14.67

(-) = no activity, paper disc = 8mm

Bacillus subtilis *Escherichia coli* *Pseudomonas fluorescens* *Xanthomonas oryzae* *Candida albicans*

Figure 2. Antimicrobial activities of isolated soil fungi (ZN-01 to ZN-10)

Identification of selected fungus ZN-10

Morphological characters of fungus ZN-10

Colonies are fast growing, suede like, violet-colored on malt extract agar, vinaceous or pale violet and pink to deep purple on PGA medium for 7 days at 25°C. The vegetative hyphae are smooth-walled, hyaline, and violet rough-walled

Microscopical characters of fungus ZN-10

Conidiophores give rise from denser mycelium, bearing branches with densely cluster phialides. Phialides developed at the end of the conidiophore were in whorls and swollen at their bases, gradually tapering into a slender neck. Conidia are divergent chains, oval to ellipsoidal in shape, and smooth walled, lacking septum, violet/purple.

On the basis of morphological-microscopical characters and references key (Luangsa *et al*., 2011; Perdomo *et al*., 2013 & 2017; Dan *et al*, 2015; Sunil *et al*., 2015), it is assumed that ZN-10 strain may be *Purpureocillium* sp.

Classification -

Kingdom - Fungi

Division - Ascomycota

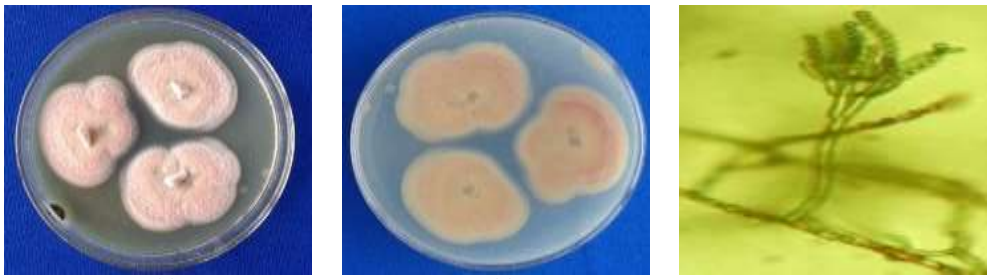
Class - Sordariomycetes

Order - Hypocreales

Family - Ophiocordycipitaceae

Genus - *Purpureocillium*

Species - *Purpureocillium* sp.



Morphology of fungus ZN-10 (Surface view) | Morphology of fungus ZN-10 (Reverse view) | micrograph of fungus ZN-10

Figure 3. Colony of morphology and micrograph of selected fungus ZN-10 (400

Discussion

In the study of isolation of soil fungi, ten different fungi were isolated from two different soil samples collected at Patheingyi Township in Mandalay Region. The isolation of soil fungi was undertaken by chemical treatment dilution method (Hayakawa and Kobayashi, 2005) were used for the isolation of soil fungi. The reported by the soil analytical data of the Department of Agriculture (Land Use) analysis of these different soil were show that the acid condition pH range (5.6 and 6.3). The soil texture was determined soil sample No.1 and No.2 soil type are Clay loam respectively.

Four fungi were collected at Yay Kyi Village and six fungi were collected at Shin Taw Gone Village. The morphological characters of four fungi (ZN-02, ZN-03, ZN-04 and ZN-05) were white colors, ZN-01 was greenish yellow color, ZN-06 was edge white, center black color, ZN-07 was brown color, ZN-08 was cream color, ZN-09 was edge white, center green color and ZN-10 was pink color in surface view. ZN-02, ZN-03 and ZN-06 were white color, ZN-04, ZN-05 and ZN-09 were cream color, ZN-01 was greenish yellow color, ZN-07 was black color, ZN-08 was orange color and ZN-10 was pink color in reverse view. In the preliminary study of ten

soil fungi were tested the paper disc diffusion assay on five test organisms. Among them, four fungi (ZN-01, ZN-04, ZN-06, and ZN-10) against on *B. subtilis*, six fungi (ZN-01, ZN-02, ZN-04, ZN-05, ZN-06 and ZN-10) against on *C. albicans*, three fungi (ZN-01, ZN-07 and ZN-10) against on *E. coli*, three fungi (ZN-01, ZN-04 and ZN-10) against on *P. fluorescens*, four fungi (ZN-01, ZN-05, ZN-07, and ZN-10) against on *X. oryzae*.

Especially, ZN-01, ZN-02, ZN-04, ZN-05, ZN-06, ZN-07 and ZN-10 showed the antimicrobial activity against on all test organisms. Moreover, ZN- 03, 08 and 09 cannot show the antimicrobial activity against on all test organisms. ZN-10 showed highest antibacterial activity against on *P. fluorescens*. The selected fungus was isolated from soil sample. No.2. The soil was collected from the place of Shin Taw Gone Village. Soil type is clay loam, pH-6.3 and N 21˚ 58′ 5.023″ and E 96˚ 10′5.487″. While selected fungus ZN-10, it was observed that colonies are fast growing, suede like. The vegetative hyphae are smooth-walled, hyaline. Conidiophores give rise from denser mycelium and bearing branches with densely cluster phialides. Phialides developed at the end of the conidiophore were in whorls and shapes are swollen at their bases, gradually tapering into a slender neck. Conidia are divergent chains, oval to ellipsoidal in shape, and smooth walled, lacking septum. On the basis of morphological-microscopical characters and references key (Luangsa *et al*., 2011; Perdomo *et al*., 2013 & 2017; Dan *et al*., 2015; Sunil *et al*., 2015), the selected fungus ZN-10 may be identified as *Purpureocillium* sp.

Eilers *et al*., (2012) reported that soil microbial abundance and diversity are highest in the top 10 cm and decline with depth. Soil samples were collected from 0-15 cm depth after removing the surface soil for the isolation of fungi. Wingfield *et al*., 2023 were stated that soil samples collected from Pattani Province, Thailand. Twenty-four fungi were isolated among them N-01, N-02, N-04, N-05, N-06, N-07, N-10, N-19 and N-24 were against on *E. coli* and *C. albicans*. In the present study, ZN-01, ZN-02 and ZN-04 were isolated from Yay Kyi Village, Patheingyi Township, Mandalay Region, that exhibit the antimicrobial activity against on *E. coli* and *C. albicans*. The morphological character of N-18 is similar to ZN-02. This is an agreement with the observation of Wingfield *et al*., 2023. Bebric *et al*., 2012 were stated that soil samples collected from Serbia. One hundred-eighteen fungi were isolated among them S- 6, S-8, S-11, S-12, S-17, S-18 and S-24 were against on *B. subtilis* and *X. oryzae*. In the present study, ZN-05, ZN-06, ZN-07 and ZN-10, were isolated from Shin Taw Gone Village, that exhibit the antibacterial activity against on *B. subtilis* and *X. oryzae*. This is an agreement with the observation of Beric *et al*., 2012.

Bhattacharyya and Jha, 2012 were described the endophytic fungi *Purpureocillium* sp. showed the antimicrobial activity against on *X. oryzae.* Kar and Chakraboty., 2018 reported that isolated fungus *Purpureocillium* sp. from the soil showed that the antimicrobial activity against on *P. fluorescens.* Dash and Dangar, 2020 were stated that isolated fungus *Purpureocillium* sp. from the soil showed that against on *B. subtilis, C. albicans, E. coli.* The results of the present work agree with Dash and Dangar, 2020.

Conclusion

The isolated fungus *Purpureocillium* sp. will further investigation to clarify the identification of isolated fungus up to species level and ages of culture, sizes of inoculums, effect of carbon source, nitrogen source, temperature, fermentation periods and time course. Majority of fungi produces secondary metabolites which may be beneficial towards pharmaceutical chemist

as these metabolites are widely used in medicine and agriculture. The results seen in the present study also support the medicinal usage as antibacterial agents in new drugs for therapy infection diseases caused by pathogens and undergo further pharmacological screening that can be used as sources for new drugs.

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ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC FUNGI FROM *GLOBBA ARRACANENSIS* KURZ.

Eaindra Chan Myae*

Abstract

Glabba is a genus of plants in the ginger family and contains about 100 species. In this research work, the plant samples (leaves and rhizomes) of *Globba arracanensis* Kurz. were collected from Nat-Taung-Pyin Hill, Pauktaw Township in Rakhine State in September, 2023. *G. arracanensis* Kurz. is only found in Rakhine State and it is now known not to be extinct. The morphological and microscopical characters of all fungal strains were carried out at Microbiology Laboratory, Department of Botany, Dagon University. The colony appearances of isolated fungal strains were irregular, circular and rhizoid. The colony margins of isolated strains were entire, filamentous, undulate and lobate. The elevations of isolated strains were raised, flat and pulvinate. The surface and reverse colors of isolated fungal strains were white, whitish pale yellow, whitish orange, light orange, whitish green, whitish yellow. whitish grey. The colony characters and microscopic characters of isolated fungi are very closed to *Penicillium*, *Rhizopus*, *Rhizoctonia*, *Cladosporium* and *Aspergillus* species. Strains Eaindra-5, Eaindra-6, Eaindra-9, Eaindra-12 and Eaindra-13 were identified as *Penicillium* species, strains Eaindra-8 and Eaindra-11 as *Aspergillus* species, strain Eaindra-3 as *Cladosporium* species, strain Eaindra-10 as *Rhizopus* species and strains Eaindra-2 and Eaindra-14 as *Rhizoctonia* species. These isolated strains possessed good antimicrobial activity on some pathogenic organisms so that this activity will be presented in the next paper.

Keywords: Endophytes of *Globba arracanensis* Kurz.

Introduction

Globba arracanensis Kurz. belongs to the genus *Globba* in the family Zingiberaceae. *Globba* is the third largest genus of the Zingiberaceae with 100-110 species in the world (Williams *et al*., 2017). *Globba arracanensis* Kurz. was found in north of Akyab (now called Sittwe) in the Kolodyne River Valley of Arracan (now called Rakhine) State in Myanmar by Kurz in 1869.

Endophytic fungi may promote the growth of their host plant by producing phytohormones or by increasing the plant's resistance to various stresses, and they can produce pesticides to protect plants from herbivores. There are many active and biologically active substances produced grouped into different categories due to the relationship between endophytes and their hosts (Hashem *et al*., 2023).

Several antimicrobial compounds produced by endophytic fungi are important in their effectiveness against pathogens that have developed resistance to antibiotics. Endophytic fungi produce biologically active secondary metabolites, such as terpenes, alkaloids, monoterpenoids, peptides, and polyketides. Fungal endophytes are used to control a wide range of human health issues, such as the production of antibiotics, antifungal, antiviral, anticancer, lytic enzyme, and degradation of toxins (Hashem *et al*., 2023).

The aim of present study is isolation, characterization and identification of endophytic fungi from *Globba arracanensis* Kurz.. The objectives are to study morphology of the selected plant, to isolate endophytic fungi from the collected plant, to investigate the morphological and microscopical characters of isolated fungi and to identify the possible genera of isolated fungi.

* Department of Botany, Dagon University

Materials and methods

Morphological Study of Selected Plant

The specimens of *Globba arracanensis* Kurz. were collected from Nat-Taung-Pyin Hill, Pauktaw Township in Rakhine State during the flowering period (June to November). After collection, the plants were identified with the help of literatures Wu *et al*., (Bordelon and John Kress (2009), Hundley and Chit Ko Ko (1987) and Kress *et al*., (2003).

Isolation of Endophytic Fungal Strains from *Globba arracanensis* Kurz .

Endophytic fungal strains were isolated from the leaves and rhizomes of *Globba arracanensis* on four different media. Isolation of endophytic fungal strains were carried out by the following schemes: (Phay, 1997).

1. The plant samples were washed under running tap water for fifteen minutes.
2. The plant samples (leaves and rhizomes) were cut into about 1.5 cm pieces.
3. These pieces were sterilized by soaking in 75% ethanol for 2 min.
4. Then, pieces of rhizomes were sterilized by soaking in 5.3% sodium hypochloriode for 1 minute.
5. These parts were dried on sterilized paper and then they were placed on appropriate medium.

Figure.1 Isolation of endophytic fungal strain from plant

Composition of culture media (Atlas, 1993)

(1) Nutrient Agar Medium (NA)

Nutrient Agar	=	3.5 g
Distilled Water	=	100 ml
Agar	=	1.0
pH	=	7

(2) Sucrose/Yeast Extract Medium (SY)

Sucrose	=	1.0 g
Yeast extract	=	0.3 g
Distilled Water	=	100 ml
Agar	=	2.5 g
pH	=	7

(3) Nutrient Agar Medium (NA)

Glucose	=	1.0 g
Yeast extract	=	0.3 g
Distilled Water	=	100 ml
Agar	=	2.5 g
pH	=	7

(4) Sucrose/Yeast Extract Medium (SY)

Lactose	=	1.0 g
Yeast	=	0.3 g
Distilled Water	=	100 ml
Agar	=	2.5 g
pH	=	7

Morphological Characters of Isolated Fungi

Isolated endophytic fungal strains grown on slant culture were transferred into the plates containing four different media. Then, these plates were incubated at 30˚C for 3-7 days. The morphological and colonial characters such as colony appearance, margin, surface and reverse colours and elevation of all isolated strains were recorded as revealed in the reference of Dubey and Maheswari (2014).

Microscopical Characters of Isolated Fungi

The microscopical characters of all isolated strains *Globba arracanensis* Kurz. (GA-1 to GA-14) were carried out under light microscope with high magnification at Department of Botany, Dagon University. The main characters of hyphae, mycelia, sporangiophores, spores, color formation on upper as well as lower surface were comparatively studied. These are compared to those of fungi with available literatures such as Barnett (1969), (1998).

Results

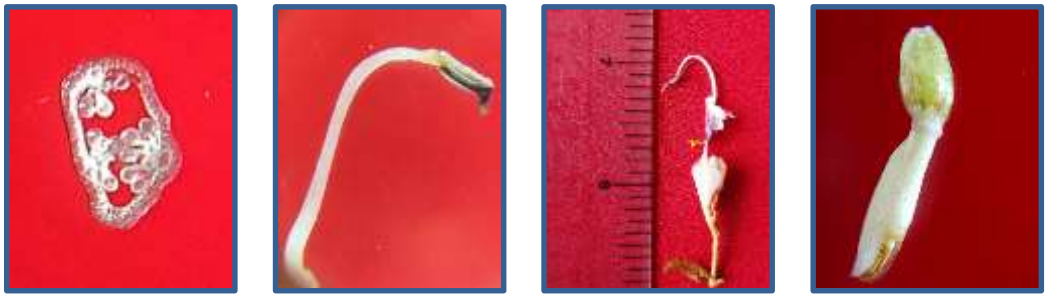
Morphological Study of Selected Plant

Scientific Name	:	*Globba arracanensis* Kurz.
Family	:	Zingiberaceae
Myanmar Name	:	Waso-pan
Flowering and Fruiting Period	:	June to November

The native range of this species is Bangladesh to North West of Myanmar. Plant are perennial herbs with leafy pseudostem and deciduous, stem about 40-80 cm long and curving upward, rhizome branched. Leaves simple, alternate, sheathing petiole; lamina ovate-elliptic, margin entire, base slightly rounded to acute, the tip highly accuminated form a thread like, about 25-45 x 5-10 cm. Inflorescence terminal on the leafy pseudostem about 10-18 cm long, about 4-9 flowers in each cincinnus. Flower bracteate, bracts are persistent, lalic color, bracteolate, bracteoles are also persistent and light lalic color, sessile, complete, bisexual, irregular, zygomorphic, trimerous, epigynous. Sepal (2+1), synsepalous, campanulate, apex obtusely 3-lobed, persistent. Petals (1+2), tube slender, lobe oblong, lalic color. Androceium 1+ 2^{st} + 2^{st}, fertile stamen 1, filament white, anther dithecous, dechiscance by longitudinal slit; 2 petal like lateral stominodes, and white to lalic white color, labellum reflexed, adnate to filament to form a slender tube above lateral staminodes and corolla tube; dark purple with yellow spots at the tip and center. Carpel (3), syncarpous, tricarpellary, many ovule in the locule, parietal placentation; style long, inserted in the fertile filament, stigma capitate, ovary inferior. Fruit capsule with persistent calyx.

Habit Leaf Rhizome Inflorescence

Figure (2)a *Globba arracanensis* Kurz.



Ovules Stamen Flower Fruit

Figure (2)b *Globba arracanensis* Kurz.

Isolation of Endophytic Fungi

In the present works, fourteen isolated strains were maintained into the pure culture and designated as *Globba arracanensis* Kurz. Eaindra-1 to Eaindra-14.

Table (1) Isolation of fungi from leaves and rhizomes of *Globba arracanensis* Kurz.

Strain from leaves	Strain from rhizomes	Culture medium
Eaindra-1	Eaindra-8 and Eaindra-9	Nutrient Agar
Eaindra-2	Eaindra-10 to Eaindra-12	Sucrose/Yeast Extract Medium
Eaindra-3	Eaindra-13	Glucose/Yeast Extract Medium
Eaindra-4 to Eaindra-7	Eaindra-14	Lactose/Yeast Extract Medium

Morphological and Microscopical Characters of Isolated Fungi

The morphological characters of isolated fungal strains were carried out at Microbiology Lab, in the Department of Botany, Dagon University. The colony appearances of isolated fungal strains Eaindra-1, Eaindra-2, Eaindra-5, Eaindra-6, Eaindra-7, Eaindra-8, Eaindra-9, Eaindra-11, Eaindra-12 and Eaindra-13 were irregular, strains Eaindra-3, Eaindra-4 and Eaindra-10 were circular, strain Eaindra-14 was rhizoid.

The margin of isolated fungal strains Eaindra-1, Eaindra-2, Eaindra-3, Eaindra-6, Eaindra-7, Eaindra-10 and Eaindra-14 were filamentous, strains Eaindra-4, Eaindra-5 and Eaindra-13 were entire, strains Eaindra-8, Eaindra-9 and Eaindra-11 were lobate and strain Eaindra-12 was undulate.

The elevation of isolated fungal strains Eaindra-1, Eaindra-2, Eaindra-3, Eaindra-4, Eaindra-8, Eaindra-9, Eaindra-11, Eaindra-12 and Eaindra-13 were raised, strains Eaindra-5, Eaindra-6, Eaindra-7 and Eaindra-14 were flat and strain Eaindra-10 was pulvinate.

The surface and reverse colors of isolated fungal strains Eaindra-4, Eaindra-5, Eaindra-6, Eaindra-12 and Eaindra-13 were white and whitish pale yellow. Strains Eaindra-2 and Eaindra-7 were white and whitish orange and strain Eaindra-1 was white and light orange. The colors of strain Eaindra-10 was white and strain Eaindra-14 was whitish grey. Strain Eaindra-11 were white and whitish grey. Strain Eaindra-3 was white and whitish yellow. The colors of strain Eaindra-8 was whitish green and whitish orange and strain Eaindra-9 was whitish green and whitish pale yellow.

Microscopical Characters of Isolated Fungi

The microscopical characters of isolated mangrove fungi are the same to the genera of *Penicillium, Aspergillus*, *Rhizopus* and *Rhizoctonia.* These strains were identified as strains Eaindra-5, Eaindra-6, Eaindra-9, Eaindra-12 and Eaindra-13 as *Penicillium* species*,* strains Eaindra-8 and Eaindra-11 as *Aspergillus* species, strain Eaindra-3 as *Cladosporium* species, strains Eaindra-2 and Eaindra-14 as *Rhizoctonia* species and strain Eaindra-10 as *Rhizopus* species. Strains Eaindra-1, Eaindra-4 and Eaindra-7 were being unable to identify their genus level. So, they were assumed as unidentified isolates.

Scientific classification

Penicillium		
Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Eurotiomycetes
Order	:	Eurotiales
Family	:	Trichocomaceae
Genus	:	***Penicillium***

Aspergillus		
Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Eurotiomycetes
Order	:	Eurotiales
Family	:	Aspergillaceae
Genus	:	***Aspergillus***

Rhizoctonia		
Kingdom	:	Fungi
Division	:	Basidiomycota
Class	:	Agaricomycetes
Order	:	Cantharellales
Family	:	Ceratobasidiaceae
Genus	:	***Rhizoctonia***

Rhizopus		
Kingdom	:	Fungi
Division	:	Zygomycota
Class	:	Zygomycetes
Order	:	Mucorales
Family	:	Mucoraceae
Genus	:	***Rhizopus***

Cladosporium		
Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Dothideomycetes
Order	:	Capnodiales
Family	:	Davidiellaceae
Genus	:	***Cladosporium***

Morphological and Microscopical Characters of All Fungal Strains

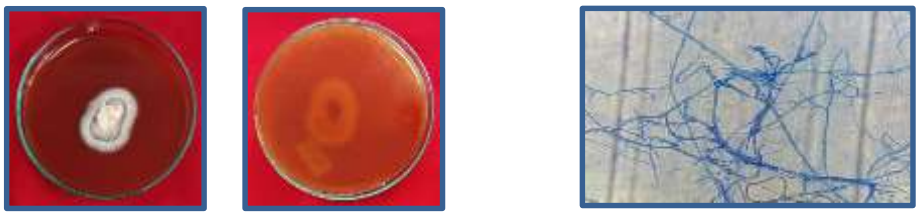


Figure (3) Morphological and microscopical characters of isolated endophytic fungus Eaindra -(1)

Most mycelia are thin without septate. They are highly branched at the tip. Conidiophores are absent. Conidia are not produced. This fungal strain (Eaindra-1) was not identified as shown in Figure (3).

Figure (4) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(2)

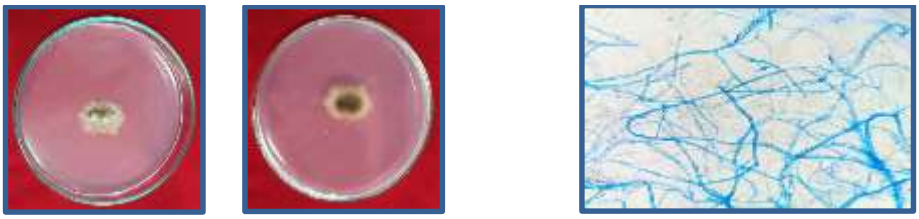


Figure (5) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(14)

Mycelium white with long cells, septa of branch (right angle) set off from main hypha. Conidiophores are connected by mycelial threads, and spores lacking. These strains (Eaindra-2 and Eaindra-14) were identified as *Rhizoctonia* species as shown in Figures (4 and 5).

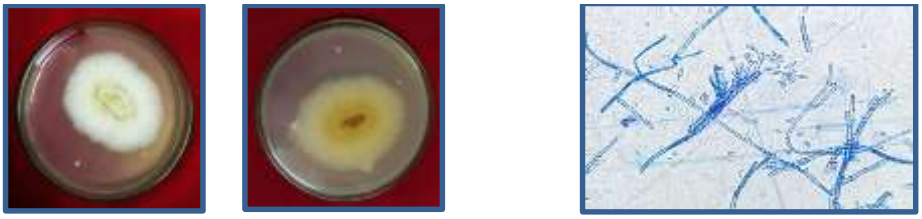


Figure (6) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(3)

Mycelia are thin with septa. Condiophores are long, branched variously near the apex. Condia are light, various shapes (oval, cylindrical, irregular, spherical) and sizes. This fungal strain (Eaindra-3) was identified as *Cladosporium* species as shown in Figure (6).

Figure (7) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(4)

Eaindra Mycelia are thin, and some are branched but some are not branched at the tip. Conidiophores are continuous with mycelium. Conidia are long oval shape at the tip of mycelium. This fungal strain (Eaindra-4) was not identified as shown in Figure (7).

Figure (8) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(5)

Figure (9) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(6)

Figure (10) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(9)

Figure (11) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(12)

Figure (12) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(13)

Conidiophores arising from the hypha singly at the apex, ending in a group of phialides; conidia Hyaline, 1- celled, mostly globose, in chains. These four strains (Eaindra-5, Eaindra-6, Eaindra-9, Eaindra-12 and Eaindra-13) were identified as *Penicillium* species as shown in Figures (8, 9, 10, 11 and 12).

Figure (13) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(7)

Mycelia are highly branched with septate, some are globose at the tip. Conidia are not produced. This fungal strain (Eaindra-7) was not identified as shown in Figure (13).

Figure (14) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(8)

Figure (15) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(11)

Conidiophores long, upright, terminating in a large globose swelling, bearing phialides which are radiating from entire surface. Conidia: 1- celled, globose, produced basipetally. These strains (Eaindra-8 and Eaindra-11) may be identified as Aspergillus species as shown in Figures (14 and 15).

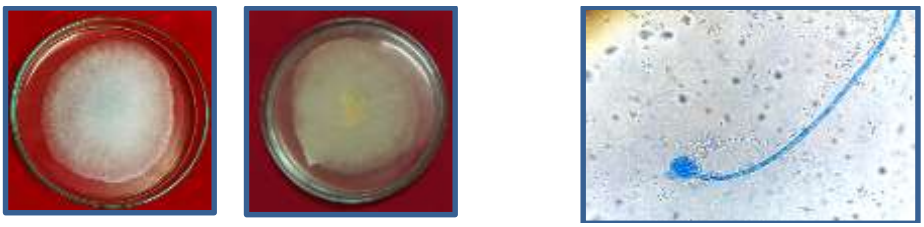


Figure (16) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(10)

Broad have no or very few, septa, usually are unbranched. Conidiophores: round sporangiophores are long, many oval, colorless spore. This strain (Eaindra-10) may be identified as *Rhizopus* species as shown in Figure (16).

Discussion and conclusion

Glabba is a genus of plants in the ginger family and contains about 100 species including about 17 species in Myanmar. In this research work, the plant samples (leaves and rhizomes) of *Globba arracanensis* Kurz. were collected from Nat-Taung-Pyin Hill, Pauktaw Township in Rakhine State in Septmber 2023.

In this study, fourteen endophytic strains were isolated from Rhizomes of *Globba arracanensis* Kurz. (Family Zingibraceae). Lutfia and Munir (2019) have isolated endophytic fungi from rhizome of *Globba pendula* Roxb. Soe Soe Yu Hnin (2018) isolated endophytic fungal strains

from rhizomes of the selected plant from the family Zingibraceae. Saw Ohnmar *et al.* (2020) studied morphology and phytochemical and antimicrobial activity of *Globba arracanensis* Kurz. But, there was no the previous record for endophytes from *Globba arracanensis* Kurz. in Myanmar.

In this study, the morphological characters of *Globba arracanensis* Kurz. were mentioned, and the colony appearance of isolated fungal strains were circular, irregular and rhizoid. The margins of isolated fungi were entire, filamentous, undulate and lobate.

In this study, the elevation of isolated fungal strains was raised, flat and pulvinate. These characters were in agreement with the statements of Dubey and Maheshwari (2014). The surface and reverse colors of isolated fungal strains were the same to the statement reported by Kyawt Kyawt Aung (2014), Soe Soe Yu Hnin (2018) and Yee Yee Thu *et al*., (2016).

In this study, five *Penicillium* spp., two *Aspergillus* spp., two *Rhizoctonia* spp., one *Cladosporium* spp., one *Rhizopus* sp., and three unknown species were isolated from *Globba arracanensis* Kurz. Many researchers have isolated bioactive fungi such as *Penicillium* sp., *Aspergillus* sp., *Cladosporium* spp., *Rhizoctonia* sp., *Rhizopus* sp., etc. The microscopical characters of isolated endophytic fungi were agreed with the statements of Barnett (1969).

Barnett *et al.* (1998) have stated that conidiophores of *Penicillium* are arising from the hypha singly at the apex, ending in a group of phialides; and conidia are hyaline, 1- celled, mostly globose or ovoid, in chains. Ariantari *et al*., (2019) have stated that endophytic *Penicillium* species were isolated from roots of the medicinal plant *Zingiber officinale* to produce indole diterpenoids. Urooj *et al*., (2021) have reported endophytic *Penicillium* species and *Pseudomonas monteilii* in inducing the systemic resistance in okra against root rotting fungi and their effect on some physiochemical properties of okra fruit.

Otero *et al.,* (2002) have reported that 108 *Rhizoctonia* like fungi were isolated from nine Puerto Rican orchids. Ma *et al*., (2004) have isolated endophytic *Rhizoctonia* sp. from *Cyanodin dactylon* to produce Anti-Helicobacter pylori metabolites. The genus *Rhizoctonia* is comprised of a highly divergent group of sterrile fungi that still share similar characteristics in their anamorphic (asexual) state, namely they remain vegetative, producing no asexual spores (Harveson, 2013).

Barnett *et al.* (1998) have stated that conidiophores of *Aspergillus* spp. are long, upright, terminating in a large globose swelling, bearing phialides which are radiating from entire surface, and conidia are 1- celled, globose, produced basipetally. Hartanto *et al*., (2019) isolated *Aspergillus* species from rhizome of *Alpinia* sp. (Zingiberaceae) in Hutan Sibayak, North Sumatera. Liu *et al*., (2019) have mentioned that an endophytic *Aspergillus flav*us was isolated from a toxic medicinal plant, *Tylophora ovata* to produce sesquiterpene.

Berisch *etal.*(2012) stated that the vegetative hyphae were erect, straight, branched or unbranched. Some conidia were produced in branched acropetal chains in *Cladosporium* sp. Alvaro *et al*. (2022) isolated endophytic fungus *Cladosporium* sp. (AC-1) from the leaves of *Annona cacans* L.

El-Zawawy *et al*. (2023) have isolated a new endophytic fungus *Rhizopus oryzae* AUMC14899 for the production of L-tyrosine and its biomedical applications. They investigated that the genus *Rhizopus* exhibited strong antibacterial and anti-biofilm activities against multidrug-resistant Gram-negative and Gram-positive bacteria.

In conclusion, fungi naturally produce antibiotics to kill or inhibit the growth of microbial diseases. Endophytic fungi have been known as excellent source of antimicrobial agents. Endophytic fungi have antibiotic acativity as they can produce the bioactive compounds. In this research, some of fungal strains isolated from leaves are the same genera to fungal strains from rhizomes of *Globba arracanensis* Kurz. All endophytic strains (six *Penicillium* species, two *Aspergillus* species, two *Rhizoctonia* species, one *Cladosporium* sp., one *Rhizopus* species and three unknown species) possessed good antimicrobial activity. This activity will be presented in the next paper.

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FERMENTATION STUDIES OF *RHIZOCTONIA* SP. ISOLATED FROM THE LEAVES OF *COMBRETUM INDICUM* (L.) R. A. DEFILIPPS.

Wint Yati[1], Soe Soe Yu Hnin[2]

Abstract

An endophytic fungi *Rhizoctonia* sp. was isolated from the leaves of *Combretum indicum* (L.) R. A. DeFillips. In the present study, fermentation (one day old) of age of inoculum was the best for fermentation. In the size of inoculum optimization, fermentation (2%) showed the best antimicrobial activity on six test organisms and fermentation (pH 5) of pH utilization was the best for the production of bioactive compounds according to the results of inhibitory zones of their antimicrobial activity against six test organisms. In the study of extraction of metabolites by using different solvents system, the extracts with methanol, ethanol, ethyl acetate and n-hexane showed the high activity on six test organisms: *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas* sp. and *Staphylococcus aureus*. Among them, ethyl acetate extract showed the best antimicrobial activity.

Keywords: Endophytes, *Rhizoctonia* sp., antimicrobial activity

Introduction

Endophytes are an endosymbiotic group of microorganisms, often bacteria or fungi that colonize the intercellular or intracellular locations of plants (Pimentel *et al*., 2011; Singh and Dubey, 2015). Medicinal plants are known to harbor endophytic microorganisms, which are found to play an important role in the production of pharmaceutically important compounds. Endophytic fungi are generally considered superior because of their ubiquitous and diverse nature. They produce many secondary metabolites greater than other endophytic microorganisms (Zhang *et al*., 2006).

The aims of this study were to evaluate fermentation studies of isolated fungal strain *Rhizoctonia* sp. and to conduct extraction of metabolites using five different solvents.

Materials and methods

Collection of plant samples

The leaves of *Combretum indicum* (L.) R. A. DeFilipps were collected from the University of Yangon Campus. The collected plant material was recorded by photographs and identified by Wu and Raven, 2007. Myanmar name was recorded by Kress *et al*. 2003.

Isolation of endophytic Fungi (Suto,1999)

The leaves of plant were washed in running tap water for five minutes. The leaves were cut into small pieces (3cm). These parts were sterilized by soaking in 75% ethanol for 15 seconds. Then, these parts cut into smaller pieces (1cm) and dried on sterilized paper. After that, they were placed on agar plates containing sucrose-yeast extract medium (SY medium) and nutrient agar medium (NA medium) supplemented with chloramphenicol (100µg / L) to inhibit bacterial growth. These plates were incubated at room temperature for 3-7 days and transferred to new plates. Then, isolated fungal strains were transferred into slant culture of test tubes.

Agar well diffusion method

The agar plates containing test organisms were punched to make the wells (8 mm in diameter) using sterile cork borer and filled with the stock solution and then these plates were

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incubated at room temperature for 24 hours. After incubation, the diameters of the growth inhibition zones surrounding the wells were measured in mm. These zones indicate the presence of antimicrobial activities which inhibit the growth of test organisms selectively. (Collins, 1965).

Fermentation studies

(i) Age of inoculum of *Rhizoctonia* sp.

One day old, two days old and three days old of seed cultures were transferred into 50 ml fermentation flasks containing 25 ml of nutrient agar (NA) medium. They were incubated for seven days. Then, these fermented broths were checked for their inhibitory activities by agar well diffusion method. (Strobel and Sullivan, 1999).

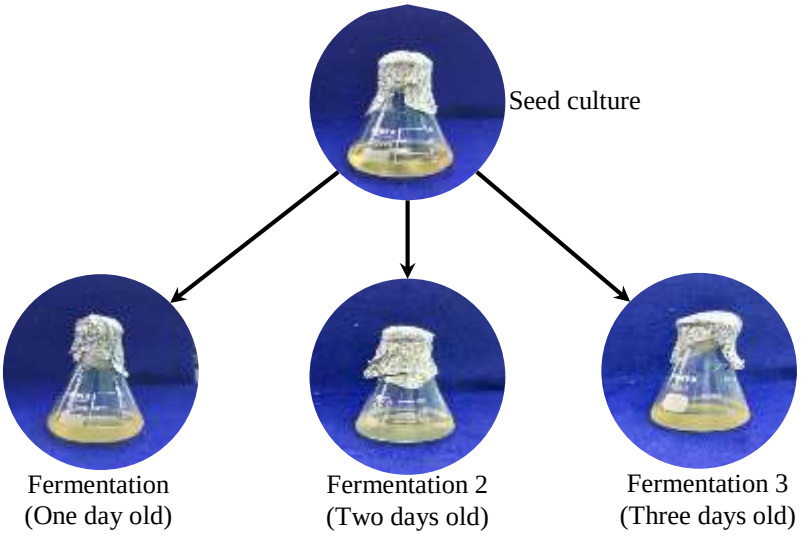


Figure 1. Seed culture and fermentation for age of inoculum

(ii) Size of inoculum of *Rhizoctonia* sp.

The proper cultivation and transfer (size of inoculum) are essential to produce bioactive metabolites. A piece from fungal plate culture of *Rhizoctonia* sp. was inoculated into 125 ml of conical flasks containing 100 ml of nutrient agar (NA) medium. The flasks were incubated at room temperature for one day. After one day, the seed cultures (1.0%, 1.5%, 2.0%, 2.5% and 3.0%) were transferred into five conical flasks (125 ml) containing 100 ml of fermentation medium as shown in Figure 2. The fermentation was carried out for seven days. (Monaghan *et al*., 1999)

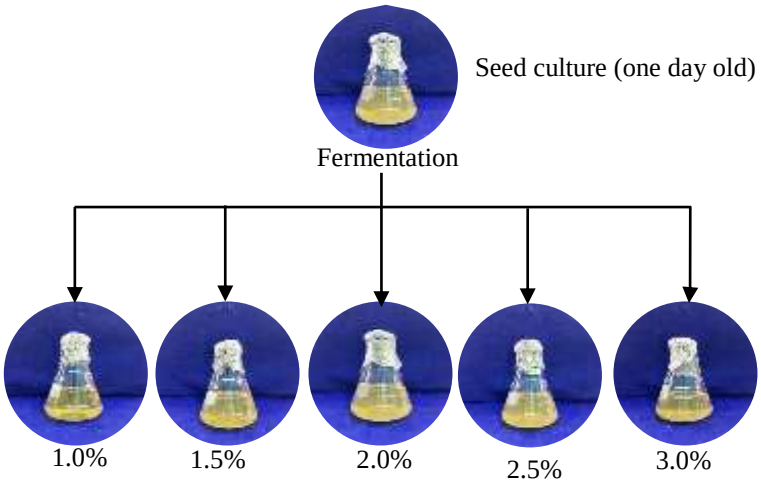


Figure 2. Seed culture and fermentation for size of inoculum

(iii) pH utilization of *Rhizoctonia* sp.

For the seed culture, a piece from fungal plate culture of *Rhizoctonia* sp. was inoculated into 125 ml of conical flask containing 100 ml of fermentation medium (Nutrient agar medium) and then flasks were incubated at room temperature for one day. Five 125 ml conical flasks containing 100 ml fermentation medium were adjusted at pH 4, 5, 6, 7, 8 and autoclaved. The seed culture (2%) was transferred to each fermentation flask with pH 4 to 8 and fermentation was carried out for one day. After one day, five fermentation flasks were checked their antimicrobial activity. (Monaghan *et al*., 1999).

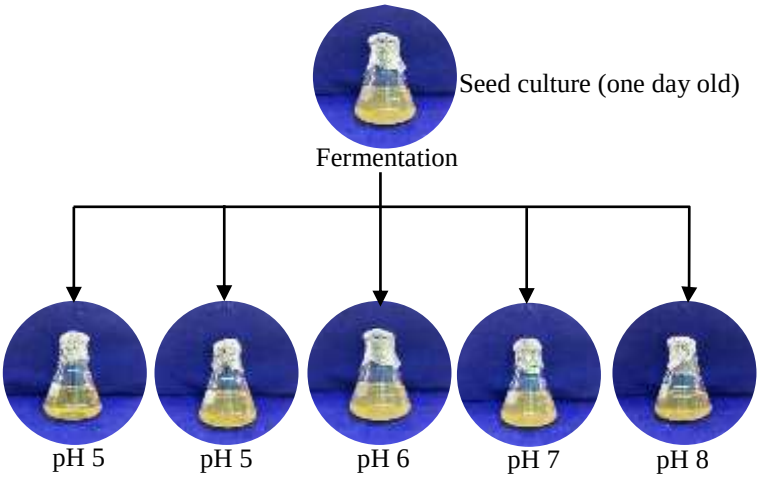


Figure 3. Seed culture and fermentation for pH of inoculum

Extraction and antimicrobial activity of bioactive compounds from *Rhizoctonia* sp.

A piece from fungal plate culture of *Rhizoctonia* sp. was cultured in 300 ml Erlenmeyer flasks containing 150 ml of fermentation medium (NA medium) and incubated at room temperature, 180 rpm for one day. Then, the media was harvested by centrifugation at 5000 rpm for 20 min. The supernatant was transferred and equal volume of five different solvents (ethanol, methanol, ethyl acetate, n-hexane and petroleum ether) were separately added to the filtrate. The mixture was shaken for 30 min and placed in water bath at 50°C to evaporate the aqueous and organic layer of crude extract and obtain a gummy crude extract. The crude extract was re-dissolved in a small volume of each solvent (1mg/1ml) and screened for their antimicrobial activity on six test organisms. The plates were incubated at room temperature for 24 hours and checked for their inhibitory zones. (Ahmed, 2007)

Results

Scientific classification

Scientific name:	***Combretum indicum*** (L.) R.A. DeFilipps
Synonym:	*Quisqualis indica* L.
Common Names:	Dawe-hmaing-nwe, Mawk-nang-nang, Rangoon creeper
Family:	Combretaceae

Outstanding characters

Lianas to 8 m tall. Branchlets brownish yellow pubescent. **Petiole** 5-9 mm, densely brown pilose when young; **leaf blade** mostly oblong-elliptic or elliptic, 5-18 × 2.5-7 cm, abaxially sometimes brown pilose, adaxially glabrous except slightly brown pilose on midvein, finely white verruculose, rarely tomentose on both surfaces, base obtuse, apex acuminate to shortly caudate;

lateral veins in 7 or 8 pairs. **Inflorescences** lax; bracts deciduous, filiform-linear to ovate, 3-12 mm, brown pilose. **Flowers** fragrant. **Calyx** tube 5-9 cm, yellow pilose; lobes deltoid, 2-3 mm, apex acute or shortly acuminate but not cuspidate. **Petals** opening white, later turning yellowish abaxially and reddish adaxially, obovate to oblanceolate, 10-24 × 4-10 mm, apex rounded to obtuse. **Fruit** red when young, greenish black or brown when ripe, fusiform, or narrowly ovoid, sharply 5-ridged, 2.7-4 × 1.2-2.3 cm, glabrous, apex mucronate.

Figure 4. Habit of *Combretum indicum* (L.) R. A. DeFilipps

Antimicrobial activity of isolated endophytic fungus *Rhizoctonia* sp.

In this study, isolated endophytic fungi *Rhizoctonia* sp. showed the best antimicrobial activities against *Agrobacterium tumefaciens, Bacillus pumilus, Candida albicans, Escherichia coli, Pseudomonas* sp. and *Staphylococcus aureus.*

Morphological and microscopical characters of isolated fungus *Rhizoctonia* sp.

In morphological character, the surface colour of *Rhizoctonia* sp. was gray, its reverse colour is black and the margin was undulate. The microscopical character was that cell of mycelium usually long, septa of branches usually set off from the main hyphae; asexual fruit bodies and conidia absent; sporodochium-like bodies and chlamydospore-like cells in chains. Therefore, according to Barnett, 1998 description, this fungus may be *Rhizoctonia* sp.

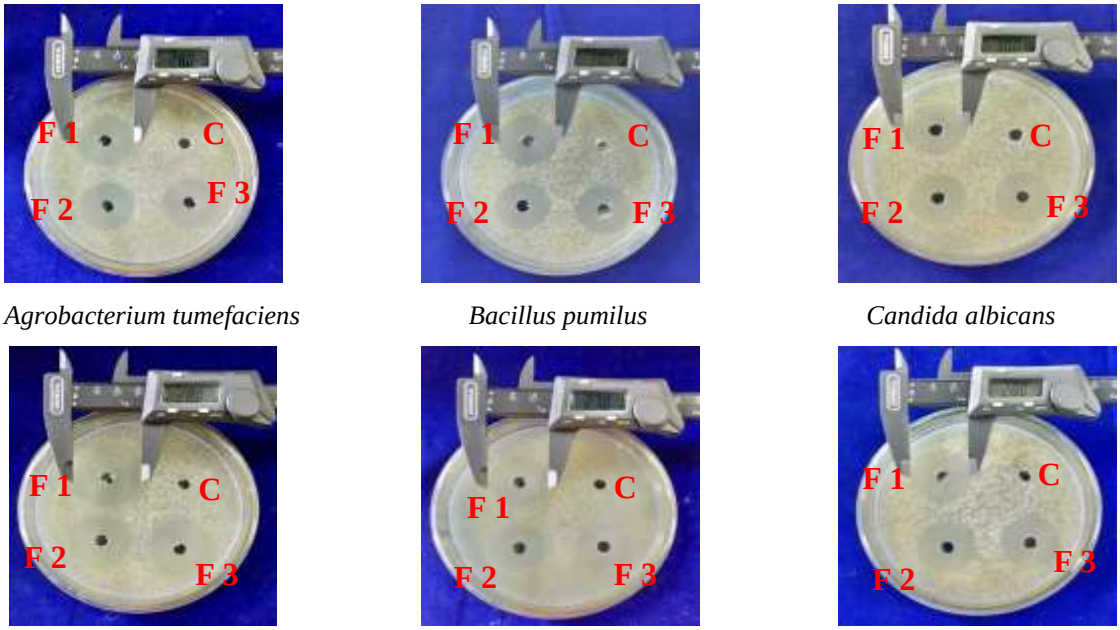
Fermentation studies of isolated fungus *Rhizoctonia* sp.

(i) Age of inoculum

In age of inoculum, fermentation 1 (One day old) showed the better activity than fermentation 2 and fermentation 3. It showed the highest activity on *Candida albicans, Micrococcus luteus* and *Staphylococcus aureus* at 1st and 2nd days. It also showed the result with high inhibition rate at 1st day on *Pseudomonas* sp., 4th and 5th days on *Agrobacterium tumifaciens* and 3rd day on *Bacillus pumilus.*

Table 1. Effect of age of inoculum on antimicrobial activity against six test organisms

Days / Test organisms	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Agrobacterium tumefaciens	17	22	27	30	30	29	28
Bacillus pumilus	23	31	32	30	30	30	30
Candida albicans	32	32	30	30	30	30	27
Micrococcus luteus	30	30	28	27	26	24	24
***Pseudomonas* sp.**	31	30	28	28	27	27	27
Staphylococcus aureus	30	30	29	28	28	28	27



Agrobacterium tumefaciens *Bacillus pumilus* *Candida albicans*

Micrococcus luteus *Pseudomonas sp.* *Staphylococcus aureus*

Figure 5. Effect of age of inoculum on antimicrobial activity against six test organisms

(F1=Fermentation 1; one day old, F2= Fermentation 2; two day old, F3=Fermentation 3; three day old)

(ii) Size of inoculum

In the study of size of inoculum optimization, among the fermentation (1.0%, 1.5%, 2%, 2.5% and 3.0%), 2% of fermentation was suitable to produce the bioactive compound. It showed the highest antimicrobial activity on *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas sp*., *Staphylococcus aureus*.

Table 2. Effect of size of inoculum on antimicrobial activity against six test organisms

Days / Test organisms	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Bacillus pumilus	29	27	27	27	24	22	19
Bacillus subtilis	29	29	28	27	27	26	26
Candida albicans	30	27	25	25	21	18	13
Micrococcus luteus	32	30	30	28	28	25	25
***Pseudomonas* sp.**	31	30	28	28	25	20	20
Staphylococcus aureus	29	28	28	28	26	26	25

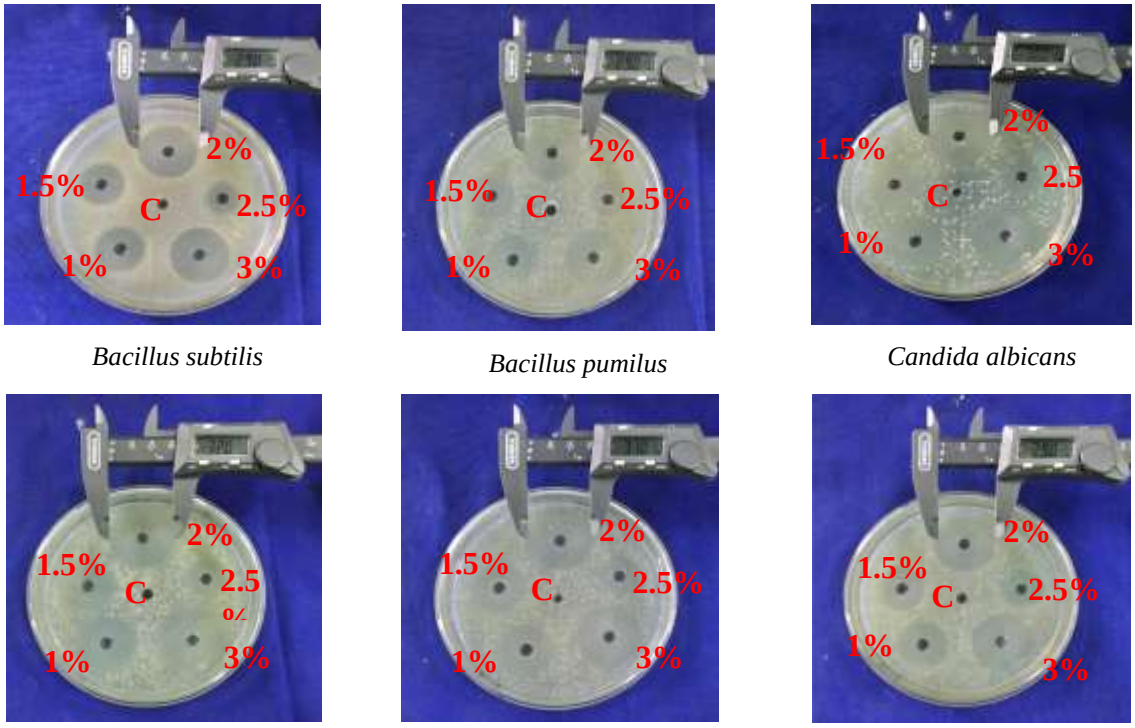


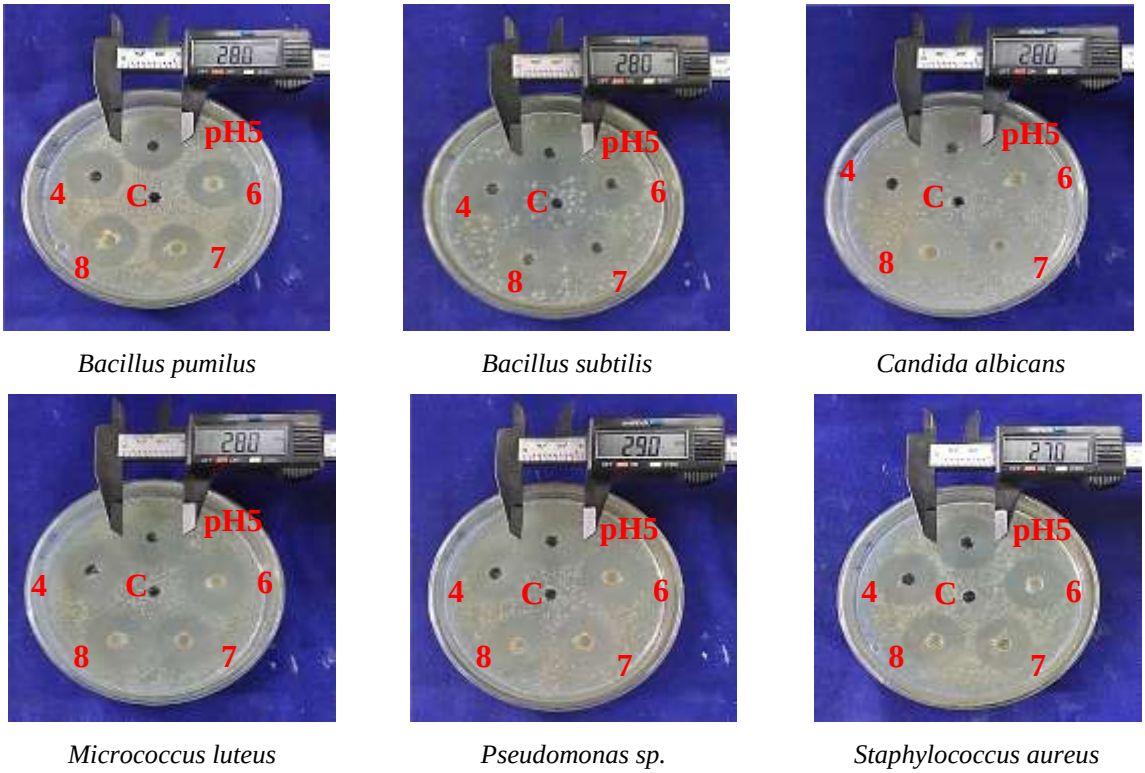
Figure 6. Effect of size of inoculum on antimicrobial activity against six text organisms

(iii) Effect of different pH

Among pH 4, 5, 6, 7 and 8 of fermented broth of *Rhizoctonia* sp., pH 5 was the best for extraction of the bioactive compounds from fermented broth according to the result of inhibitory zones against six test organisms. Fermentation with pH 5 showed the highest antimicrobial activity on *Bacillus pumilus*, *Candida albicans* and *Pseudomonas* sp. at 4th day. The best antimicrobial activity against *Bacillus subtilis* was found in pH 5 at 2nd, 3rd and 4th days. It also showed the result with high inhibition rate at 3rd and 4th days on *Micrococcus luteus* and at 4th and 5th days on *Staphylococcus aureus*.

Table 3. Effect of pH of inoculum on antimicrobial activity against six test organisms

Days / Test organisms	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Bacillus pumilus	25	25	27	28	25	25	25
Bacillus subtilis	26	28	28	28	27	26	26
Candida albicans	22	25	27	28	26	25	25
Micrococcus luteus	25	26	28	28	26	26	25
***Pseudomonas* sp.**	24	24	28	29	27	27	27
Staphylococcus aureus	23	24	26	27	27	25	25



Bacillus pumilus *Bacillus subtilis* *Candida albicans*

Micrococcus luteus *Pseudomonas sp.* *Staphylococcus aureus*

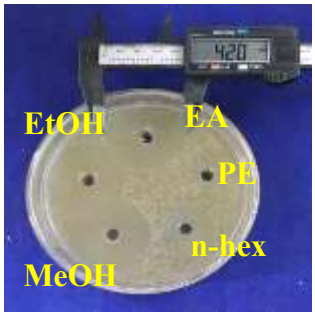
Figure 7. Effect of pH of inoculum on antimicrobial activity against six test organisms

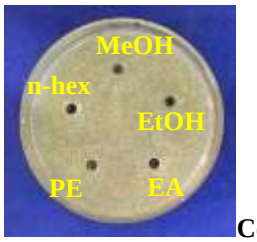
Extraction and antimicrobial activity of bioactive compounds from *Rhizoctonia* sp.

In the study of extraction of metabolites by using different solvent system, the extracts with methanol, ethanol, ethyl acetate, petroleum ether and n-hexane showed the highest activity on six test organisms: *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas* sp. and *Staphylococcus aureus*. Among them, ethyl acetate extract showed the best activity on six test organisms. Therefore, ethyl acetate was the best to produce bioactive metabolites for *Rhizoctonia* sp. (Ahmed, 2007)

Table 4. Antimicrobial activity of different extracted metabolites produced by *Rhizoctonia* sp.

Solvents / Test organisms	Methanol	Ethanol	Ethyl acetate	Petroleum ether	n-hexane
Bacillus pumilus	40.0	40.0	42.0	-	26.0
Bacillus subtilis	41.0	40.0	42.0	20.0	22.0
Candida albicans	40.0	41.0	43.0	26.0	20.0
Micrococcus luteus	40.0	40.0	40.0	30.0	26.0
***Pseudomonas* sp.**	40.0	40.0	44.0	-	24.0
Staphylococcus aureus	42.0	40.0	43.0	-	20.0



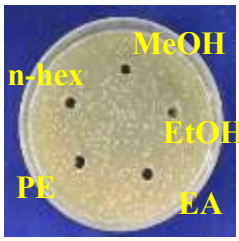


Control

Bacillus pumilus

Control

Bacillus subtilis

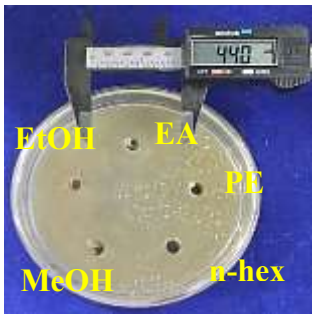


Control

Candida albicans

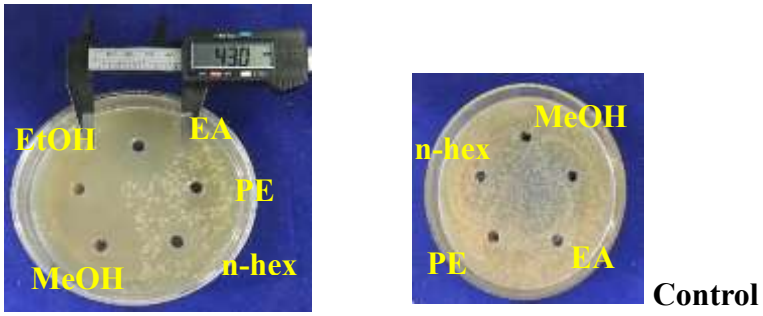
Control

Micrococcus luteus



Control

Pseudomonas sp.



Staphylococcus aureus

Figure 8. Effect of solvent on extraction of metabolites on six test organisms

Discussion and conclusions

In the present study, *Rhizoctonia* sp. was isolated from the leaves of *Combretum indicum* (L.) R. A. DeFilipps. In the study of fermentation, fermentation (one day old) of age of inoculum was the best for fermentation. In the size of inoculum optimization, fermentation (2%) showed the best antimicrobial activity on six test organisms and fermentation (pH 5) of pH utilization was the best to produce bioactive compounds. The antimicrobial activity of different extraction of the metabolites was carried out by using different solvent system: methanol, ethanol, ethyl acetate, petroleum ether and n-hexane. Among them, the extracts with ethyl acetate showed the high inhibition activity on *Bacillus subtilis*, *Bacillus pumilus*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas* sp. and *Staphylococcus aureus*.

In Myanmar, Aye Pe (2001), Ni Ni Win (2011), Kyawt Kyawt Aung (2014), Kyi Kyi Khine (2014), Phoo Wint Yee Thaw (2015), Soe Soe Yu Hnin (2018), Hnin Wit Mhon (2018), and Kay Thwe Lwin (2021) have isolated many endophytic fungal (including *Rhizoctonia* sp.) and bacterial strains from different plant species to isolate the bioactive compounds and had good antimicrobial activity on *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus*.

Yu Myat Maw (2016) found that *Rhizoctonia* sp. isolated from the leaves of *Ipomoea* sp. showed highest activity at pH 5 on *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Malassezia furfur*, *Salmonella typhi* and *Staphylococcus aureus*. Shah *et al*., (2017) proved that the antimicrobial activities of N-hexane, chloroform, ethanol and aqueous extracts of leaves, flowers, roots, and stems of *Quisqualis indica* against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bascillus subtilis*.

Dutta *et al*., (2019) studied that the aqueous and ethyl acetate extracts of leaves, stems and flowers of *Quisqualis indica* Linn. showed antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli.* Kay Tha Ye Soe Win (2015) investigated that the ethanol, ethyl acetate and aqueous extract of leaves of *Quisqualis indica* exhibited antibacterial activity against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Malassezia furfur*.

Kamber *et al*., (2014) found that the inhibitory effect of leaf and flower extracts of *Combretum indicum* were effective against *Staphylococcus aureus*. Zahidul *et al*., (2017) investigated that the antimicrobial activity of different extract (petroleum ether, methanol and aqueous) of *Quisqualis indica* leaves against different types of bacterial strains both gram positive and gram negative bacteria.

In conclusion, *Rhizoctonia* sp. indicated the highest antimicrobial activity on test organisms. The best fermentation condition was 2% of one day old seed culture and pH 5 to produce bioactive metabolites. *Rhizoctonia* sp. showed the highest antimicrobial activity on

different test organisms and the good fermentation results and indicated the highest inhibition activity in the extraction of secondary metabolites. Therefore, *Rhizoctonia* sp. should be chosen for further investigations.

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EFFECT OF DIFFERENT FERTILIZERS ON GERMINATION AND SEEDLING GROWTH OF *VICIA FABA* L. (FAVA BEAN) IN LABORATORY EXPERIMENT

Khaing Khaing[1], Khin Soe Aye[2], Shwe Sin Win[3]

Abstract

The present study was carried out to determine the effect of different fertilizers on germination and seedling growth of *Vicia faba* L. (Fava bean) at Department of Botany, Yadanabon University from August 2022 to January 2023. In laboratory experiment, the seeds of fava bean were treated with T_1 (Cowdung $4gl^{-1}$), T_2 (*Spirulina* $4gl^{-1}$), T_3 (*Nostoc* $4gl^{-1}$) and Control ($0gl^{-1}$) using Completely Randomized Design (CRD) with five replications. These results showed that, $4gl^{-1}$ of *Spirulina* treatment was the best germination percentage and maximum mean value of seedling growth of *Vicia faba* L. Therefore, the present research indicated that *Spirulina* ($4gl^{-1}$) suspension fertilizer has in effective role of *Vicia faba* L. in laboratory experiment.

Keywords: Fertilizer, Cowdung, *Spirulina, Nostoc*, Fava bean

Introduction

Vicia faba L. appears to have organized in north Africa and Southwest Asia and extensively cultivated elsewhere. Broad beans have a long traditional of cultivation in old world agriculture, being among the most ancient plants in cultivation and also among the easiest to grow. It is believed that along with lentils, peas and chickpeas, they became part of the eastern Mediterranean diet in around 6000 BC or earlier. They are still often grown as a cover crop to prevent erosion because they can over winter and as a legume, they can fix nitrogen in the soil. Myanmar is standing as a lead country of pulses production among ASEAN member countries and second largest exporter in the world. Major exportable cultivar of pulses is green gram, black gram, pigeon pea, soybean, fava bean, cowpea and kidney bean. Cultivation of pulses, with relatively less expenses in cost of cultivation and due to the increasing demand for domestic consumption and export, has increased substantially from 1.8 million acres in 1988-89 to 8 million acres in 2003-04 (MOAI, 2006).

Organic fertilizers, on the other hand, provide beneficial effects to the soil and also increase availability of nutrients, which helps to maintain the quality and yield of crops and are less expensive than inorganic fertilizers (Thy and Buntha, 2005). Organic fertilizers are not only the source of organic matter and nutrient, but also the booster of microbial population, physical, biological and chemical properties of the soil (Albiach *et al.,* 2000). The application of plant growth regulators with an effective nitrogen fixation plant potential is one of the effective ways of increasing the productivity of legumes. Microbial products from soil organisms are expected to make important contributions to production of food in coming years. Microalgae will also make important contributions to agriculture. In the near-term, the efficiency of cyanobacterial biofertilizer for rice will be improved (Kots and Mykhalkiv, 2001).

Cowdung has long been recognized as the most desirable animal manures because of its high nutrient and organic matter content. Addition of cow dung increases the organic carbon content of degraded soil which may lead to the increasing activity of beneficial soil microorganisms

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as well as the fertility status of soil by increasing the availability of nutrients for the plants from soil. Cow dung significantly increased the growth and yield of plants (Gudugi, 2013).

Just as *Nostoc* performs photosynthesis, it also carries out another unique activity often associated with leguminous plants. *Nostoc* takes nitrogen gas from the atmosphere and 'fixes' it into a form that plants and animals can use. Whereas legumes partner with rhizobia bacteria in the soil to fix nitrogen, *Nostoc* colonies produce specialized nitrogen-fixing cells called heterocysts (Jordan Franklin, *et al.* 2018). *Nostoc muscorum* are important for the nutrient cycling of carbon and nitrogen within the soil ecosystems in which they are found. The process of fixing atmospheric nitrogen contributes plant-available nitrogen to the soil, improving plant growth.

Spirulina grows naturally in alkaline lakes containing sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$), other minerals and a source of fixed nitrogen. These lakes are found on every continent including Antartica and very often lie near volcanoes within the caldera of the volcano. They are found also in deserts which receives mineralized water runoff periodically from mountains. There are many countries where *Spirulina* grow or has grown in the past. Few selected sites were Africa (Algeria, Chad, Sudan, Ethiopia, Kenya, Tanzania, Zambia, and Madagacar), Asia (India, Myanmar, Sri Lanka, Pakistan, Thailand, and Azerbaijan), South America (Peru, Mexico, Urguay, Bolivia), North America (Caliifornia, Haiti, Dominican Republic) and Eruope (France and Hungary) (Fox, 1996).

In Myanmar, *Spirulina* was found nonspecifically in 4-natural alkaline lakes. *Spirulina* is found all the year round although peak blooming season occurs in summer months. Abundance of *Spirulina* is especially noticeable in Twyan Taung Lake where surface blooms may form thick mats filling more than half the lake area in summer months (Min Thein, 1987). The effects of algal suspension with various concentrations were improved to the germination of some vegetables. Cherkezov and Christov (1996) reported that the lowest concentration of *Spirulina* suspension led to highest stimulation in carrot and onion seeds. Khin Pyone Lwin (1987) stated that Ye Kharr lakes are a series of volcanic creater lakes with bicarbonate and carbonate salts and it is a shallow lake with its salt content mainly as sulphate. The relative efficiency of 10-blue green algae in promoting the growth and yield of rice was found in Tun Chun (1982). Thet Naing Htwe (2008) studied the effect of *Spirulina* on the germination and growth of chick pea, soybean and butter bean. Khaing Khaing (2012) found that the effect of *Spirulina* on the germination, growth, yield and nutritive value of *Phaseolus lunatus* L. (lima bean). Shwe Yee Win Maung Maung (2014) had observed the effect of *Nostoc* on germination growth and yield of Yonbade. The aims and objectives of the present research were to investigate the best application rate of different fertilizers for fava bean and to analyze the effects of different fertilizers on percentage of germination, shoot and root length of fava bean.

Materials and Methods

Laboratory Experiment

In the present study, laboratory experiment was conducted at the Department of Botany, Yadanabon University, from August 2022 to January 2023. Cowdung biomass was collected from See Mee Htun Village, Amarapura Township. *Nostoc* biomass was bought from Zay Cho markets. *Spirulina* powder (Lod No. 391) was obtained from Myanmar Pharmaceutical Factory, Ye kharr, Sagaing Region. Morphological character of fava bean (*Vicia faba* L.) was obtained from Hundley and Chit Ko Ko (1961), Dassanayake (1980-2000) and *Spirulina* and *Nostoc* were from Prescott

(1962). The experimental design were Complete Randomized Design (CRD) with five replications. The plastic petridishes (16.2 cm in diameter and 5 cm high) were used in this study. Cowdung, *Spirulina* and *Nostoc* powder were weighted by using digital balance, according to w/v ratio and different fertilizers, such as (T_1, Cowdung; T_2, *Spirulina* and T_3, *Nostoc*) were conduced for this experiment.

Laboratory Experiment with Cowdung, *Spirulina* and *Nostoc* Suspension

In this experiment, Cowdung, *Spirulina* and *Nostoc* powder (4 g) were weight. It took about 24 hours. Therefore, different fertilizers of Cowdung, *Nostoc* and *Spirulina* suspension were obtained. Before treatment, the seeds were presoaked in water for 6 hrs., 12 hrs. and 18 hrs. The 18 hrs presoaked was the best for germination. Therefore, the seeds of *Vicia faba* L. were soaked in different fertilizers of Cowdung, *Spirulina* and *Nostoc* suspension for 18 hours and control was soaked in pure water. After treatment twenty-five fava bean seeds were placed on blotting paper in each plastic petridishes for each treatment fifteen ml of purified water were sprayed twice a day to keep the moisture. All of these plastic petridishes were kept under natural condition and room temperature ranging from 22-25°C. Five replications were made at the same condition. The germinating seeds were counted on the 3rd, 6th, 9th day. The length of the shoots and roots (cm) were measured on the 9th day after sowing by using ruler (Figure 1).

Germination Percentage (%)

Germination percentage of fava bean (*Vicia faba* L.) in laboratory experiment was counted. Germination rate of each treatment was expressed in percentage.

$$\text{Germination percent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Figure 1. Preparation of laboratory experiment

A. Fava bean seeds
B. Fava bean seeds soak in different fertilizers
C. Different fertilizers
D. Experimental layout in Complete Randomized Design (CRD)

Results

Morphological Character of Fava Bean

Scientific name : *Vicia faba* L.
English name : Broad bean: Horse bean; Fava bean
Local name : Pe let ma; Tayoke pe gyi
Family : Fabaceae

Annual erect herbs, up right, about 30 cm high, stem unbranched, quadrangular glabrous, pale green and soft. Leaves unipinnate compound, paripinnate, alternate, stipulate and petiolate; stipules triangular, glabrous, pale green and foliaceous, exstipellate; petiole slightly flattened, glabrous and pale green; leaflets 4-6 broadly elliptic to oval, pale green and fleshy. Inflorescences axillary short raceme, 1-6 flowered; peduncle flattened, caniculate above, glabrous and green. Flower bisexual, zygomorphic, white with dark purple markings; bracts minute and deciduous; pedicels flattened, softy tomentose and pale green. Calyx 5-lobed; tube oblique, glabrous and pale green, lobes acuminate, lower lobes longer than others, glabrous and pale green. Corolla papilionaceous exserted; standard elliptic, glabrous and white with brownish vein above, wings oblong with long claw, glabrous and white with dark-violet blotch; keels ovate with short claw, glabrous and white. Stamens 1+(9), diadelphous; staminal tube, glabrous and white; anther dithecous, uniform, minute, basified and longitudinally slits. Ovary oblong, tomentose and pale green; unilocular with 2-4 ovuled, marginal placentae; style terete, tomentose and pale green; stigma simple, glabrous. Pod oblong, tomentose, dehiscent, green, with 3-4 seeded. Seeds ovoid to oblong, smooth and green (Figure 2).

Figure 2. A. Habit of *Vicia faba* L. (Fava bean)
B. Inflorescence of *Vicia faba* L. (Fava bean)

Laboratory Experiment

In this study, *Vicia faba* L. seeds were with different fertilizers at Cowdung, *Spirulina* and *Nostoc* suspension ($4gl^{-1}$) were treated on the germination, shoot and root length were shown in Table (1) and Figure (1). In this result, the best germination percentage of *Vicia faba* L. 77.00 % was found in *Nostoc* suspension treatment $4gl^{-1}$ on 3 DAS.

The best mean germination percentage of Cowdung, *Spirulina*, *Nostoc* and control were 76.40 %, 85.10 %, 81.40 % and 70.60 % at 6 DAS. And then mean germination percentage of *Spirulina* suspension was 95.10 % and control was 78.20 % on 9 DAS (Table 1 and Figure 3 and 5).

The mean shoot length of *Vicia faba* L. (Tayoke pe gyi) seed was treated with different suspension (4 gl^{-1}) (Cowdung, *Spirulina, Nostoc*) on the highest mean shoot length were found 18.34 cm and that of control was 12.64 cm(Table 2 and Figure 4)

At different fertilizers suspension (4 gl^{-1}), (Cowdung, *Spirulina, Nostoc*) on the mean root lengths were found 9.17 cm, 11.85 cm, 10.66 cm and control was 8.02 cm respectively on 9 DAS. The germination percentage, the mean shoot and root length of *Vicia faba* L. (Tayoke pe gyi) at T_2 ($4gl^{-1}$) were higher than the other treatment and control in Table 2 and figure 4 and 6.

Table 1. Effect of different fiertilizers on germination percentage of *Vicia faba* L. (Laboratory experiment)

Control and Treatments	Mean germination % ± sd		
	3 DAS	6 DAS	9 DAS
Control	64.20 ± 8.180	70.60 ± 3.314	78.20 ± 3.472
T_1 (Cowdung)	69.70 ± 2.162	76.40 ± 5.316	82.20 ± 6.951
T_2 (Spirulina)	75.20 ± 5.334	85.10 ± 9.334	95.10 ± 9.284
T_3 (Nostoc)	77.00 ± 9.921	81.40 ± 9.637	87.00 ± 7.618

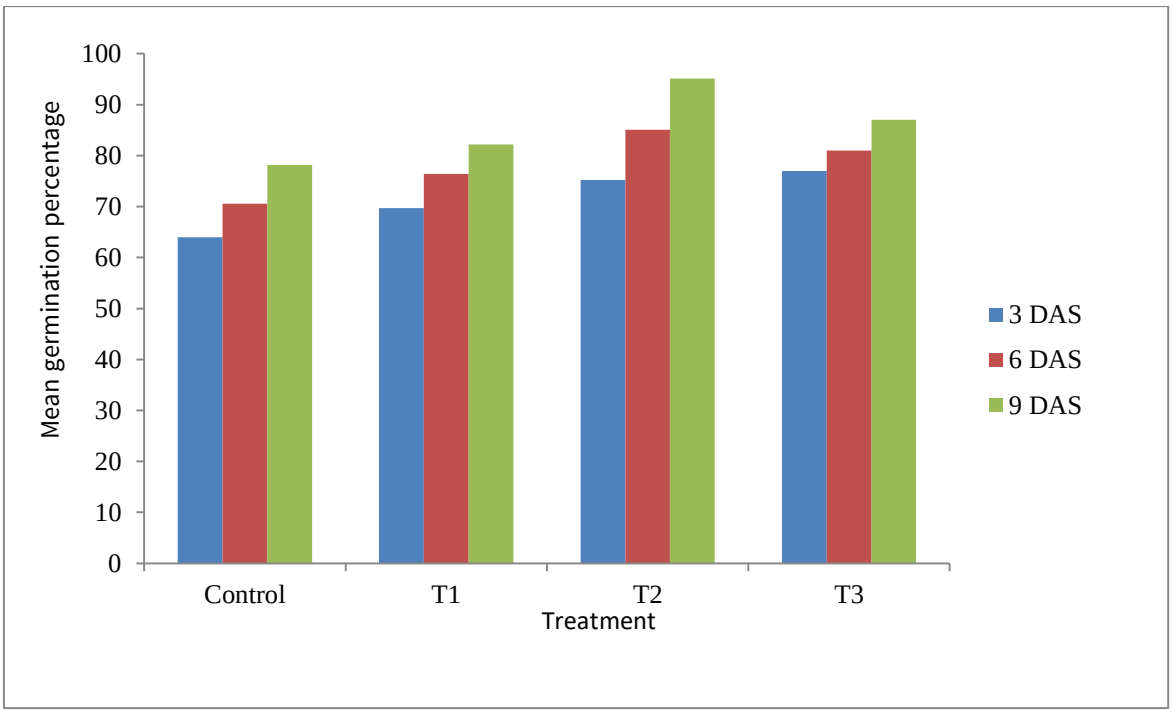

Figure 3. Comparison of different fertilizers on germination percentage of *Vicia faba* L. (Laboratory experiment)

Table 2. Effect of Cowdung(T_1), *Spirulina*(T_2) and *Nostoc*(T_3) suspension on mean shoot length and root length of *Vicia faba* L. (Laboratory experiment)

Nostoc Suspension Treatment (gl^{-1})	9 DAS	
	Mean shoot length (cm) ± sd	Mean root length (cm)± sd
Control	12.64 ± 0.442	8.02 ± 1.544
T_1	15.32 ± 0.915	9.17 ± 1.362
T_2	18.34 ± 0.718	11.85 ± 1.168
T_3	16.77 ± 0.754	10.66 ± 2.573

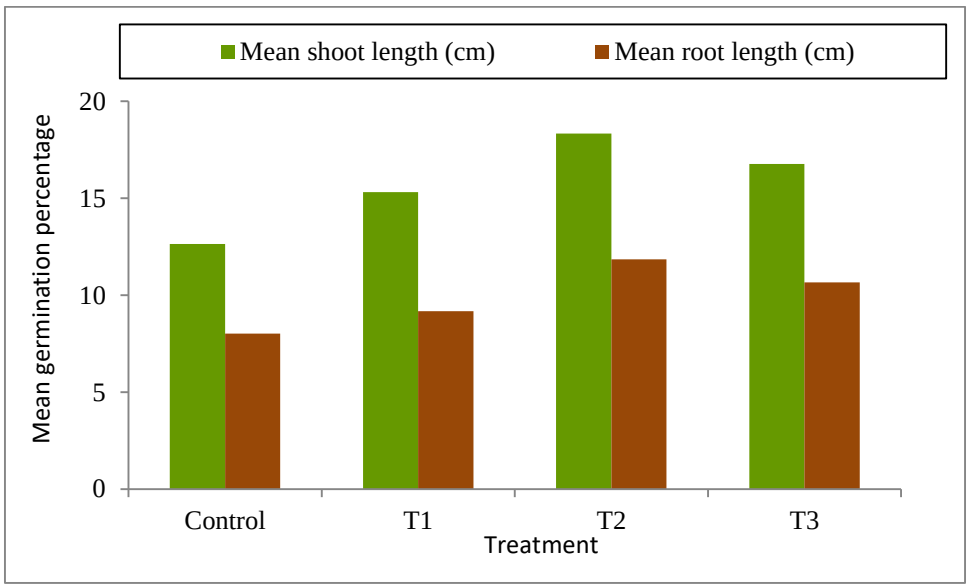

Figure 4. Comparison on different fertilizers on mean shoot length and root length of *Vicia faba* L. at 9 DAS (Laboratory experiment)

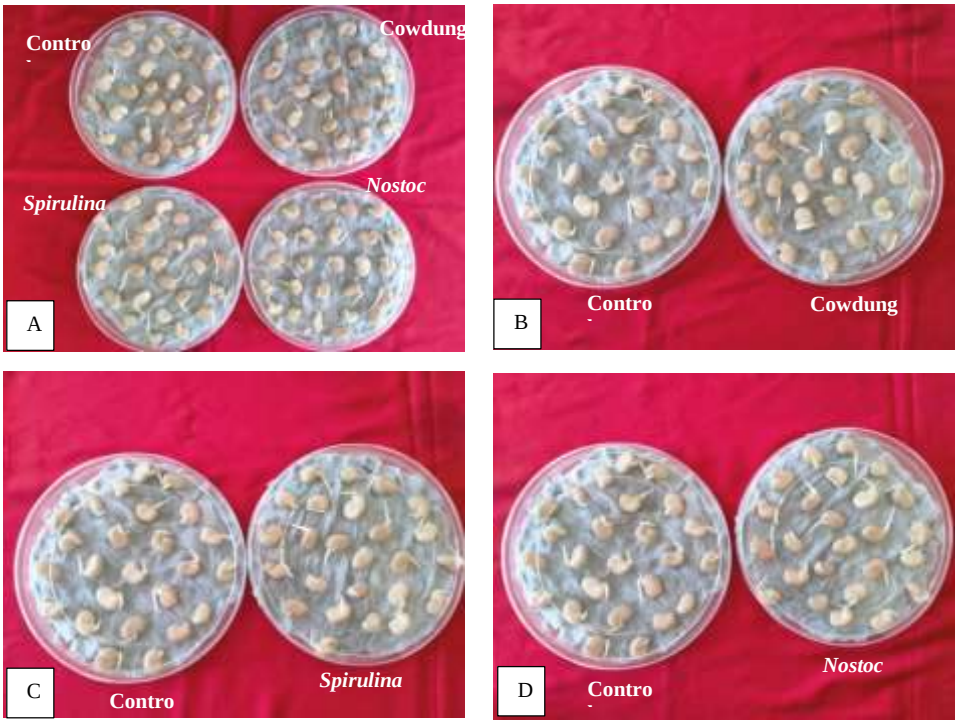

Figure 5. Effect of different fertilizers on germination percentage of fava bean at 3 DAS in laboratory experiment

A. Control and different fertilizers
B. Control and Cowdung suspension (4 gl-1)
C. Control and Spirulina suspension (4 gl-1)
D. Control and Nostoc suspension (4 gl-1)

Figure 6. Effect of control and different fertilizers on shoot and root length of fava bean at 9 DAS in laboratory experiment

A. Control and different fertilizers
B. Control and Cowdung suspension (4 gl-1)
C. Control and Spirulina suspension (4 gl-1)
D. Control and Nostoc suspension (4 gl-1)

Discussion and Conclusion

The effect of Cowdung, *Spirulina* and *Nostoc* on *Vicia faba* L. (Tayoke pe gyi) showed the best germination and seedling growth in 4 gl^{-1} treatment. Due to the comparative result of shoot and root length (cm) *Spirulina* suspension 4 gl^{-1} produced the highest efficient of shoot and root length. *Spirulina* suspension (4 gl^{-1}) was found 95.10 % germination and control was found 78.20 % germinated on 6 DAS.

The highest mean shoot length of *Vicia faba* L. treatment with *Spirulina* suspension (4 gl^{-1}) was 18.34 cm and that of control was 12.64 cm and the highest mean root length of *Spirulina* suspension (4 gl^{-1}) on fava bean was 11.85 cm but control was 8.02 cm on 9DAS. The present results showed that the application of *Spirulina* suspension on fava bean is beneficial.

Khaing Khaing (2012) observed that 2 gl^{-1} *Spirulina* suspension was the best germination and shoot and root length of *Phaseolus lunatus* (L.). In addition, Thida Aye (2011) reported that 3 gl^{-1} of *Nostoc* fertilizer suspension was the best for germination rate and shoot length of mustard. The present finding was agreed with Tin Tin Maw (2012) presented that *Spirulina* suspension (4 gl^{-1}) was the best germination and shoot and root length of *Vigna mungo* (L.) Hepper.

The present studies showed that, 4 gl^{-1} *Spirulina* suspension fertilizers had the best germination percentage, shoot length and root length of *Vicia faba* L. (fava bean) higher than control. The using of Cowdung, *Spirulina* and *Nostoc* suspension obtained enhancement in the germination and seedling growth of fava bean. However, *Spirulina* suspension was better than other fertilizers.

It can be concluded that *Spirulina* biomass could give a potential algal biofertilizer in germination and seedling growth of *Vicia faba* (Fava bean).

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ANTIMICROBIAL ACTIVITY OF ENDOPHYTES STRAIN ISOLATED FROM DIFFERENT PARTS OF *NYPA FRUTICANS* (WURMB) THUNB.

Yee Yee Nwe[1], Ei Pyae Myo Win[2], Aye Thet Pan Min[3], Hpue Mon Thant[4]

Abstract

In this study, the samples of Nypa fruticans (wurmb)Thunb. were collected from Toung-Goke Township in Rakhine State. Eleven entophytic fungi were isolated from different parts (leaves, fruit and inflorescences) of Nypa fruticans (wurmb) Thunb. on five different media. The morphological and microscopically characters of isolated strains were carried out at the Microbiology Laboratory, Department of Botany, Dagon University. The colony appearances of isolated fungal strains were circular, irregular and filamentous. The margins of isolated fungal strains were curled, filamentous, and entire. The elevation of isolated fungal strains was raised, flat and convex. The reverse colors of isolated fungal strains were milky white, pale yellow, white, pale orange, pale green, pale brown and orange. The cultural characters of isolated fungal strains were white, deep black, pale orange, pale green, whitish green, greenish white, creaming white. Eleven isolated fungal strains were identified into possible Aspergillus, Fusarium, Cladosporium, Rhizoctonia, Penicillium and Mucor species. Strains NF-1 and 6 were identified as Aspegillus specie. Strains NF-2,4 and 9 were identified as Fusarium species. Strains NF-3 may be as Mucor species. Strains NF-7 may be Cladosporium species. Strains NF-5 may be Rhizoctonia specie. Strains NF-8 was as Penicillium sp. The antimicrobial activity was tested against eight test organisms by using paper disc diffusion method and fermentation was carried out for 7 to 10 days. The fermented broths of all isolated strains NF-5 and 8 showed good antimicrobial activity on the nine day. Secondary metabolites of eleven active strains were extracted with ethyl acetate and butanol. Their antimicrobial activity was also examined on eight test organisms. The crude extracts of two strains (NF-5 and 8) were showed excellent antimicrobial activity on seven test organisms.

Keywords: Endophytic fungi, antimicrobial activity, Nypa fruticans (wurmb) Thunb.

Introduction

Endophytes are microorganisms living within the tissue of a plant as endosymbionts, without causing symptoms of disease. Some of them are mutualistic symbionts with advantageous effects on their host, such as improved growth or resistance against disease or environmental stress, and are being used as microbial inoculants.

At the most basic level, endophyte simply means the location of an organism, with “endo” means “inside” and “phyte” means “plants”.An endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant for at least part of its life cycle without causing apparent disease. Endophytic fungal may also be used as biopesticide to prevent pathogen in plants Forchetti *et al*., (2007). Endophytic fungal normally live on intercellular spaces that contain carbohydrates, amino acids, and high amounts of inorganic nutrient. One notable endophyte with medicinal advantage to humans was discovered by Strobel (2004).

Endophytic fugal have been increased worldwide attention because of the search for new or raw biologically active compounds. The ability of endophytes to produce a great range of secondary metabolites such as antibiotics, bioinsecticides, fine chemicals, and enzymes had

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indicated some convincing results in combating pathogens and even cancer cell-lines in animals and humans Atmosukarto et al., (2005).

Nipa palm (Nypa fruticans) is a mangrove palm that thrives naturally in river estuaries and brackish water environment in which salt and fresh water mingle (Nguyen, 2014). N. fruticans is the only species present in the genus Nypa. Endophytes colonizing inside plant tissues contribute to the fitness of host and in return, they gain nutrient and protection from the host. Endophytic fungal produce a wide range of phytohormones, such as auxins, cytokinins and the gibberellins. In addition, endophytic bacteria help to enhance the nutrient ability and fix nitrogen for plants. For instance, plant hormones made by endophytic microorganisms seem to be necessary for bryophyte development. Hornschuh, et al., (2002).

Tan and Zou (2001) reported that the bioactive natural products from endophytes are promising resources for medicine, agriculture and industry Different kinds of Alkaloids are gave to plant by endophytes. Some of these alkaloids raise plants' resistance to environmental stress, and some are growth-promoting compounds.

Although many endophytic strains have been found, commercial endophytic inoculants for agriculture wider adoption of endophyte inoculants has been used by cheap synthetic fertilizers, variable responses by the endophyte depending on host genotype and environment, competition from endogenous microbes, host genotype specificity, poor establishment, and persistence. Endophytes possess significant potential to improve agriculture, but this will require further discovery of novel endophytes, genetic improvement of endophytes and their hosts, standardized testing, and formulation. Novel genes and metabolites from endophytes represent an additional largely primary resource for future agricultural biotechnology. (Website-2)

Mangroves are aggregations of ecological community with a competency to grow in extreme environmental conditions. Endophytic microorganisms alive within the plants in a symbiotic relationship where both the plants and the endophytes experience benefits.

In this investigation, altogether eleven endophytic fungal were isolated into pure culture by using five different media. Eleven isolated fungal strains are subjected into the examination microscopical character, to identify the possible genera of isolated fungal strains, and to examine antimicrobial activity of isolated fungal strains.

Materials and methods

Collection of Plant Materials

In the present research, the healthy plant parts (leaves, fruits and inflorescences of Nypa fruticans (wurmb) Thunb. were collected from Taunggoke Township in Rakhine state. The collected specimens were identified with the help of available literature. (Hundely and Chit Ko Ko, 1998; Kress, et al., 2003). Plant identification was checked with the international plant name index and world Checklist of Selected Plant Families (2022). The samples were taken and the experiments were carried out from March 2022. Samples were placed in clean plastic bags, brought to the laboratory and used for further experimental purpose.

Media for isolating endophytic Fungi

The choice of the growth medium is crucial as it directly affects the number and type of endophytic fungi that can be isolated from the leave, fruit and inflorescence 1. Nutrient agar Medium (Atlas,1993) 2. Potato Dextro Agar Medium 3. Sucrose, Yeast extract and Agar medium

4. Glucose, Yeast extract and Agar Medium 5. Lactose, Yeast extract and Agar were used for the isolation of endophytic fungi. Since there is no component in media which can suppress the growth of endophytic bacteria, so the media used for the isolation of endophytic fungi were supplemented with antibacterial agent, chloramphenicol at a concentration of 0.001 mg of each to suppress bacterial growth.

Isolation of Entophytic Fungal Strains from

Isolation of endophytic fungal strains was carried out by the scheme (Lee and Kim 2002)

Morphological Characters of Isolated Fungal Strains

The morphological and colonial characters such as colony appearance, margin, surface and reverse colours, and elevation of all isolated strains were recorded as revealed in the reference of Dubey and Maheswari, (2014).

Microscopical Characters of Isolated Fungal Strains

The microscopical characters of all isolated strains (NF-1 to NF-11) were carried out under light microscope with high magnification at Department of Botany, Dagon University. The main characters of hyphae, mycelium, sporangiophores, spore, color formation on upper as well as lower surface were comparatively studied. These are compared to those of fungi with available literatures such as Barnett, (1969).

Test Organisms

All endophytic fungi isolates were screened for antimicrobial activities. The indicator microbe included Agrobacterium tumefaciens, Bacillus subtilis, Bacillus pumilus, Candida albicans, Escherichia coli, Malassezia furfur, Pseudomonas sp., Xanthomonous sp., and Staphylococcus aureus.

Fermentation of isolated Fungal and antimicrobial activity test of liquid fermented

Nutrient, Potato Dextore, Sucrose/Yeast, Glucose/Yeast, Lactose/Yeast media were also used in this test. Each medium was heated to boiling and sterilized by autoclaving at 121 °C for 15 minutes. Each fungal isolate that has been purified in previous experiments was inoculated on the liquid medium and then fermented. The fermentation process was done in a 250 ml Erlenmeyer flask. Incubation is for 72-120 hours at a temperature of 25-27 °C on a rotary shaker incubator for fungus at a speed of 90 rpm. The ferment broth was tested its antimicrobial activity against microbes using paper disc method. Paper discs dipped in the ferment broth and planted in the medium NA containing eight test organisms and incubated at a temperature of 37 °C for 18-24 hours. Barriers to growth were observed and measured in diameter by using digital caliper.

Extraction and isolation of crude ethyl acetate extracts and butanol extract from fungal fermentation broths

Each of the pure cultures was re-cultivated on the selected media at 28°C for 5 to 7 days. Three pieces (0.5×0.5 cm^2) of mycelia agar plugs were inoculated into 500 ml Erlenmeyer flasks containing 300 ml the selective broth (NB, PDB, YSB, YGB and YLB) and incubated at room temperature for ten days under stirring conditions. The active constituents were extracted from both filtrate and mycelia after separation from the fermented culture broth. One part of the filtrate was extracted two times with equal volume of n-butanol and another part with ethyl acetate. The mycelia were extracted with only acetone. All extract was concentrated by removing the solvents

under reduced pressure at 35-40°Cat oven. The extract residue was stored at 4°C as stock solution for antimicrobial bioassay. The crude ethyl acetate and butanol extracts of the NF-1,2,3,4,5,7,8,9,10 and 11 endophytic fungi were tested for their antibacterial activity against eight test organisms.

Results

Morphological Characteristics of Collected Plant Samples

Scientific Name - Nypa fruticans (wurmb) Thunb.

Myanmar Name - Ye-Ohn-Thee

Family - Arecaceae

Habit Inflorescence Leaves Fruits

Figure 1. Habit of the Plants, Leaves, Fruits and Inflorescence

Screening of Endophytic Fungal Strains

In the present works, twelve isolated fungi designate as NF-1 to NF-11 were maintained into the pure culture for further studies. Designated fungal strains NF-1 and 7 were obtained from inflorescences, NF-3, 4, 8 and 9 were from leaves and NF-2,5,6,10 and 11 were isolated from fruits.

Table 1. Isolation of Eleven Endophytic Fungal Strains

Isolated Strains	Sources
NF-1 and NF-7	Inflorescences
NF-3, 4, 8 and 9	Leaves
NF-2, 5, 6, 10 and 11	Fruits

Table 2. Colony and morphological characterization of isolated fungi

Isolated strain	Surface color	Reverse color	Colony apperance	Margin	Elevation
NF-1	Gray	White gray	filamentous	Filiform	Raise
NF-2	White	White	Circular	Curled	Raise
NF-3	white	Light yellow	irregular	curled	Raise
NF-4	White	White	Circular	Curled	Raise
NF-5	white	Light yellow	Circular	Curled	Convex
NF-6	gray	White grey	Circular	Curled	Raise

Isolated strain	Surface color	Reverse color	Colony apperance	Margin	Elevation
NF-7	Light yellow	Light yellow	Circular	Curled	Raise
NF-8	Light grey	Light yellow	Irregular	undulate	Raise
NF-9	white	Light Yellow	rhizoid	Curled	Raise
NF-10	White	white	filamentous	Filamentous	Convex
NF-11	White	White	Circular	Filamentous	Raise

Possible Genus of Isolated Fungi According to Imperfect Fungi (Barnet ,1969)

Morphological and Microscopical character of strain NF-1

The surface color of NF-1 was pale green. Its reverse color was pale green as shown in Figure 2. Endophytic fungal strain from Inflorescences Nypa fruticans **(**Inflorescences) on NA medium.

Division - Ascomycota
Class - Ascomycetes
Order - Aspergillales
Family - Aspergillaceae
Genus - ***Aspergillus***

Surface View Reverse View Microscopical character

Figure 2. Morphological and Microscopical character of isolated Fungi (NF-1)

Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia (phialospores) hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid, in dry basipetal chains. This strain may be identified as Aspergillus sp. as shown in Figure 2.

Morphological and Microscopical character of strain NF-2

The surface color of NF-2 was white. Its reverse color was white as shown in Figure 3. Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on NA medium.

Division - Ascomycota
Class - Sordariomycetes
Order - Hypocreales
Family - Nectriaceae
Genus - ***Fusarium***

Figure 3. Morphological and Microscopical character of isolated Fungi. (NF-2)

Mycelium extensive and cotton-like in culture, often with some tinge of pink, in the mycelium on medium; conidiophores variable, branched irregularly, single or grouped into sporodochia; conidia (phialospore) hyaline, variable, principally of two kinds; parasitic on higher plants. These strains may be identified as Fusarium species as shown in Figure 3.

Morphological and Microscopical character of strain NF-3

The surface color of NF-3 was pale orange. Its reverse color was pale orange as shown in Figure 4 Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YS medium.

Division - Mucoromycota
Class - Zygomycetes
Order - Mucorales
Family - Mucoraceae
Genus - ***Mucor***

Figure 4. Morphological and Microscopical character of isolated Fungi (NF-3)

Typically exhibits rapid growth, producing globose sporangia on sporangiophores that are entire solitary or branched. This strains may be identified as Mucor specie as shown in Figure (4)

Morphological and Microscopical character of strain NF-4

The surface color of NF-4 was white. Its reverse color was white as shown in Figure (5). Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YS medium.

Division - Ascomycota
Class - Sordariomycetes
Order - Hypocreales
Family - Nectriaceae
Genus - ***Fusarium***

Figure 5. Morphological and Microscopical character of isolated Fungi (NF-4)

Mycelium extensive and cotton-like in culture, often with some tinge of pink, in the mycelium on medium; conidiophores variable, branched irregularly, single or grouped into sporodochia; conidia (phialospore) hyaline, variable, principally of two kinds; parasitic on higher plants. These strains may be identified as Fusarium species as shown in Figure (5).

Morphological and Microscopical character of strain NF-5

The surface color of NF-5 was pale green. Its reverse color was orange as shown in Figure 6. Endophytic fungal strain from Inflorescences Nypa fruticans **(**fruits) on YS medium.

Division - Basidiomycota
Class - Agaricomycetes
Order - Cantharellales

Family - Ceratobasidiaceae

Genus - ***Rhizoctonia***

Figure 6. Morphological and Microscopical character of isolated Fungi (NF-5)

Asexual fruit bodies and spores lacking; sclerotia brown or black, variable in form, frequently small and loosely formed, among and connected by mycelia thread; hyphae with long cell, septa of branch set off form main hypha. This strain may be identified as Rhizoctonia s sp. as shown in Figure (7).

Morphological and Microscopical character of strain NF-6

The surface color of NF-6 was deep black. Its reverse color was pale brown as shown in Figure 7. Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on YS medium.

Division - Ascomycota

Class - Ascomycetes

Order - Aspergillales

Family - Aspergillaceae

Genus - ***Aspergillus***

Figure 7. Morphological and Microscopical character of isolated Fungi (NF-6)

Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia (phialospores) hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid, in dry basipetal chains. This strain may be identified as Aspergillus sp. as shown in Figure (7).

Morphological and Microscopical character of strain NF-7

The surface color of NF-7 was central pale green, margin white. Its reverse color was pale green as shown in Figure 8. Endophytic fungal strain from Inflorescences Nypa fruticans **(**inflorescence) on YG medium.

Division - Basidiomycota

Class - Dothideomycetes

Order - Capnodiales

Family - Davidiellaceae

Genus - ***Cladosporium***

Figure 8. Morphological and Microscopical character of isolated Fungi (NF-7)

Conidiophores tall, branched variously near the apex, clustered; conidia light, 1 or 2 celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon – shaped; often in simple or branched acropetalous chains; saprophytic. This strain may be identified as Cladosporium species as shown in Figure (8).

Morphological and Microscopical character of strain NF-8

The surface color of NF-8 was central green, margin white. Its reverse color was milky white as shown in Figure 9. Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YG medium.

Division	-	Ascomycota
Class	-	Eurotiomycetes
Order	-	Eurotiales
Family	-	Trichocomaceae
Genus	-	***Penicillium***

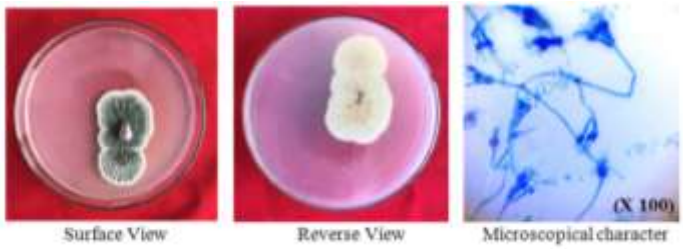

Figure 9. Morphological and Microscopical character of isolated Fungi (NF-8)

Conidiophores arising from the mycelium singly, branched near the apex to form a brush-like, conidia-bearing apparatus; conidia hyaline or brightly colored in mass, 1-celled, mostly globose produced basipetally.

Morphological and Microscopical character of strain NF-9

The surface color of NF-9 was central milky, margin white. Its reverse color was pale yellow as shown in Figure (10). Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YG medium.

Division	-	Ascomycota
Class	-	Sordariomycetes
Order	-	Hypocreales
Family	-	Nectriaceae
Genus	-	***Fusarium***

Figure 10. Morphological and Microscopical character of isolated Fungi (NF-9)

Mycelium extensive and cotton-like in culture, often with some tinge of pink, in the mycelium on medium; conidiophores variable, branched irregularly, single or grouped into sporodochia; conidia (phialospore) hyaline, variable, principally of two kinds; parasitic on higher plants. These strains may be identified as Fusarium species as shown in Figure 10.

Morphological and Microscopical character of strain NF-10

The surface color of NF-10 was white. Its reverse color was pale orange as shown in Figure 11. It was not identified as shown in Figure (11). Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on YL medium.

Figure 11. Morphological and Microscopical character of isolated Fungi (NF-10)

Morphological and Microscopical character of strain NF-11

The surface color of NF-11 was white. Its reverse color was white as shown in Figure 12. Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on YL medium.

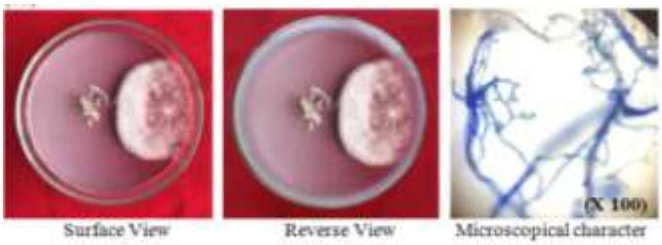

Figure 12. Morphological and Microscopical character of isolated Fungi (NF-11)

It was not identified as shown in Figure 12.

Microscopical Characters of Isolated Fungi

The microscopical characters of isolated entophytic fungi are the possible genera of Aspegillus, Cladosporium, Fusarium, Mucor, Penicillium and Rhizoctonia. These strains were identified as strains NF-1 and 6 as Aspegillus species, strain NF-7 as Cladosporium species, strains NF-2,4 and 9 as Fusarium species, strain NF-3 as Mucor, strain NF-8 as Penicillium, strain NF-5 as Rhizoctonia, strains NF-10 and 11 were being unable to identify their genus level. So, they were assumed as unidentified isolates.

Antimicrobial Activity of Fermented Broths of Isolated Entophytic Strains

At 8^{th} day fermentation strain NF-2 inhibited high activity against eight test organisms except Bacillus subtilis. Strain NF-5 stated moderate activity against on Agrobacterium tumefaciens, Bacillus subtilis, Malassezia furfur, Staphylococcus aureus, Xanthomous sp. Strain NF-8 state activity against all test organisms except Bacillus pumilus, Candida albicans, Escherichia coli. Strains NF-1, 4, 10 and NF-11 indicated activity Agrobacterium tumefaciens, Bacillus subtilis, Staphylococcus aureus, Xanthomous sp. as shown in Figure 17.

Figure 13. Antimicrobial Activity of Fermented Broths of Eight Day Old Culture

Antimicrobial activity of fermented broths of isolated entophytic strains

At 9th day fermentation, strain NF-8 indicated very high activity against on all test organism, strain NF-5 inhabited moderate activity against except Staphylococcus aureus all test organisms. Strain NF-8 expressed activity against, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Staphylococcus aureus, Xanthomous sp. as shown in Fig. (20).

Figure 14. Antimicrobial Activity of Fermented Broths of Nine Day Old Cult

Antimicrobial Activity of Crude Extracts of Entophytic Fungal Strains

Screening of endophytic fungi from different parts of Nypa fruticans (Wurmb) Thunb. was done by using five different media as basal isolation medium. To isolate the fungi, the chloramphenicol (0.001 mg) was put into five different media for the fungi only. Eleven isolated fungi were obtained in the present screening. The isolated fungi from different parts of Nypa fruticans (Wurmb) were designated codes NF-1 to 11. The colonies of isolated strains were shown in Table 1. The photomicrograph of morphology, mycelium and spore were displayed in Figure 3 and 13. The results of morphological cultural and biochemical test were shown in Table 3 to 7. The antimicrobial activity of all isolated against eight test organisms and indicated by size of clear zone was shown in Table 4 to 7.

The second parts of the present work mainly depend on extraction of metabolites by using ethyl acetate and butanol. According to the results of antimicrobial activity the isolated fungal strains NF-1,2,3,4,5,7,8,9,10 and NF-11 were selectively used in extraction of metabolites. The isolated fungi were grown in the 200 ml of five different broths. In the case of isolated fungi, the fermentation periods were checked 7,8,9 and 10 days and the best antimicrobial activity was detected in the 9 days. After each fermentation periods, the fermented broths were extracted with 100 ml each of ethyl-acetate and butanol in different separating funnels. The resulted crude extracts were subjected in the antimicrobial test and sizes of clear zone were tabulated in Table 4 and Figure 16. In the case of fungi, not only crude extract from fermented broth but also the mycelium biomass crude extract, by acetone was also applied in the clear zone tests. In the metabolite extraction by fungi, the isolate NF-5 and 8 which may be Rhizoctonia spp. Penicillin sp. were found to give best antibacterial activity. It was recorded that the ethyl acetate NF-5 (Rhizoctonia spp.) provide 25mm clear zone against Candida albicans, 20.0 mm against Bacillus pumilus, Bacillus subtilis, Malassezia furfur and Staphylococcus aureus. Similarly, ethyl acetate extract of NF-8 (Penicillin sp.) also showed 30 mm clear zone on Agrobacterium tumefaciens and 25.0 mm against on Candida albicans. The butanol extracts of NF-5 (Aspergillus) also provided 30 mm high antimicrobial activity on Bacillus pumilus and Bacillus subtilis such as 26 mm, 25 mm, 25 mm

and 22 mm against activity Staphylococcus aureus, Agrobacterium tumefaciens, Candida albicans and Malassezia furfur.

The mycelia acetone extract of NF-5 (Rhizoctonia sp.) also show 24 mm, 23 mm, 22 mm and 20 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur, Staphylococcus aureus, Xanthomous spp. The mycelia acetone extract of NF-8 (Penicillin.) also show against activity 27 mm, 25 mm, 26 mm and 30 mm, 20 mm and 20 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur, Staphylococcus aureus, Xanthomous spp. The results were shown in Table (8). and Figure 22 to 23.

Figure 15. Antimicrobial Activity of Nine day Crude Extract of Two Entophytic Fungi NF-5 and NF-8 Against Seven Test Organism

In the metabolite extraction by fungi, the isolate NF-9,10, 11 and 4 which may be Fusarium spp. unknown, unknown, Fusarium sp. were found to give best antibacterial activity. It was recorded that the ethyl acetate NF-9 (Fusarium spp.) provide 30 mm clear zone against Candida albicans, 27.0 mm against Malassezia furfur, 26 mm on Bacillus pumilus, 25 mm on Agrobacterium tumefaciens. Similarly, ethyl acetate extract of NF-10 showed 30, 16, 20,22 and 20 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans and Xanthomous sp. The ethyl acetate extract of NF-11indicated activity against 25,15,18,16 mm on Agrobacterium tumefaciens, Bacillus pumilus, Candida albicans and Xanthomous sp. The ethyl acetate extract of NF-4 indicated activity against on Agrobacterium tumefaciens and Candida albicans. The butanol extract of NF-9 was showed 23, 20,20,20,16 and 30 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous sp. The butanol extract of NF-10 was indicated activity against 25, 20, 23, 22,20,25 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The butanol extract of NF-11 was indicated activity against 25, 26,22,25,15 and 26 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The butanol extract of NF-4 was indicated activity against 20,28, and 20 Agrobacterium tumefaciens, Bacillus pumilus and Xanthomous spp.

The mycelia acetone extract of NF-9 also shows against activity 21, 20,22,20,20 and 21 Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The mycelia acetone extract of NF-10 also shows against activity 20,19,16,16,17 and 25 Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The results were shown in Table 9 and Figure 24 and 25.

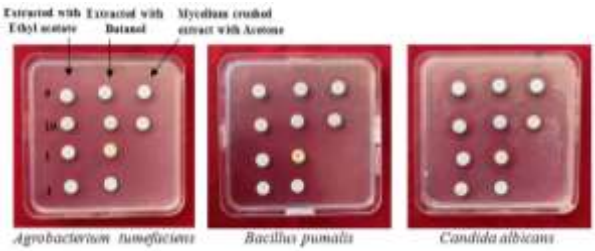

Figure 16. Antimicrobial Activity of Nine day Crude Extract of Four Entophytic Fungi NF 9,10, 11 and NF-4 Against Six Test Organisms

In the metabolite extraction by fungi, the isolate NF-2, 7,1 and 3 which may be Fusarium sp., Cladosporiumr spp., Aspegillus spp. and Mucor sp. were found to give best antibacterial activity.

It was recorded that the ethyl acetate NF-2 was indicated activity against 25,25 and 25 mm on Candida albicans, Malassezia furfur, and Xanthomous sp. The ethyl acetate NF-7 was indicated activity against 23, 23 and 25 mm on Candida albicans, Malassezia furfur and Xanthomous sp.

The ethyl acetate NF-1 was exhibited activity against 25,15 and 25mm on Candida albicans, Malassezia furfur and Xanthomous sp. The butanol extracted NF-2 was displayed activity against 20,26 and 30mm on Candida albicans, Malassezia furfur and Xanthomous sp. The butanol extracted NF-7 was indicated activity against 20, 25 and 22 on Candida albicans, Malassezia furfur, and Xanthomous sp. The butanol extracted NF-1 was indicated activity against 20,15 and 22 on Candida albicans, Malassezia furfur and Xanthomous sp.

The butanol extracted NF-3 was showed activity against 18, 25 and 22 on Candida albicans, Malassezia furfur and Xanthomous sp. The mycelium acetone extracted NF-7 was stated activity against 15, 20 and 25 on Candida albicans, Malassezia furfur and Xanthomous spp. The mycelium acetone extracted NF-1 was point to activity against 18,20 and 20 on Candida albicans, Malassezia furfur, Xanthomous spp.

The mycelium acetone extracted NF-3 was demonstrated activity against 16,25 and 15 on Candida albicans, Malassezia furfur and Xanthomous sp. as shown in Table (10) and Fig. (26 and 27).

Figure 17. Antimicrobial Activity of Nine Day Crude Extract of Four Entophytic Fungi NF-2,7,1 and NF-3 Against Two Test Organisms

Discussion and conclusion

The present investigation was 11 endophytes fungal from different parts of Nypa fruticans (wurmb) Thunb. The colony appearances of isolated fungal strains were circular, irregular and filamentous. These results were in agreement with Dubey and Maheswari, (2014). The margins of isolated fungal strains were curled, filamentous and entire. The elevation of isolated fungal strains was raised, flat and convex. These findings were in agreement with Dubey and Maheswari, (2014). The reverse colors of isolated fungal strains were white, light yellow, yellowish white, yellow,

whitish grey, brownish white and brownish gray. These results were in agreement with Davise, (1995).

In the present research among 11 isolated strains, the colony, conidiophore and conidia characteristic NF-7 was very closely to those of Cladosporium genus. Therefore, these strains were identified as possible genus Cladosporium. Zhang et al. (2014) concluded that five new compounds were isolated from the mangrove-derived endophytic fungus C.cladosporioides MA-299.

In this study Under the microscope, conidiophores arising from the mycelium singly in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid NF-6 were very closely to those of Aspergillus genus. Therefore, these strains were identified as possible genus Aspergillus. This strain may be identified as Aspergillus. Peng et al. (2022) and Gao et al. (2013) were stated that mangrove endophytic fungi of the genus Aspergillus have been reported to afford a wide range of secondary metabolites.

Chung et al., (2013) were reported that mangrove-derived endophytic fungi are rich sources for the production of structurally diverse and fascinating natural products with a variety of biological properties.

In this investigation, NF-3 was exhibits rapid growth, producing globose sporangia on sporangiophores that are entire solitary or branched. These characters were similar to Mucor. Shu-Shan Gao et al. (2016) were explained that Rhizovarins A–F, Indole-Diterpenes from the mangrove-derived endophytic fungus Mucor irregularis QEN-189.

Conidiophores are upright, simple, terminating in a globose, bearing phialides at the apex or radiading from the entire surface; globose often variously in mass, produce basipetally. These characters were very similar to those observed in NF8. Therefore, these strains may be identified as Penicillin. Hongju et al. (2016) were reported that polyketides with immunosuppressive activities from mangrove endophytic fungus Penicillin sp. ZJ-SY_2**.**

In the present study, Mycelium extensive and cotton-like in culture, often with some tinge of yellow in the mycelium on medium; conidiophores variable, slender, and simple, or stout, branched irregularly, single; conidia hyaline, variable, oblong, borne singly; oblong or slightly curved; parasitic on higher plants. These strains may be identified as Fusarium species. Zhongiing et al., (2011) were concluded that a new isoflavone from the mangrove endophytic fungus Fusarium sp. (ZZF60). Tauhidur et.al (2020) reported that Screening of endophytic fungi from mangrove plant with inhibitory activities against pathogenic bacteria or fungi might lead to potential novel natural products with higher antimicrobial activity. Similar to our study, Buatong et al. (2011) reported the extraction of fungal broth by ethyl acetate while the mycelia were extracted using methanol, hexane, acetone and ethyl acetate in sequence, respectively.

Ethyl acetate is widely used in extraction of endophytic fungal cultures (Bhardwaj et al. 2015) followed by methanol. As a solvent, ethyl acetate solvent possesses medium polarity so that it has the ability to dissolve both polar and non-polar active compounds and methanol solvent being a polar solvent can dissolve almost all organic compounds, even polar, semi polar and non-polar (Rahmawati et al. 2018). Li et al. (2017) stated that Identification and antifungal activity of compounds from the mangrove endophytic fungus Aspergillus clavatus R7.

In this study, we successfully isolated and identified 11 different species of endophytic fungi belonging to 5 different genera. All the isolates are moderately active against tested microorganisms. However, further studies could be initiated with Penicillum sp., Rhizoctonic sp., Cladosproiwm sp. and Fusarium spp. for potential bioactive compounds as our results showed promising activities with these endophytic fungi. The findings of this study also suggest that endophytes from mangrove ecosystem might be an attractive source for bio-prospecting of new antimicrobial compounds.

In our study, endophytic fungi isolate from eleven mangrove species were found to be diverse. In the present research among 11 isolated strains, the colony, conidiophore and conidia characteristic NF-7 was very closely to those of Cladosporium genus. Therefore, these strains were identified as possible genus Cladosporium.

In this study Under the microscope, conidiophores arising from the mycelium singly in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid NF 6 were very closely to those of Aspergillus genus. Therefore, these strains were identified as possible genus Aspergillus. This strain may be identified as Aspergillus.

In this investigation, NF-3 was exhibits rapid growth, producing globose sporangia on sporangiophores that are entire solitary or branched. These characters were similar to Mucor. Conidiophores are upright, simple, terminating in a globose, bearing phialides at the apex or radiading from the entire surface; globose often variously in mass, produce basipetally. These characters were very similar to those observed in NF8. Therefore, these strains may be identified as Penicillin.

In the present study, Mycelium extensive and cotton-like in culture, often with some tinge of yellow in the mycelium on medium; conidiophores variable, slender, and simple, or stout, branched irregularly, single; conidia hyaline, variable, oblong, borne singly; oblong or slightly curved; parasitic on higher plants. These strains may be identified as Fusarium species.

Ethyl acetate is widely used in extraction of endophytic fungal cultures (Bhardwaj et al., 2015) followed by methanol. As a solvent, ethyl acetate solvent possesses medium polarity so that it has the ability to dissolve both polar and non-polar active compounds and methanol solvent being a polar solvent can dissolve almost all organic compounds, even polar, semi polar and non-polar (Rahmawati et al., 2018).

Similar to our study, Buatong et al., (2011) reported the extraction of fungal broth by ethyl acetate while the mycelia were extracted using methanol, hexane, acetone and ethyl acetate in sequence, respectively.

Prihanto et al., (2011) was suggested that Isolated of Endophytic fungi from Rhizophora mucronata (Malay: bakau kurap) of the genera Penicillium, Ampelomyces, and Fusarium were showed to be active against E.coli . De Souza Sebastianes et al., (2013) were concluded that fungi from marine environments grow in habitats with unique conditions that attributed to the activation of metabolic pathways and the synthesis of distinct unknown molecules. Fox and Howlett, (2008) was concluded that production of these compounds aids in supporting the adaptation and survival of the fungi in marine ecosystems. Hence, endophytes from mangrove ecosystem are not only the bio-prospecting but also provides protection against pahogenes. An intensive search for newer and effective antimicrobial agents is needed.

This study indicates that endophytic fungi isolated from mangrove possess potential antimicrobial properties and should be further investigated to produce their active compounds as antibiotic agents.

Acknowledgements

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GROWTH AND YIELD OF CHILLI AS AFFECTED BY ORGANIC AND INORGANIC LIQUID FERTILIZER APPLICATION IN HOT SEASON OF TAUNGOO TOWNSHIP

Kyu Kyu[1]

Abstract

The planting pot experiments of chilli were carried out behind Mahogany hostel in Taungoo University Campus, Bago Region from March to July, 2022. The chilli seeds were sown in soil tray for transplanting in field. The transplanted plants were used with five treatments of organic and inorganic liquid fertilizer which including the untreated control treatment. *Capsicum frutescens* L. which belongs to family Solanaceae is referred to as chilli. These chilli plants had significant results for growth parameters, yield and yield components of chilli due to application of organic and inorganic liquid fertilizers compared to control. Among the five different treatments, the combination of organic and inorganic liquid fertilizers (T4) treatment resulted in maximum plant height, number of branches per plant, leaf number, fresh and dry weight of the plant, number of fruits per plant and total yield. The combined organic and inorganic liquid fertilizers (T4) treatment was the highest agronomic characters and set up more fruits of chilli plants in hottest season of Myanmar.

Keywords - Combined organic and inorganic liquid fertilizers, chilli, growth parameters, yield.

Introduction

Chilli are now widely cultivated in the world and are economically important as condiments, vegetables and medicines. It is cultivated mainly in home gardens or small fields and is used for seasoning foods in the daily diet (Sunil, P., *et al*,2012). Most nutrients needed by plants are supplied solely by soil. Insufficient supply of these nutrients may limit plant growth. However, agricultural crops may require more nutrients than natural vegetation (Lim T K ,2013). Organic fertilizers are derived from living things including plants and animals manures while inorganic are synthetically derived chemicals plus minerals from the earth.

Organic fertilizers are naturally available mineral sources that contain moderate amount of plant essential nutrients. Fertilizers are materials that can be added to soil or plants, in order to provide nutrients and sustain growth (Barker and Allen V, 2012*).* The application of organic fertilizers combined with inorganic fertilizers is expected to increase plant growth and the availability of nutrients in plants. Liquid organic fertilizers have the advantage of being able to increase nutrient uptake and quickly overcome nutrient deficiencies because the nutrients in it have broken down so that they are more easily to absorb efficiency (Pangaribuan, *et al*., 2017).

Chicken and cowdung manure contain NPK for almost all types of plants and crops. It brings back nutrient balance to fields organically (Telkamp, M, 2015). Chicken and cowdung manure are some of the commonest farmyard manure. They are used as fertilizers to boost plant yield and also to improve soil structure and fertility. Myanmar has a tropical to sub-tropical monsoon climate with three seasons namely hot (mid-February to mid-May), rainy (mid-May to late October) and cool (late October to mid-February). The present study was carried out to study the effect of organic and inorganic liquid fertilizers on growth and yield of chilli in hot season of

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Taungoo Township. This study aims to evaluate the effects of organic and inorganic liquid fertilizers on growth and yield of chilli in hottest season of Taungoo Township, to reduce the use of excessive inorganic fertilizers among farmers and to give the suitable information of this study to local farmers.

Materials and Methods

Time and place of field experiment

The field experiments on growth and yield of chilli plant were conducted from March to July, 2022 behind the Mahogany hostel in Taungoo University Campus, Bago Region. In this study, chilli seeds were sown from moderate hot to very hot season in Myanmar (from March to July, 2022).

Preparation of seedling for transplanting and planting pot

The chilli seeds were sown in soil tray for transplanting in field. The 30 days ages and 4 ± 1 inch plant height of transplanted chilli plants were sown in each planting pot. This study arranged 15 plants in RCD (Randomized Complete Design).

The planting pots were prepared with soil and rice husk charcoal (2 :1) to better soil aeration and soil physical condition. This cultivation consists of the 15 prepared soil planting pot with 5 treatments and 3 replications of chilli plants. Each soil planting pot was spaced 18 inches in a row. Each row was 2 ft apart to each other and a row consists 5 soil planting pots. Each soil planting pot was filled with 5Kg soil and rice husk charcoal (2: 1).

The transplant was sown in each planting pot to get one plant per soil planting pot. When chilli plants were established, the soil around the base of plants was pulverized, gaps filling, weeding, irrigation and pest management were done as per requirement.

After one week for transplanting of chilli plants, the following treatments were applied in each treated planting pot with two times interval per month.

The treatments were:

T1 - 5 kg of soil with no fertilizer (control)

T2 - 10 Kg ground chicken manure mixed with 20 liter water (Chicken manure liquid fertilizer)

T3 - 10 Kg ground cowdung manure mixed with 20 liter water (Cowdung manure liquid fertilizer)

T4 - 3g NPK mixed with1 liter water + 1 liter chicken manure liquid fertilizer + 1 liter cowdung manure liquid fertilizer (Combined liquid fertilizer)

T5 - 3g NPK mixed with 1 liter water (NPK liquid fertilizer)

Figure1. Transplanted plants and preparation of planting pots in chilli cultivation

Preparation of organic and inorganic liquid fertilizers (Dewi Putri Ratna and Kanevi Octova Paradita, 2010)

Cowdung manure was grounded to make the powder form. Then, 10Kg cowdung manure powdered forms were mixed with 20 liters water (1: 2) for making cowdung liquid fertilizer for chilli cultivation. Then, the both materials are mixed and stirred. Optimally, stirring is done for 4 hours by hand and ready to be use in this study. After that, then filtered or separated between the liquid and solid parts. The liquid, so organic liquid and solid fertilizers were stirred for a few minute before use. 1 liter cowdung liquid fertilizer was applied in each cowdung liquid fertilizer treatment from 1 week after transplanting for 2-week interval in chilli cultivation. Similarly, chicken manure liquid fertilizer was prepared in this procedure. NPK was bought in market being as inorganic fertilizers in this cultivation. 30g per liter NPK liquid fertilizer was applied in each NPK treated planting pot respectively.

Figure 2 . NPK, Chicken and Cowdung manure

Preparation for the Morphological Study

The seeds of chilli were obtained from Taungoo District and Local farmers cultivated these seeds as local variety. The morphological characters of the fresh specimens sample chilli plants were studied from habit to seed. All fresh specimens were recorded by taking the photographs in the study. Chilli plants were collected from the field and were identified by their specific morphological characters according to their literatures.

Measurement of agronomic characters

Plant height was measured starting from the base of the stem to the tip of the highest leaf by using measuring tape. The unit used for plant height measurement was inch. The number of leaves, flowers and fruits per plant were counted weekly after transplanting. Chilli fruits \weight in each treated pot were weighted by digital balance to evaluate the weight yield of chilli plant at harvested time.

Data collection and statistically analysis

Plant height, number of leaves, number of flowers and fruits were counted from 2 week after sowing in chilli cultivation. All data in each treatment were calculated and compared to evaluate the best results in this study of chilli cultivation. Watering and other cultural practices were done whenever it was necessary.

The agronomical data in this study were statistically analysed by using Cropstat software program. Treatment means were compared by using LSD (Least significant Differences) at 1% and 5% level of significant.

Climatological data collection

The climatological data such as monthly minimum temperature, maximum temperature and relative humidity during chilli cultivation was determined at Department of Meteorology and Hydrology, Taungoo township, Bago Region.

Results

Scientific name - *Capsicum frutescens* L.

English name - Chilli

Myanmar name - Kala-aww

Family - Solanaceae

Morphological Characters

Habit: Erect herbs, much branched. **Leaves**: alternate, simple, petiolate, stipulate. **Inflorescence:** axillary, solitary, cymose. **Flower:** solitary, terminal, axillary, bisexual, regular, actinomorphic, complete, pentamerous, cyclic, hypogynous. **Calyx:** (5 - 6), synpetalous, petaloid, valvate, cupshape, sepaloid,persistent, inferior. imbricate, persistent, inferior. **Corolla:** (5 - 6), synpetalous, petaloid (greenish), imbricate, inferior. **Androecium:** 5, apostemonous, petalostemonous, adnate at base to corolla tube, long anther with groove, filament short, introrse, longitudinal dehiscence, and inferior. **Gynoecium:** (2), syncarpous, bilocular, axile placentation, many ovules in each locule in T. S, style long and slender, stigma capitate, superior. **Fruits:** The berries fruit is red when mature and tapered. The fruit are narrow, conical or elliptical, 1 - 2.5cm long and 0.5cm wide and contain numerous pale yellow, flattened seeds.

Figure 3. Morphological Characters chilli

Plant height of chilli plant during growing periods

Plant heights of chilli plant were presented in Table 1. Among the different fertilizers, the plant height was the highest in combined fertilizer application from 8 week to 12 week after transplanting. All treatments including control were developed the different height to each other. Control (without fertilizer) was the shortest in all treatments. The plant height in all treatments were significantly different compared to the control treatment.

Table 1. Mean values of plant height of chilli during growing periods

Treatments	Plant height plant^{-1}of chilli plant during growing periods (inch)					
	2wk	**4wk**	**6wk**	**8wk**	**10wk**	**12wk**
Control (T_1)	3.00	8.67	12.33	14.33	17.33	19.33
Chicken manure liquid fertilizer (T_2)	5.67	12.33	15.33	20.33	26.67	30.33
Cowdung manure liquid fertilizer (T_3)	4.47	10.33	17.33	19.67	21.67	24.00
Combined liquid fertilizer (T_4)	5.50	10.33	17.33	21.33	28.33	32.00
NPK liquid fertilizer (T_5)	4.13	10.33	17.33	21.33	27.67	31.00
F-test	**	**	**	**	**	**
LSD (5%)	**0.68**	**1.14**	**0.84**	**1.29**	**1.68**	**1.58**
cv (%)	**7.9**	**5.8**	**2.8**	**3.5**	**3.6**	**3.1**

**=significant at 1% level of LSD, *=significant at 5% level of LSD, ns=not significant, wk= week after transplanting

Leaves numbers of chilli plant during growing periods

Among the different fertilizers, the maximum number of leaves was found in combined fertilizer application from two week after transplanting to harvested time. Control (without fertilizer) was the minimum number of leaves in all treatments. The detailed results were presented in table 2.

Table 2. Mean values of number of leaves of chilli plant during growing periods

Treatments	Leaves number plant^{-1}of chilli plant during growing periods					
	2wk	**4wk**	**6wk**	**8wk**	**10wk**	**12wk**
Control (T_1)	7.33	21.33	35.33	45.33	52.33	58.33
Chicken manure liquid fertilizer (T_2)	10.33	42.67	68.33	89.33	117.67	124.33
Cowdung manure liquid fertilizer (T_3)	8.33	25.67	38.67	51.67	89.67	97.33
Combined liquid fertilizer (T_4)	16.33	48.67	79.67	98.00	137.33	147.00
NPK liquid fertilizer (T_5)	9.33	31.33	52.67	71.33	100.67	106.33
F-test	**	**	**	**	**	**
LSD (5%)	**1.03**	**1.79**	**1.19**	**1.65**	**1.99**	**1.99**
cv (%)	**5.3**	**2.8**	**1.2**	**1.2**	**0.6**	**1.0**

**=significant at 1% level of LSD, *=significant at 5% level of LSD, ns=not significant, wk= week after transplanting

Number of branches in chilli plant during growing periods

Among the different fertilizers, the maximum number of branches was founded in combined fertilizer application from two week after transplanting to harvested time. Control (without fertilizer) was the minimum number of branches in all treatments. The detailed results were presented in table -3.

Table 3. Mean values of number of branches of chilli plant during growing periods

Treatments	Number of branches plant^{-1} of chilli plant during growing periods								
	4wk	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**	**11wk**	**12wk**
Control (T_1)	3.33	5.33	5.67	5.67	5.67	5.67	5.67	5.67	5.67
Chicken manure liquid fertilizer (T_2)	7.33	9.33	9.33	9.67	9.67	9.67	9.67	9.67	9.67
Cowdung manure liquid fertilizer (T_3)	2.33	2.67	4.33	4.67	6.33	6.33	6.33	6.33	6.33
Combined liquid fertilizer (T_4)	8.33	9.33	10.33	10.67	11.33	11.33	11.33	11.33	11.33
NPK liquid fertilizer (T_5)	6.33	7.33	7.33	9.00	9.33	9.33	9.33	9.33	9.33
F-test	**	**	**	**	**	**	**	**	**
LSD (5%)	**0.14**	**0.49**	**0.77**	**0.60**	**0.60**	**0.60**	**0.60**	**0.60**	**0.60**
cv (%)	**0.0**	**3.8**	**5.6**	**6.5**	**3.7**	**3.7**	**3.7**	**3.7**	**3.7**

**=significant at 1% level of LSD, *=significant at 5% level of LSD, ns=not significant, wk= week after transplanting

Number of flowers of chilli plant during growing periods

Among the different fertilizers, the maximum number of flowers was combined fertilizer application from four week after transplanting to harvested time. The number of flowers in control

(without treatment) and other treatments were a differences throughout the chilli cultivation. The detailed results were shown in Table 4 and figure 4.

Table 4. Mean of number of flowers in chilli plant during growing periods

Treatments	Number of flowers plant^{-1} of chilli plant during growing periods						
	6wk	**7wk**	**8wk**	**9wk**	**10wk**	**11wk**	**12wk**
Control (T_1)	5.33	7.33	10.33	12.33	14.67	7.67	12.67
Chicken manure liquid fertilizer (T_2)	9.33	13.33	20.33	38.33	25.33	11.67	14.33
Cowdung manure liquid fertilizer (T_3)	7.33	10.33	12.33	27.67	19.67	13.33	15.67
Combined liquid fertilizer (T_4)	13.33	18.33	22.67	56.67	84.33	20.33	23.33
NPK liquid fertilizer (T_5)	10.33	14.33	21.67	31.33	23.33	11.33	15.67
F-test	**	**	**	**	**	**	**
LSD (5%)	1.11	1.11	1.11	1.74	1.46	1.58	1.68
cv (%)	6.5	4.6	3.4	2.8	2.3	6.5	5.5

**=significant at 1% level of LSD, *=significant at 5% level of LSD, ns=not significant, wk= week after transplanting

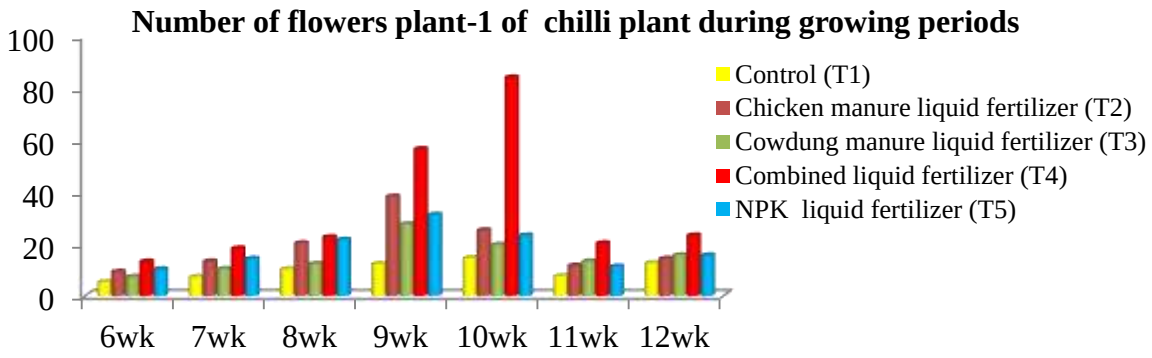

Figure 4. Mean of number of flowers in chilli plant during growing periods

Number of fruits of chilli plant during growing periods

Among the different fertilizers, the maximum number of fruits was the highest combined fertilizer application in chilli plant during the growing periods. The detailed results were shown in Table 5 and figure 5.

Table 5. Mean of number of fruits in chilli plant during growing periods

Treatments	Number of fruits plant^{-1} of chilli plant during growing periods						
	6wk	**7wk**	**8wk**	**9wk**	**10wk**	**11wk**	**12wk**
Control (T_1)	0.00	0.00	0.00	7.33	13.33	20.33	22.33
Chicken manure liquid fertilizer (T_2)	0.00	0.00	1.33	15.33	54.67	75.33	77.33
Cowdung manure liquid fertilizer (T_3)	0.00	0.00	0.00	10.33	30.33	54.67	58.33
Combined liquid fertilizer (T_4)	0.00	2.33	5.33	17.33	66.67	92.67	98.33
NPK liquid fertilizer (T_5)	0.00	0.00	0.00	12.67	50.33	69.67	75.33
F-test	**ns**	**	**	**	**	**	**
LSD (5%)	**0.00**	**0.49**	**0.94**	**1.22**	**0.73**	**1.11**	**1.11**
cv (%)	**0.0**	**55.3**	**27.8**	**5.1**	**0.9**	**0.9**	**0.9**

**=significant at 1% level of LSD, *=significant at 5% level of LSD, ns=not significant, wk= week after transplanting

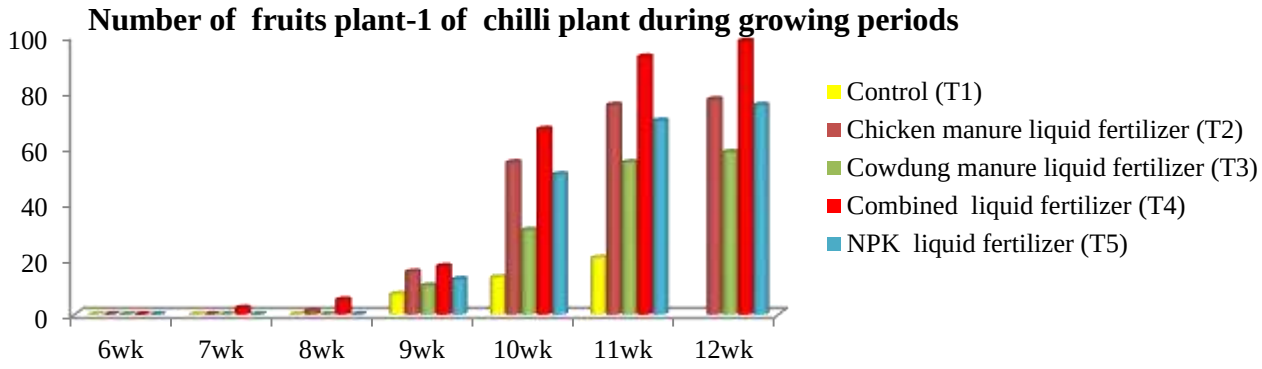

Figure 5. Mean of number of fruits in chilli plant during growing periods

Yields of chilli at harvested time

Among the different fertilizers, the highest weight of yields were combined fertilizer application in chilli plant at harvesting time. In the fruit weight of different treatments, the fruit weight of combined fertilizer application was the highest at harvesting time. The detailed results were shown in Table - 6.

Table 6. Mean of number of yields in chilli plant during growing periods

Treatments	**Yields of chilli at harvested time**		
	Number of fruits plant^{-1}	Weight of fruits plant^{-1} (g)	Weight of fruits plant^{-1} (g) /Number of fruits plant^{-1}
Control (T_1)	22.33	17.93	0.8
Chicken manure liquid fertilizer (T_2)	77.33	98.33	1.27
Cowdung manure liquid fertilizer (T_3)	58.33	75.60	1.30
Combined liquid fertilizer (T_4)	98.33	137.63	1.40
NPK liquid fertilizer (T_5)	75.33	97.67	1.30
F-test	**	**	
LSD (5%)	1.11	1.95	
cv (%)	0.9	1.2	

**=significant at 1% level of LSD, *=significant at 5% level of LSD, ns=not significant, wk= week after transplanting

Table 7. The climatological data of experimental site during chilli plant cultivation

Parameters	**Monthly mean temperature, relative humidity and rainfall from March to July, 2022**					
		March	**April**	**May**	**June**	**July**
Temperature (ºC)	Minimum temperature	26.1	30.0	27.8	24.4	25.6
	Maximum temperature	32.0	34.0	30.6	29.0	31.0
Relative humidity(%)		70.10	64.87	81.68	84.52	86.54
Rainfall (inches)		0.03	0.03	0.28	0.51	0.24

Source: Department of Meteorology and Hydrology, Taungoo

Discussion and Conclusion

This study revealed that erect herbs and much branched chilli plant with the berries fruit. The fruits are narrow, conical, 1 - 2.5cm long and 0.5cm wide and contain numerous pale yellow, flattened seeds. Based on morphological characters, scientific name is *Capsicum frutescens* L. in Solanaceae family. It agreed with C. Azurdia, 2020 and *Carvalho, 2014).* Height of Plants, number of leaves and number of branches were highest in combined liquid fertilizer (T_4) treatment of chilli plant. Irwan A W and Nurmala T, 2018 found that the combination of applying organic fertilizers and inorganic fertilizers to chilli plants can make efficient use of inorganic fertilizers as well as increase the availability of nitrogen in the soil and nitrogen uptake in plants will be minimize nutrients lost because organic fertilizers are able to bind nutrients. Moreover, number of flowers and fruits and fruits weight in combined liquid fertilizer (T_4) treatment were higher than other treatments and closely followed by chicken manure liquid fertilizer (T_2) and NPK liquid fertilizer (T_5) in chilli cultivation. The nutrient balance for plant was better growth and yield. The leaves of chicken manure liquid fertilizer (T_2) treatment were thicken and margin roll upward but formed many fruits in this treatment. Cowdung manure liquid fertilizer (T_3) treatment was higher than control and later fruit setting than other treatments. The flowers was shed from 6week to 8week in NPK liquid fertilizer (T_5) treatment and then the fruit setting was at 9week after transplanting. Combined liquid fertilizer (T_4) treatment was earlier flower initiation and fruit formation of chilli plant. The use of liquid organic fertilizers is expected to reduce the use of inorganic fertilizers in order to achieve environmentally friendly and sustainable agriculture.

The transplanted chilli plants was grown from Apirl to June in hottest season of Myanmar. The chilli plants were developed by using liquid fertilizer throughout the growing period. Rizqiani, 2006 found that liquid organic fertilizers have several benefits, including being able to encourage and increase the formation of leaf chlorophyll and the formation of root nodules in plants, thereby increasing the ability of plant photosynthesis and absorption of nitrogen from the air, can increase plant vigor so that plants become sturdy and strong, increase plant resistance to drought, weather stress and attack by disease-causing pathogens, stimulate the growth of production branches, and increase the formation of flowers and ovaries, and reduce the loss of leaves, flowers and ovaries.

In conclusion, combined liquid fertilizer (T_4) treatment was the best suitable treatment due to the improvement of plant growth parameters and yield in hot season of Myanmar. Based on the benefits and advantages of liquid organic fertilizers, the results of this study can be used as additional information to expand the use of organic fertilizers, especially liquid organic fertilizers, as a nutrient source at the farm level so as to support sustainable agriculture.



Figure 6. Five treatments of chilli plant during growing periods

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EFFECT OF *CHROMOLAENA ODORATA* AS ORGANIC FERTILIZER ON *GLYCINE MAX* (L.) MERRILL.

Myo Win[1]

Abstract

Chromolaena odorata (L.) R. M. King & H. Robiston; which was belong to the family Asteraceae and locally known as Bezat or Jamani-chon in Myanmar. The plant was collected in Mohnyin University Campus, Kachin State from June 2022 to September 2022. The collected specimen have been taken photographs and dried at room temperature for one month. The dried samples were ground in grinder to get fine powder. Experiments using a randomized complete block design (RCBD) with five replications were carried out. This study investigated the effect of various *Chromolaena* suspension on the growth and development of soybean during 2022 to 2023. The seed of soybean cultivar Y15 were tested with *Chromolaena* suspension and experimentally studied in laboratory and pot culture. Among the five different kinds of *Chromolaena* suspension treatment, the *Chromolaena* suspension 4 gL^{-1} and 6 gL^{-1} were best for growth and development of Soybean Y15 cultivar. In pot culture *Chromolaena* suspension 4 gL^{-1} and 6 gL^{-1} were much more effective in enhancement of plant height and leaf area than other treatment and control. The result showed that *Chromolaena* suspension actually promotes the growth and development of soybean Y15 variety.

Keywords: *Chromolaena odorata*, suspension, organic fertilizer

Introduction

Soybean is one of the most important food plants world-wide grown as an industrial and versatile crop (Afework & Adam, 2018; Shea *et al*., 2020). Global soybean production reached 358 million metric ton in 2019 with American producing 95 million metric tons in 2019. Brazil produced 123 million metric tons of soybean in 2019 and became a world record. They are followed by Argentina, China, India, Paraguay, Canada, Mexico and some European countries in the list of highest soybean producers. The mean protein content in these trials (41.6%) was comparable to the global average protein content, for example, Brazil (40.9%), USA (41.4%) and China (42.1%). The oil content in the trials (19.1%) was higher than the oil content in Brazil (18.7%), USA (18.8%) and China (16.8%) (Grieshop and Fehey, 2001).

Soy-based nutritious food products such as tofu, soymilk, soy sauce, miso, etc. have been developed for human consumption while oil extracted soy meal is used as a nutritious animal feed. Besides its use for domestic purposes, soy oil finds multifarious uses in industries related to production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides and cosmetics. Recently, use of soy oil as biodiesel has opened up another possibility of renewable sources of energy for industrial uses. Soybean occupies a premier position among agricultural crops, being the most important source of good quality concentrated proteins as well as vegetable oil. Seeds of soybean used in Asia and other part of world for many centuries to prepare a variety of fresh, fermented and dried foods (Probst and Judd 1973).

Myanmar is an agricultural country bordering China a part of the Belt and Road Initiative and that has the potential to penetrate the large soybean market and enter the global stage. This is why we need to make effective preparation for soybean production. We can divide this into cultivation, industrial processing, and quality aspects. Soybean seeds have been put to a wide range of uses throughout the world, partly because of its high nutritional values and partly for its good

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quality oil. Moreover, soybean oil is used for salad, dressing, cooking and margarine. Therefore, the improvement of soybean production becomes an important section in both tropical and temperate countries. Soybean is the most important source of food and feed, versatile crop and produces an abundant supply of protein and oil (Yin Yin Thant 2008).

Fertilizer supply nutrients needed by crops. Fertilizers can produce more food and cash crops of better quality. Natural organic fetilizer (Biofertilizer) that helps to keep in the soil with all nutrients and living required for the benefits of the plants. Therefore, agricultural scientists have been suggested that biofertilizer shoul be used in place of chemical fertilizer, to avoid long-term negative effects of chemical fertilizer on the soil (Parr *et al.,* 1990).

Chromolaena odorata is a perennial succulent or semi-woody shrub belonging to the Asteraceae family or Compositae (known as the aster, daisy, or sunflower family) is the largest family of flowering plants represented by about 950 genera and 20,000 specxies over the globe (Mahbubur 2013). The common name for these plants are Siam weed, devil weed, epatorium, Jack-in-the bush, king weed,paraffin weed (Vijayaghavan et al., 2017).

C. odorata shoot contains 1.26% Nitrogen, 0.67% Phosphorous, 1.08% Potassium, 2.33% Calcium and 0.005% Magnesium (Olabode *et al*., 2007). *C.odorata* grows in wild bushes without any organized cultivation. Indeed, it is considered to be a weed in most farming systems. Siam weed biomass has a reasonably high nutrient content (2.56% N, 0.38% P, 2.41 % K). Siam weed has grown wild had the potential to be used as a source of organic materials for the production of high biomass (Ojeniyi *et al*., 2012). Siam weeds are very difficult to control and cause many problems in various agriculture and plantations (Karian *et al*., 2017).

Chromolaena odorada leaves were also a rich source of mineral elements such as Ca, Na, K, Fe, Mn, Zn, Cu, P and Mg. Leaves also yield alkaloids, flavonoids, saponins, cyanogenic glycosides, tannins and phytic acid (Nwinuka, *et al*., 2008)

Chromolaena plant biomass possess good microbial association in the rhizosphere and upon using this in compositing and supplying to the main field would favor good growth by encouraging useful microbes in soil. Further, use of weed composts also enhances the soil organic carbon which helps in increasing plant growth promoting rhizobacter in the soil. These were known to produce phytohormones and vitamins apart from nitrozing fixing by free living microbes organisms to the growing crop plants. All these biological features improved plant high, leaf number and biological factors by using weed composed favoured the soil chemical and biological properties which lead to higher nutrient uptake by the crop. All these resulted in higher agronomic efficiency of applied nitrogen. (Kumar, 2004)

The aim of the present study was to test the effects of application of organic fertilizer in various rates on growth and development. The objective of this research were to study the effect of *Chromolaena odorata* suspensions as organic fertilizer on soybean and to determine the importance suitable dose of organic fertilizer application in order to improve growth and development.

Materials and Methods

Laboratory Experiment

Collection of the *Chromolaena odorata* (L.) plants had been done from Mohnyin University Campus, Kachin State from June 2022 to September 2022. The collected specimens

were taken photographs, and dried at room temperature allowed to keep one month. The dried samples were grounded in a grinder to get fine powder. Various weights (2g, 3g, 4g, 5g and 6g) of plant powder were mixed in pure water for 24 hours, and then different concentration of suspensions were obtained.

Laboratory experiment with *Chromolaena odorata* Suspesion

The soy bean seeds were soaked in pure water and mixed with different percentages of *Chromolaena odorata* powder T_1 (2%), T_2 (3%), T_3 (4%), T_4 (5%) and T_5 (6%).

Pot Experiment

Experiments were conducted at Mohnyin University Campus from December 2022 to March 2023. In this experiment, soybean seeds were presoaked in various concentration ($2gL^{-1}$, $3gL^{-1}$, $4gL^{-1}$, $5gL^{-1}$ and $6gL^{-1}$) of *Chromolaena* suspension for 24 hours. Control was soaked in water for 24 hours. In the experiments Randomized Complete Block Design (RCBD) was used with five replications. The soil was sterilized under the sun for 3 days. The prepared soil was placed into the polyethylene (PE) bags. Each seed was placed in PE bag. Each soybean cultivars were included 30 PE bags and each bag was consisted one seed.Each treament was regularly watered with 500 ml once a day.

Pot Experiment Design Randomized Complete Block Design (RCBD)

Replication – 5

Control = T0 = 0 gL^{-1}(C) Cultivar Yezin -15

Treatment = T1 = $2gL^{-1}$

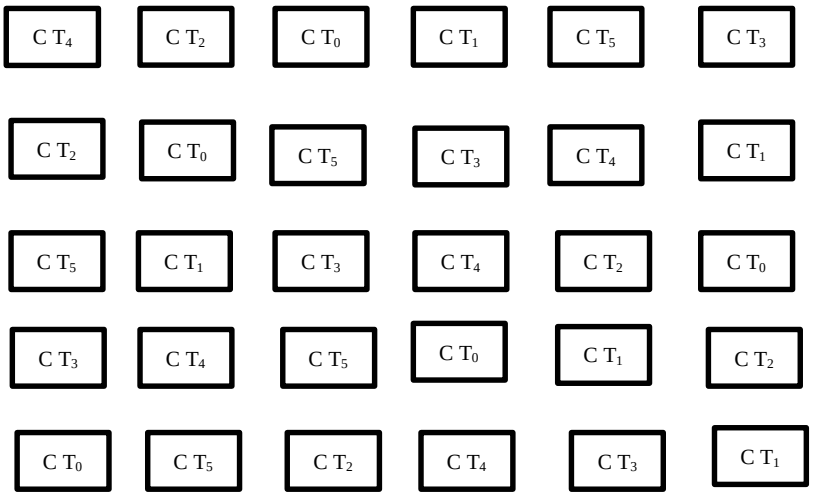
T2 = $3gL^{-1}$

T3 = 4 gL^{-1}

T4 = 5 gL^{-1}

T5 = 6 gL^{-1}

Experimental Layout (CY_{15})



Results

Morphological Characters of *Glycine max* (L.) Merrill.

Family name	:	Fabaceae
Scientific name	:	*Glycine max* (L.) Merrill.
English name	:	Soybean, Soya
Myanmar name	:	Peboke
Flowering period	:	July to September

Annual herbs, about 1m high; stems and branches terete, gray-brownish hairy. Leaves trifoliolate pinnately compound; stipules acute, about 3 mm long, pubescent, persistent; petioles terete, furrowed above, 12.5- 2.0 cm long. pubescent; stipels setaceous, 1.0- 3.0 mm long, pubescent, persistent; leaflets broadly ovate, 7.0- 12.0 cm by 4.0- 7.5 cm, pubescent with lax hairs to glabrescent, cuneate or rounded at the base, entire along the margin, acute or occasionally obtuse at the apex. Inflorescence axillary raceme, few-flowered, usually 1-flower at a node. Flowers bisexual. zygomorphic, about 0,5 cm in diameter, purple; bracts lanceolate, striate, 4.2- 5.5 mm long, bracteoles linear-lanceolate or setaceous, 2.0 - 3.0 mm long; pedicels short., Calyx tubular, 5-lobed; tube about 0.3 mm long, lobes acute-acuminate, about 0.3 mm long. glabrous within, villous without. Corolla papilionaceous, exserted; standard squarish, orbicular, about 5.0 mm by 5.0 mm; wing obovate to oblong, 5.0 - 7.0 mm by 2.0- 3.0 mm; keel oblong, about 4 mm long, pale purple, glabrous. Stamens 10, diadelphous; filament filiform, about 3 mm long, white, glabrous; anthers ovoid, about 1mm long, dithecous, basifixed, dehiscent by longitudinal slit. Ovary oblong. about 2.0 mm long, green, pubescent; style short, about 1 mm long, white, pubescent, stigma simple. Fruits pod, oblongoid, 2.5- 5.0 cm by 0.8- 1.0 cm, green, yellowish- brown when ripen, bristly hairy. Seeds globose, glabrous, pale yellow. (Figure 1, E,F)

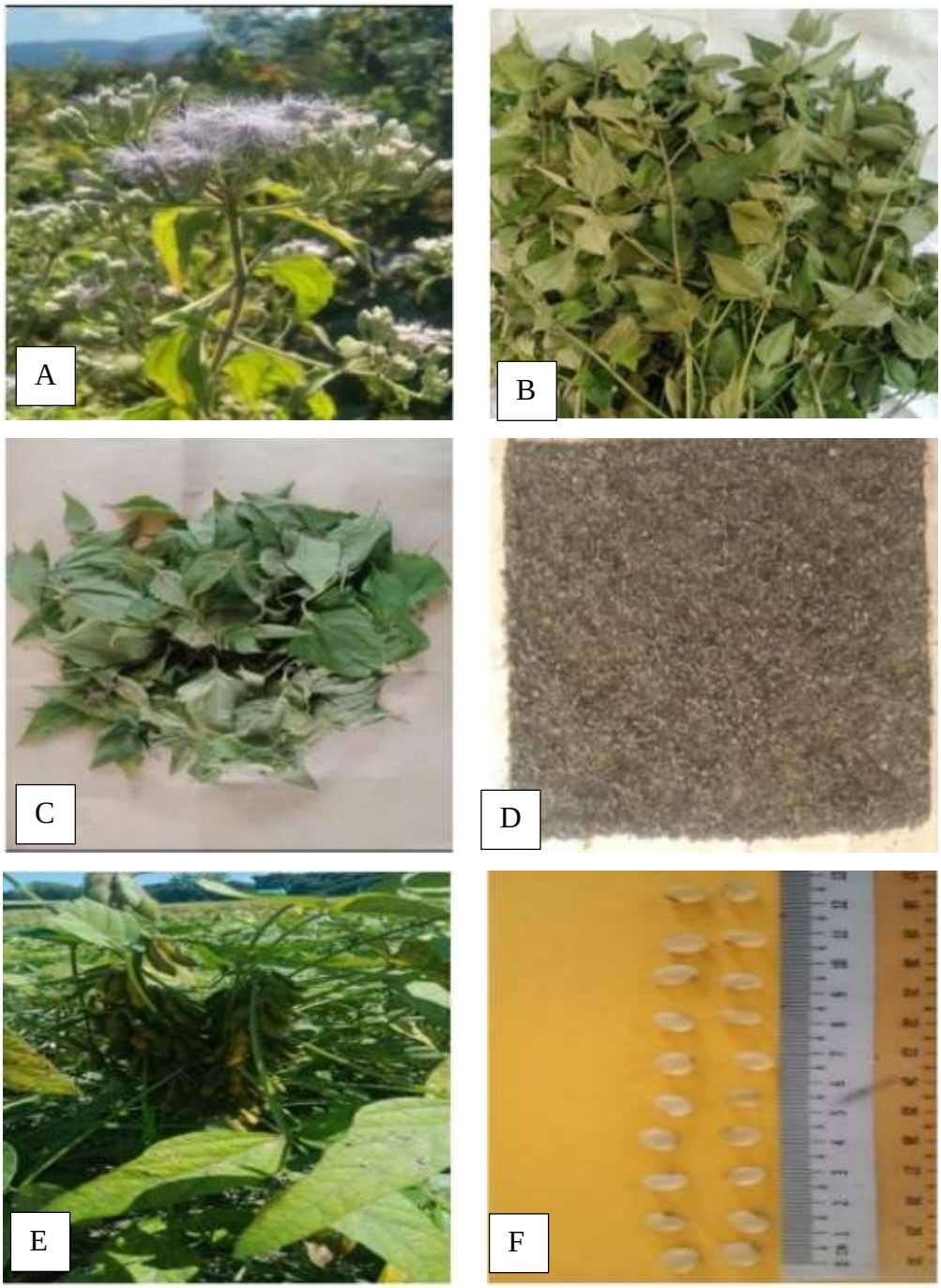


Figure 1. Morphological Characters of *Chromolaena odorata* (L.) and *Glycine max* (L.)

A. Habit of *chromolaena odorena*
B. Stems and Leaves
C. Leaves of *Chromolaena odorena*
D. Dry Leaves of *Chromolaena odorena*
E. *Glycine max*
F. Y 15 Seeds(*Glycine max)*

The results show that doses of *Chromolaena odorata* suspension significantly affected of the plant growth and development. In addition, *Chromolaena* suspension at a dose of $4gl^{-1}$/pot and $6gl^{-1}$/pot produced higher plant height, leaf size , length of petiole and number of pods compared to other treatments and control. (Table 1, 2, 3, 4)

Table 1. The effect of *Chromolaena odorata* suspension on plant height of soybean cultivar (Yezin-15) at different sampling dates

Treatments	1 Week	2 Week	3 Week	4 Week	5 Week	6 Week	7 Week
T1 (2 g l^{-1})	6.15 ab	10.50 bc	15.84 cd	18.01 c	24.55 c	27.40 cd	27.50 cd
T2 (3 g l^{-1})	6.20 ab	11.35 abc	16.75 bc	19.30 bc	26.10 bc	28.90 bc	29.05 cd
T3 (4 g l^{-1})	6.30 ab	11.65 ab	17.40 b	19.97 b	27.55 b	30.20 ab	30.95 b
T4 (5 g l^{-1})	6.45 a	10.80 abc	15.70 cd	18.85 bc	26.35 bc	28.60 bcd	29.35 bc
T5 (6 g l^{-1})	6.65 a	12.10 a	19.70 a	22.00 a	30.05 a	31.70 a	33.05 a
T0(control)	5.80 b	10.25 c	15.00 d	17.80 c	24.15 c	27.20 d	27.35 d
F-Test	n.s	n.s	**	**	**	**	**
LSD (5%)	0.60	1.39	1.35	1.63	2.34	1.62	1.89
CV%	7.32	9.49	6.13	6.40	6.71	4.22	4.86

n.s = (> 0.05) Non- Significant, ∗ = Significant at 5% (≤ 0.05), ∗∗ = Significant at 1% (≤ 0.01)

Table 2. The effect of *Chromolaena odorata* suspension on leaf size of soybean cultivar (Yezin-15)

Treatment	First Leaf		Second Leaf	
	Length (cm)	Wide (cm)	Length (cm)	Wide (cm)
T1 (2 g l^{-1})	3.89 ab	2.53 bc	3.38 cd	1.97 bc
T2 (3 g l^{-1})	4.29 a	2.81 a	3.66 bc	2.14 b
T3 (4 g l^{-1})	4.33 a	2.75 ab	4.02 ab	2.22 ab
T4 (5 g l^{-1})	4.17 ab	2.80 a	3.67 bc	2.11 bc
T5 (6 g l^{-1})	4.26 a	2.88 a	4.37 a	2.43 a
T0(control)	3.74 b	2.44 c	3.08 d	1.83 c
F-Test	n.s	*	**	* *
LSD (5%)	0.49	0.27	0.57	0.29
CV%	9.00	7.52	11.70	10.38

n.s = (> 0.05) Non- Significant, ∗ = Significant at 5% (≤ 0.05), ∗∗ = Significant at 1% (≤ 0.01)

Table 3. The effect of *Chromolaena odorata* suspension on leaf size of soybean cultivar (Yezin-15)

Treatment	Third Leaf		Fourth Leaf		Fifth Leaf		Sixth Leaf	
	Length (cm)	Wide (cm)	Length (cm)	Wide (cm)	Length (cm)	Wide (cm)	Length (cm)	Wide (cm)
T1 (2 g l^{-1})	3.74 cd	2.21 bc	3.31 c	1.97 bc	4.550 c	2.45 c	3.92 c	2.20 bc
T2 (3 g l^{-1})	4.05 bcd	2.45 abc	3.56 bc	2.08 bc	5.40 ab	2.92 ab	4.58 a	2.38 b
T3 (4 g l^{-1})	4.39 ab	2.55 ab	3.99 ab	2.19 b	5.26 ab	2.82 ab	4.22 abc	2.60 a
T4 (5 g l^{-1})	4.29 abc	2.58 ab	3.87 ab	2.10 b	4.92 bc	2.74 bc	4.07 bc	2.37 b
T5 (6 g l^{-1})	4.81 a	2.84 a	4.39 a	2.52 a	5.46 a	3.16 a	4.47 ab	2.66 a
T0(control)	3.57 d	2.10 c	3.24 c	1.80 c	4.66c	2.45 c	3.82 c	2.13 c
F-Test	**	*	**	**	**	**	*	**
LSD (5%)	0.5706	0.40	0.55	0.30	0.49	0.37	0.46	0.22
CV%	10.44	12.48	11.13	10.58	7.37	10.06	8.34	6.85

n.s = (> 0.05) Non- Significant, ∗ = Significant at 5% (≤ 0.05), ∗∗ = Significant at 1% (≤ 0.01)

Table 4. The effect of *Chromolaena odorata* suspension on petiole and number of pod per plant of soybean cultivar (Yezin-15)

Treatment	First Petiole	Second Petiole	Third Petiole	Fourth Petiole	Fifth Petiole	Sixth Petiole	Pods/plant
	Length (cm)	Length (cm)	Length (cm)	Length (cm)	Length (cm)	Length (cm)	
T1 (2 g l^{-1})	0.89 cd	4.35 cd	5.50 b	6.65 b	3.87ab	2.57bc	8.80 b
T2 (3 g l^{-1})	1.09 abc	5.05 bc	5.20 b	8.00 a	4.29a	2.75ab	10.80 ab
T3 (4 g l^{-1})	0.99 bcd	5.40 b	6.65 a	7.90 a	4.34a	2.81a	12.60 a
T4 (5 g l^{-1})	1.17 ab	5.15 b	6.70 a	7.80 a	4.17ab	2.80a	10.80 ab
T5 (6 g l^{-1})	1.26 a	6.45 a	7.00 a	8.05 a	4.26a	2.88a	13.40 a
T0(control)	0.75 d	4.25 d	5.15 b	6.52 b	3.73b	2.43c	7.80 b
F-Test	**	**	*	*	n.s	*	**
LSD (5%)	0.26	0.70	0.10	0.98	0.49	0.27	3.03
CV%	19.22	10.40	12.51	9.91	9.00	7.52	21.4

n.s = (> 0.05) Non- Significant, ∗ = Significant at 5% (≤ 0.05), ∗∗ = Significant at 1% (≤ 0.01)

Discussion

Application of *Chromolaena odorata* residues is expected to benefit both the soil and the crop. The soil was predominantly sandy and slightly acidic, low in nitrogen content, two phosphorus content and a high quantity of potassium (Ogundare *et al.* 2013)

Ogundare (2011) reported that combination of *Chromolaena odorata* residues and mineral fertilizer (Urea) showed promising potential in conserving soil fertility and improve the yield of maize in the study area. The better maize growth observed for *Chromolaena odorata* residues plus were fertilizer. The effects of *Chromolaena* residues and urea fertilizer on yield and yields components maize. (Ogundare *et al.* 2015)

The application of *C. odorata* weed compost at various doses resulted in higher total nitrogen available P, and available K when compared to N.P.K. Phonska treatment and without fertilization. In addition, *C odorata* weed compost at a dose of 666 grms/pot produced higher nitrogen uptake, phosphate leaf, and leaf potassium compared to other treatment *C. odorata* at a dose of 444 grams/ pot gave the highest content of nitrogen, phosphate, and potassium in Lettuce plants. Compared to inorganic fertilizer, cow manure, and without fertilizer treatments. (Alima Prapto, 2020). Siam weeds compost application of 20 tons ha^{-1} can substitute urea of 200 kg ha^{-1}, while increasing the yield of chili paper (Setyowati *et al*., 2014). The application of Siam weed compost of 10 tons ha^{-1}showed the highest yield in upland rice by 2.97 tons ha^{-1} and increase yield by 91.75 % compared to without application of Siam weed compost (Suryanto *et al*, 2020)

Chromolaena odorata is an invasive plant the potentially organic manure. This factors examined were doses (2, 3, 4, 5 and 6 gl^{-1}). The result showed that the *Chromolaena* suspension was more effective than control. The application of *Chromolaena suspension* 4 gl^{-1} and 6 gl^{-1} give more effective than other treatment and control. The optimum dosage of *Chromolaena* suspension application for soybean were 4 gl^{-1} and 6 gl^{-1}, respectively, with a maximum growth and development.

Finally, according to the present study, the utilization of Chromolaena suspension as a natural fertilizer which is not harmful to both living things and environment have been established.

Conclusion

From the present investigation, it can be concluded that *Glycine max* (L). Merr. Soybean cultiver (Y15) gave the recommended dose of *Chromolaena* suspension T3 (4 gL^{-1}) and T5 (6 gL^{-1}). The application of *Chromolaena* suspension significitly increased plant growth and development. *Chromolaena* suspension at a dose of 4gL^{-1} and 6 gL^{-1} gave the highest growth and development in soybean plants compared to control. Thus it is suggested that *Chromolaena* suspension is one of the most important tools production and for maintaining soil productivity. It is recorded that their treatments with organic fertilizer have higher values when it is compared to without fertilizer.

Enhancement of growth and yield of soybean by *Chromolaena* suspension treatment indicates that *Chromolaena* acts as a fertilizer similar to chemical fertilizers. Free availability of *Chromolaena* to farmers would greatly reduce cost of crop production and also lower cost of products for consumes.

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ISOLATION OF ENDOPHYTIC FUNGI FROM *VITEX TRIFOLIA* L. AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS

Thwe Thwe Oo[1], Cho Cho[2], Tin Myo Hlaing[3], Cho Zin Swe[4]

Abstract

Endophytic fungi (6 strains) were isolated from the leaf segments of *Vitex trifolia* L. collected from Kamayut Township in Yangon region. All the characters of vegetative and reproductive parts of the selected plants were checked with the help of available literatures. The leaves of *Vitex trifolia* L. were surface sterilized and the isolation of endophytic fungi was done on the sucrose yeast extract medium at 30°C for 3-7 days. All 6 different strains were sub-cultured into separate SY agar slants. The antimicrobial activity of 6 isolated endophytic fungi were investigated against 6 kinds of test organisms by paper disc diffusion method. Six types of endophytic fungi were observed to possess very high antibacterial activity. The selected strains T2, T3, T5 and T6 was extracted and isolation of bioactive compound from each selected broth culture by using TLC, UV and IR.

Keywords: *Vitex trifolia* L. leaves, endophytic fungi, TLC, UV and IR.

Introduction

Vitex trifolia L. is belong to the family Lamiaceae (Verbenaceae). It is also called chaste tree in English and jalanirgundi in Sanskrit, Nichinda in Hindi, and Manjingzi in Chinese. This plant is found in the tropical and subtropical regions around the world including India, Sri Lanka, China and Indonesia, Australia, and Singapore, East Africa and introduced to many islands in the Central Pacific and Hawaii. Meena Ajay Kumar, *et al*; (2011), Shashank Tiwari and Shreya Talreja, (2020). *Vitex trifolia* L. is a shrub or small tree, pungent smell. Hooker, (1897), Kirtiker and Basu. (1933), Backer,(1965), Julissa Rojas-Sandoval , (2022).

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, fruits, roots, seeds etc. The most important bioactive compounds from medicinal plants include terpenes, alkaloids, phenolic compounds, steroidal compounds and flavonoids. Maroof Ahmed, *et al*; (2012).

This plant is known to possess various active constituents such as essential oils, diterpenes, Vitetrifolins with several pharmacological properties such as antipyretic, antibacterial, works against asthma and allergic diseases, anti-inflammatory and sedative for headache, rheumatism and for common cold and as anti-trypanosomal in Asian countries. Several oils were extracted from the leaves of the plant that showed considerable mosquito repellent activity. The plant is a Chinese folk medicine for the treatment of cancers, evaluated by sulforhodamine B, which is widely used in Chinese folk medicine. The fruit extracts of *V. trifolia* exhibited antipyretic, analgesic, and anti-inflammatory activity. Anita Rani and Anupam Sharma, (2013).

The leaves are traditionally made into decoction for oral inflammation, or externally applied as a poultice for rheumatic pain and sprains and exhibited moderate inhibition of both Gram-positive and Gram-negative bacteria. The flowers are administered orally as infusion for treating intermittent fever accompanied by vomiting and thirst, while the stems are used for

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dysentery. The roots are used as antiemetic, expectorant. Meena Ajay Kumar *et al*; (2011). Ning Wee Hai, *et al*; (2020).

Endophytes are microbes which colonize living, internal tissues of plants without causing any harm to their host. These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites. The endophytic fungi play important physiological and ecological roles in their host life. Khan Rezwana, *et al;* (2010). Today around 40% of modern medicines are plant-derived because of fewer side effects. Plants generate various secondary metabolites, which may be broken down into the chemical categories of phenolics, terpenes, and alkaloids .Sharma and Singh(2021).

In this study the morphological characters of plant sample and isolation of endophytic fungi from *Vitex trifolia* L. leaves ,antimicrobial activity and then characterization of bioactive compound from selected strains The main objectives of research work were to study morphological characters of *Vitex trifolia* L.to investigate the isolation of endophytic fungal strains from *Vitex trifolia* L. leaves and antimicrobial activity of endophytic fungi, to study the preparation for evaluation of bioactive compounds from isolated fungi.

Materials and Methods

Collection of Plant Samples

The plant sample of *Vitex trifolia* L. (Kyaung pan-galay) were collected from Kamayut Township ,in Yangon region. All vegetative and reproductive parts of the collected sample were identified and studied with the help of available literatures Hooker, (1885), Kirtiker and Basu. (1933), Backer,(1965), Julissa Rojas-Sandoval, (2022) at Department of Botany, University of Yangon.

Isolation of endophytic fungi strains

The plants were washed in running water for fifteen minutes. These parts were sterilized by soaking in 75% ethanol for two minutes. Then they were sterilized by soaking in 5.3% sodium hypochlorite for one minute. After that, these parts were sterilized by soaking in 75% ethanol for fifteen seconds. These parts were dried on sterilized paper and then they were placed on agar plates containing nutrient sucrose yeast extract medium (Sucrose 10.0g, Yeast extract 3.0g, NaCl 0.5g, $CaCO_3$ 0.1g, Agar 18.0g, Distilled water 1L and pH 6.8±0.2) Strobel and Sullivan, (1999). Then, these plates were incubated at 30°C for 3-7 days and transferred to new plates. Then, isolated fungal strains were transferred into slant culture of test tubes Phay, (1997), Carbungco *et al.*, (2017) To suppress the growth of bacteria 0.001g of chloramphenicol was added to the above medium. Similarly, to deter the growth of fungi and yeasts about 0.001g of nystatin was added to isolation medium.

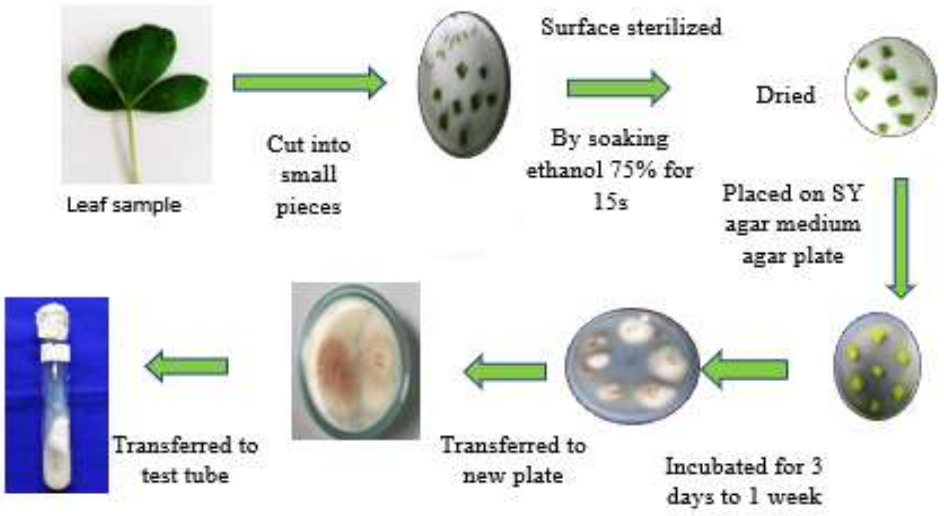


Figure 1. Isolation of Endophytic fungi from *Vitex trifolia* L.

Fermentation (broth culture)

The isolated fungal strains grown on slant culture were transferred into conical flasks containing 100 ml of SY broth (Sucrose 10.0g, Yeast extract 3.0g, NaCl 0.5g, $CaCO_3$ 0.1g, Distilled water 1L and pH 6.8±0.2). Then, isolated fungal strains were grown in broth media and these flasks were placed in chamber at room temperature for 3-7 days. Colony forms were showed on the surface of the broth media Strobel and Sullivan, (1999). Next step, experimentation of endophytic fungal activity against on test organisms on the culture media.

Antimicrobial activity test (Paper disc diffusion assay), Phay (1997)

After solidification, paper discs impregnated with broth samples were applied on the test plates and these plates were incubated at 30°C for 24-48 hrs. After 24-48 hrs clear zones (inhibitory zone) surrounding the test discs were measured. These zones indicate the presence of the bioactive compounds which inhibit the growth of test organisms.

Extraction of bioactive compound

The isolated individual endophytic fungi were inoculated in 150ml of conical flask containing sterilized SY broth medium. The flask was incubated at 28°C room temperature for 20 days with periodical shaking. After incubation period the fermented medium was filtered by using sterile qualitative filter paper ϕ 12.5cm and the filtrate was preserved and the mycelium part was removed. For the extraction of secondary metabolites, equal volume of filtrate and equal volume of ethyl acetate solvent was taken in the separating funnel and shaken continuously for 30 minutes and allowed to stand for 5 minutes until the formation of two immiscible layers. The upper portion of the solvent was separated and kept for evaporation. The culture filtrate was extracted thrice with ethyl acetate. Then the solvent phase was evaporated at 40°C. The residue was redissolved in methanol for subsequent separation and the crude extract was analyzed by thin layer chromatographic separation. Nithya and Muthumary, (2011), Sheeba *et al*, (2019).

Thin Layer Chromatography (TLC)

In TLC analysis, 6.5cm×1.5cm square pieces of TLC plates cut out from 20cm×20cm plate produced by Bezogen Von CAMAG (Germany) was used. The platting particle size of silica gel was 60 F254. The 20 days fermented broth of T2, T3, T5 and T6 was extracted with ethyl acetate

and about 0.5 µl was loaded onto TLC plate. After drying, the TLC plate was put into the chamber containing the solvent system of Petroleum ether : water: Ethyl acetate (9 : 3 : 2),Chloroform : Methanol (8 : 2),Hexane : Chloroform :Methanol (3 :2 :1), Methanol :ethyl acetate : Petrolium ether (9 : 3 : 1) in ascending condition. After 20-30 minutes the TLC plate was taken out and dried. Then the sport of metabolite was developed by using the spray containing vanillin: ethanol: sulphuric acid (5:2:0.5). The R_f value of the spot was calculated by using the following formula.

$$R_f = \frac{\text{Distance of compound from origin}}{\text{Distance of solvent from origin}}$$

Characterization Techniques

UV, FTIR and investigation of the ethyl acetate endophytic fungi extract and mixing with methanol solvent solution were carried out. The UV -visible spectrophotometer was recorded between 250-600 wavelength number by using (Evolution 220 UV-visible spectrophotometer). The mixing solution were subjected to FTIR (Nicolet summit PRO spectrophotometer in the range 450 - 4500 Spectrometry). The FTIR spectrum was recorded between 4500-500 cm^{-1} using the KBr pellet mode, at Department of Chemistry, University of Yangon.

Results

Scientific Name – *Vitex trifolia* L.
Myanmar Name – Kyaung Pan -kalay
English Name – Indian Wild pepper
Family – Lamiaceae /Verbenaceae

Outstanding characters

Habit perennial, shrub, stem long, erect, hairy present, aromatic smell present. Leaf simple,3- trifoliate, opposite, short petioles, salivary hair present, exstipulate, ovate-oblong, serrate, acute. Inflorescence terminal panicle cyme. Flower pedicellate, bracteate, bracts small, ebracteolate, bisexual, zygomorphic complete, pale purple, hypogynous. Calyx (5), synsepalous, triangular acute, sepaloid, valvate. Corolla (5), syn petalous, bilabiate, corolla tube, petaloid (pale purple), upper lip 2-lobes, lower lip 3 lobes, imbricate. Androecium stamens 4, didynamous, epipetalous, anther dithecous, introrse, dorsifixed. Gynoecium 2, bicarpellary, syncarpous, ovary bilocular (early stage), tetra in mature stage, ovary superior, axile placentation one ovule in each locule, style long, stigma bifid. Fruit globose, salivary hair present.

Figure 2. Habit and Inflorescence of *Vitex trifolia* L.

Six isolated endophytic fungi from *Vitex trifolia* L.

Table 1. Cultural Characters and Colony Morphology of Six isolated Endophytic Fungi

Strains	Cultural characters		Colony Morphology		
	Front view	Reverse view	Form	Elevation	Margin
T1	white	white, light yellow at the center	circular	umbonate	entire
T2	light grey	Pink, black at the center	filamentous	umbonate	filiform
T3	white	orange ,white at the margin	filamentous	flat	curled
T4	white	pink	circular	crateriform	undulate(wavy)
T5	white	white, brown at the center	circular	umbonate	filiform
T6	light green	white	circular	flat	entire

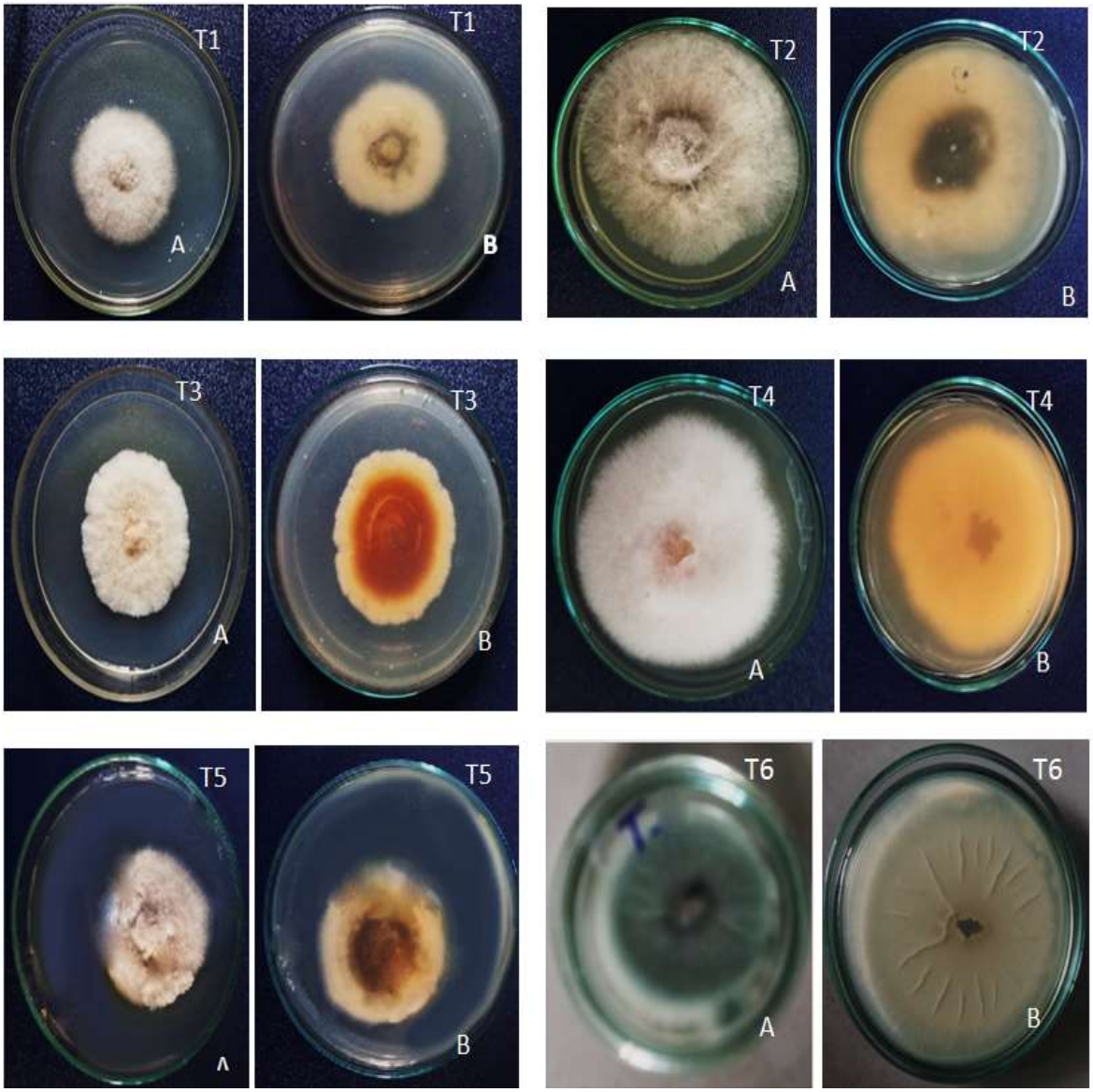


Figure 3. A. Front View, B. Reverse View of isolated Endophytic fungi T1,T2,T3,T4,T5 and T6.

Table 2. Test organisms and diseases

Test organisms	Code number	Diseases
Bacillus subtilis	JAP-0225025	Endocarditis, pneumonia, septicemia
Bacillus pumilus	IFO- 905571	Food poising the symptoms that resulted from infection included dizziness, headache, chill, back pain, stomach cramp, and diarrhea
Candida albicans	IFO-1060	Skin infection, vaginal candidiasis, alimentary tract infection, urogenital infection.
Micrococcus luteus	NITE-83297	Skin disease
Pseudomonas spp.	IFO-94307	Urinary tract infection, respiratory system infection, dermatitis, bone and joint infections, gastrointestinal infections. skin infection.
Staphylococcus aureus	ATCC-12877	Blood stream infection, burns, abscesses, skin disease, food poison wound infection, staphylococcal pneumonia.

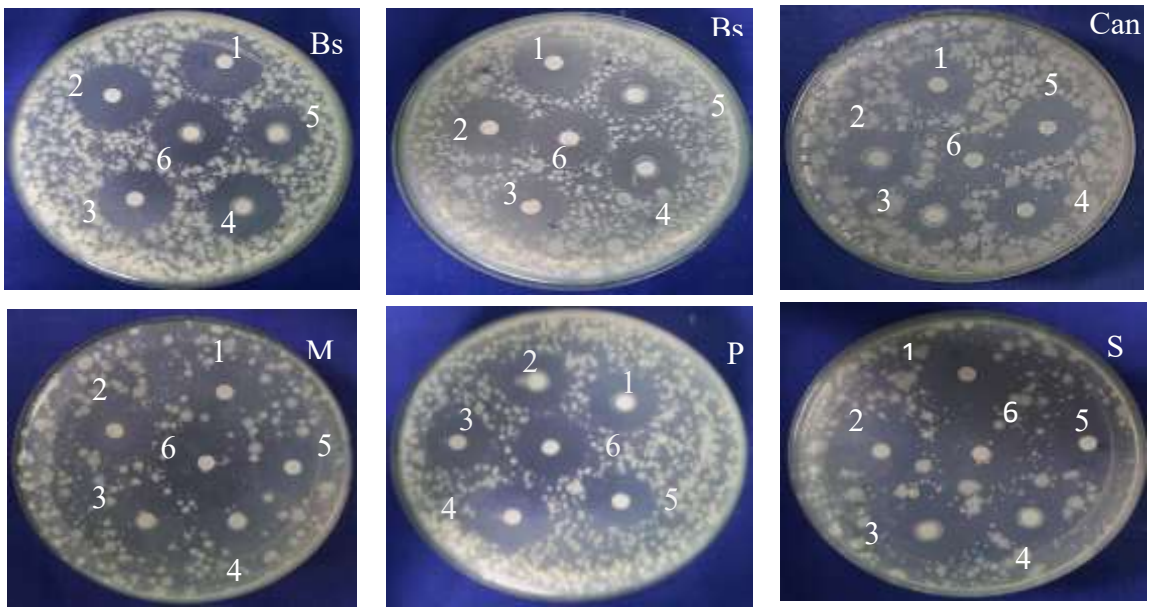
Screening for Antimicrobial Activity by paper disc diffusion method

In this screening it was observed that fungi T1, T2, T3, T4, T5 and T6 showed the antimicrobial activities on *Bacillus subtilis*, *Bacillus pumilus*, *Candida albicans*, *Micrococcus luteus*, *Staphylococcus aureus* and *Pseudomonas* spp. All strains were showed very high antimicrobial activity on six test organisms.

Table 3. Antimicrobial activities of isolated endophytic fungi from *Vitex trifolia* L. (Numbers shown in Tables are size of clear zones in mm)

Test organisms / Strains	***Bacillus subtilis***	***Bacillus pumilus***	***Candida albicans***	***Micrococcus luteus***	***Pseudomonas* spp.**	***Staphylococcus aureus***
T1	30	28	24	29	24	25
T2	30	30	26	27	26	26
T3	27	28	25	25	25	30
T4	28	26	22	27	23	30
T5	29	23	29	28	26	30
T6	30	25	26	30	27	30

Paper disc size = 6mm, 10mm-12mm weak activity, 13mm-17mm high activity, 18mm – above very high activity



BS= *Bacillus subtilis*, BP= *Bacillus pumilus*, Can= *Candida albicans*, M=*Micrococcus luteus*, P= *Pseudomonas* spp., S= *Staphylococcus aureus*

Figure 4. Antimicrobial activities of isolated endophytic fungi from *Vitex trifolia* L.

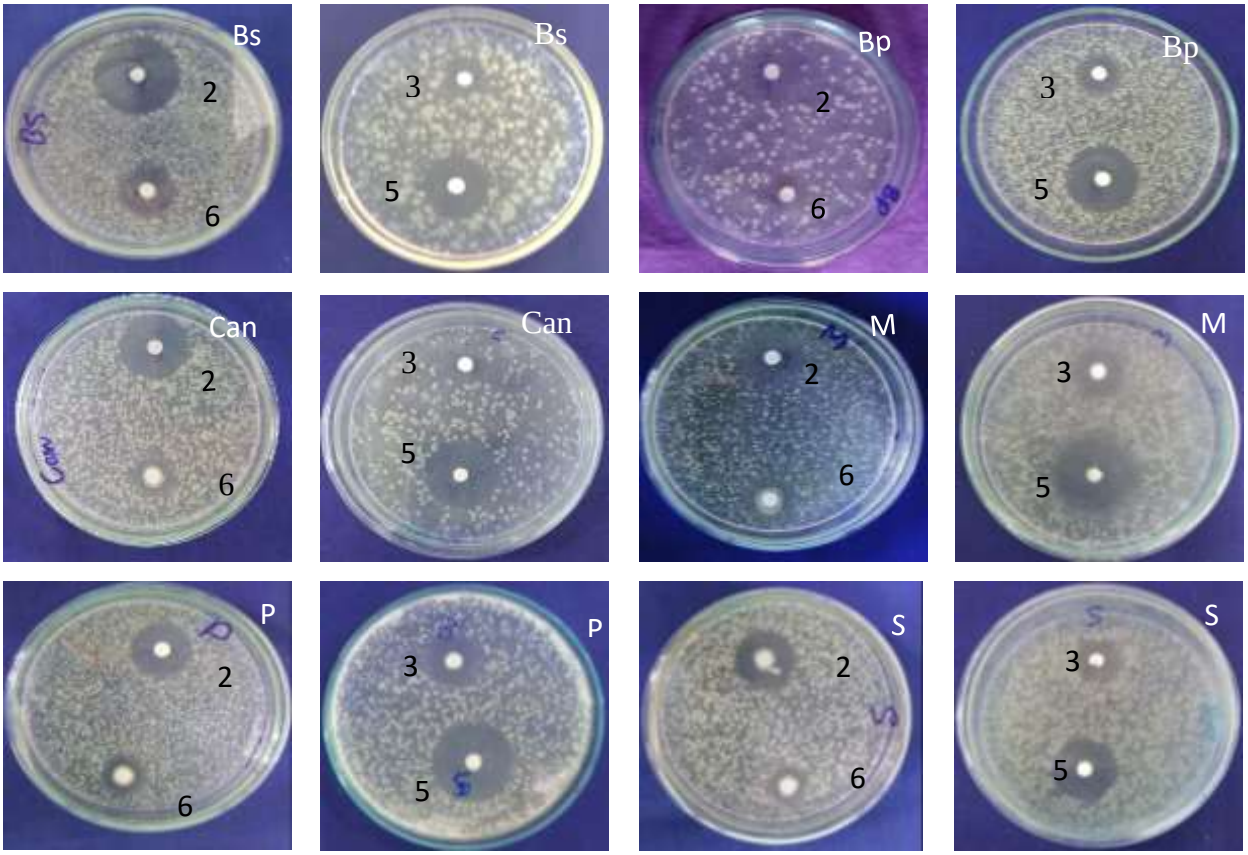
Screening for Antimicrobial activity of ethyl acetate extract from selected fungi T2,T3,T5 and T6 by paper disc diffusion method

In this experiment, only four isolates which revealed very high antimicrobial activity were selected for further investigation. They are T2, T3, T5 and T6. T2 extract was showed highest activity on *Bacillus subtilis, Bacillus pumilus Micrococcus luteus, and Pseudomonas* spp. T5 extract was found highest activity on all organisms and the rest of the extracts were showed high and very high activity on six test organisms.

Table 4. Screening for Antimicrobial activity of ethyl acetate extract from selected fungi T2,T3,T5 and T6 by paper disc diffusion method (Numbers shown in Tables are mm.)

Strains / Test of organisms	T2	T3	T5	T6
Bacillus subtilis	33	17	26	23
Bacillus pumilus	31	17	29	23
Candida albicans	23	20	27	20
Micrococcus luteus	27	23	36	23
Pseudomonas spp.	27	22	30	20
Staphylococcus aureus	22	19	26	16

Paper disc size = 6mm, 10mm-12mm weak activity, 13mm-17mm high activity, 18mm – above very high activity.



BS= *Bacillus subtilis*, BP= *Bacillus pumilus*, Can= *Candida albicans*,

M= *Micrococcus luteus*, P= *Pseudomonas* spp., S= *Staphylococcus aureus*

Figure 5. Antimicrobial activity of ethyl acetate extract from isolated endophytic fungi T2,T3,T5 and T6

Preliminary detection of metabolite in the crude extract of endophytic fungi T2,T3,T5 and T6 by Thin Layer Chromatography

Solvent system T2= Petroleum ether : Water: Ethyl acetate (9 : 3 : 2)

T3 = Chloroform : Methanol (8 : 2)

T5 = Hexane : Chloroform :Methanol (3 :2 :1)

T6 = Methanol :Ethyl acetate : Petrolium ether (9 : 3 : 1)

Spraying = Vanillin : Ethanol :Sulphuric Acid (5:2:0.5)

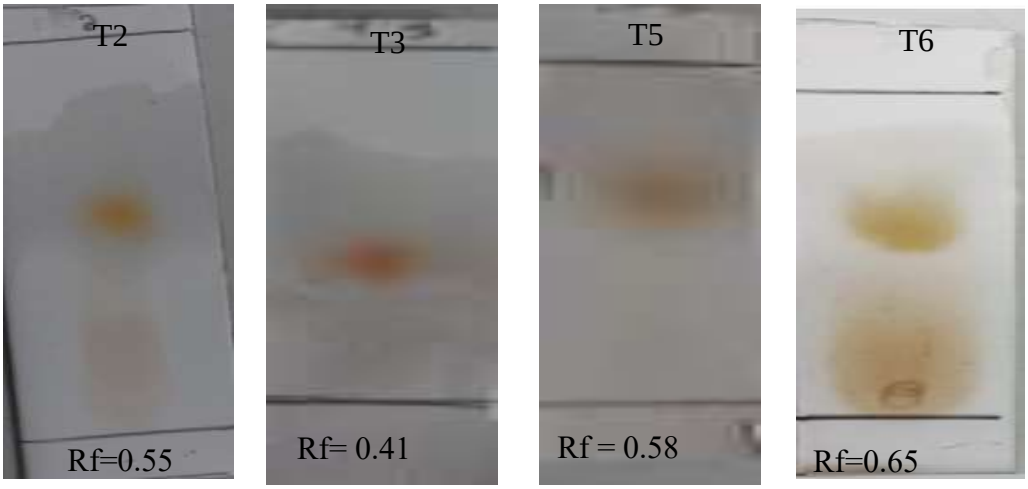


Figure 6. Crude bioactive compound from isolated endophytic fungi T2, T3, T5 and T6 by Thin Layer Chromatography (TLC)

Analysis of selected extract T2, T3, T5 and T6 by UV visible spectroscopy

The UV visible analysis data the wavelength number range 200 - 600 nm were recorded data of four selected extract sample. T2 was showed the wavelength number of (261, 283 and 303 nm), T3 showed the (290 and 403 nm), T5 was observed 297 and T6 was showed (294, 324 and 344 nm). This result was showed that the methanolic soluble compound on UV data.

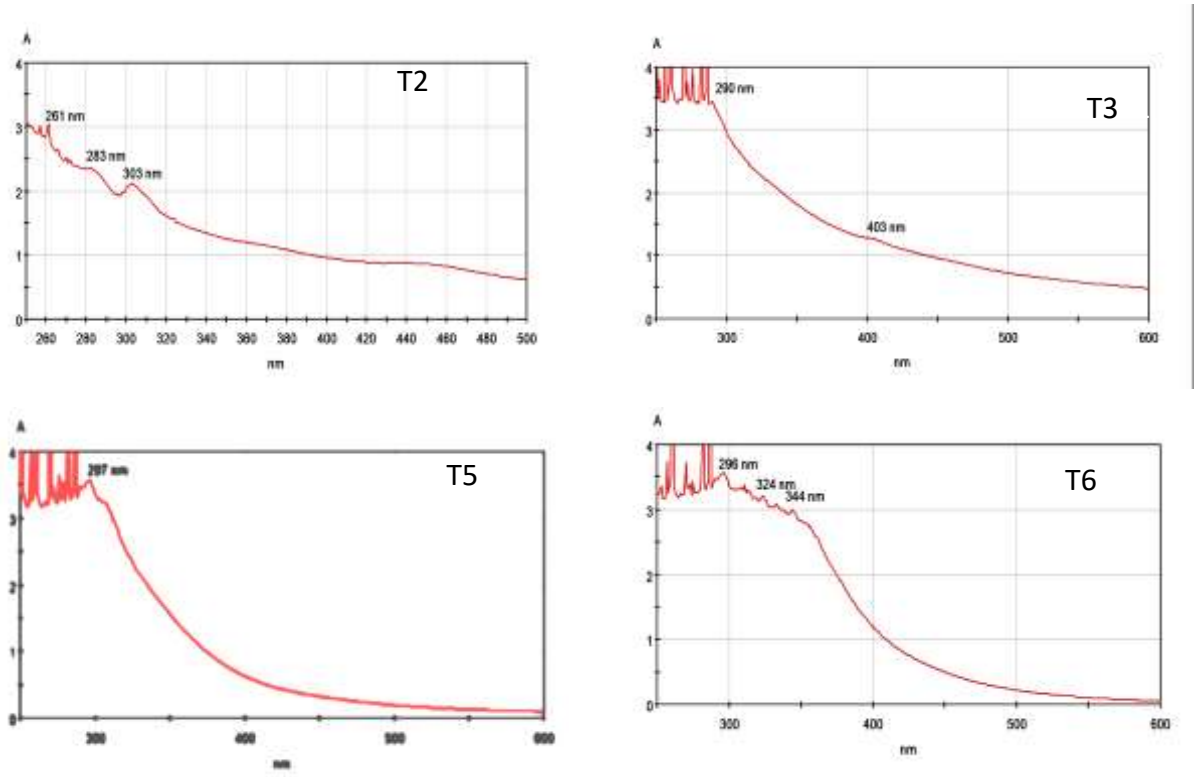


Figure 7. The UV data of four selected strains (T2,T3,T5,T6)

Infra-red spectral data

The FTIR spectra of the investigated T2, T3, T5and T6 ethyl acetate sample extract and mixing with methanol solvent which solution analysis of the synthesized have showed in Fig 8 The IR spectrum data was recording by using Nicolet IS 50 FTIR spectrophotometer in the range 500 - 4500 cm^{-1} with the FTIR spectrophotometer. The determination of FTIR spectrum was obtained from the following data. The main peaks and their assignment to functional groups of methanol mixing sample. The results showed characteristic strong absorption bands at 3351,3339.,3339., 3360, -OH stretching in alcohol, 2954, 2843 for -CH stretching for CH_3 group,2120,2115$cm^{-1}$for -C≡C stretching for acetyl group, 1643.1642,1634 for -C=O stretching for keto-enol system and at 1457,1453,1450, 1411$cm^{-1}$for C = C stretching for aromatic ring 1015 for C-O stretching in alcohol, 610,607,559 (C-N banding out of plane) respectively.

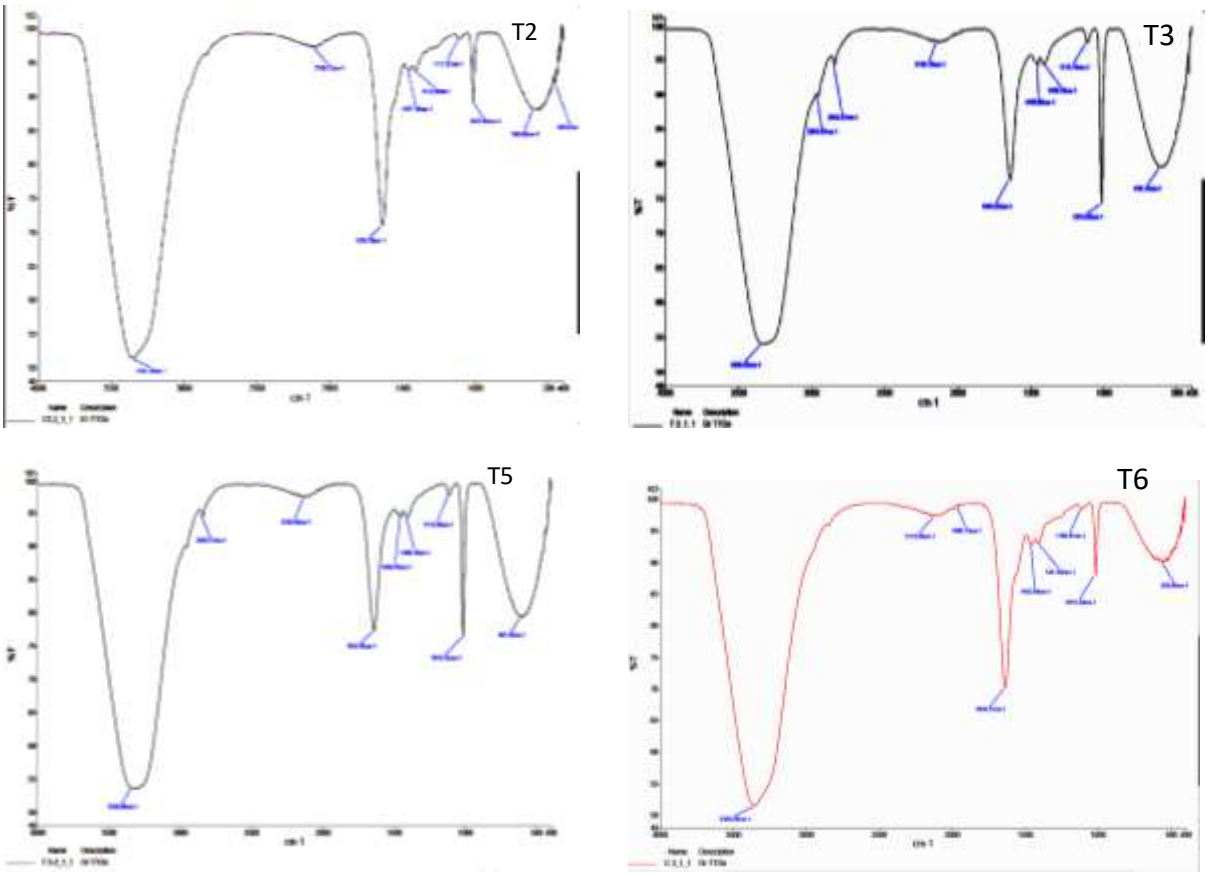


Figure. (8) FTIR spectral data of T2,T3,T5 and T6 sample extract solution

Discussion

Vitex trifolia L. plants were cultivated in tropical and subtropical area. Perennial sub-shrub, dioecious, salivary pubescent, pungent smell present, bilabiate tubular flower, purple. The fruit globose. According to this characters are in agreement with those mention by previous authors (Hooker (1885), Kirtiker and Basu. (1933). Backer and Bakhuizen, (1965), Julissa Rojas-Sandoval, (2022). Leaf , simple,3- trifoliate, opposite, short petioles, leaflet sessile , bracts small, , pale purple, triangular acute are agreement with those mention by Julissa Rojas-Sandoval , (2022).

The six isolated endophytic fungi (T1, T2, T3, T4, T5and T6,) from the leaves of *Vitex trifolia* L. Six strains were transferred to new plates (pure culture) and broth culture. And then fermentation process after one week, the fermented broth of endophytic fungi were tested with six

test organisms (*Bacillus subtilis*, *Bacillus pumilus*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas* spp., *Staphylococcus aureus*.). The first day, the isolated strains of T1, T5 and T6 were showed the highest antimicrobial activity on all test organisms. After second day, (T2,T3,) and T4 were observed the highest antimicrobial activity on (*Micrococcus luteus*) and *Candida albicans* . After three days, the antimicrobial activity indicated by size of clear zone was observed to be decreased in all isolates.

These crude bioactive compound was distinctly showed only one spot R_f value T2(0.55),T3 (0.41) ,T5(0.58) and T6(0.68) on thin layer chromatography (TLC) plate by using the solvent system of Petroleum ether : water: Ethyl acetate (9 : 3 : 2), Chloroform : Methanol (8 : 2), Hexane : Chloroform :Methanol (3 :2 :1),Methanol :ethyl acetate : Petrolium ether (9 : 3 : 1).the four extracted sample were showed the wavelength number of T2 (261,283,303), T3(290,403),T5(297), T6 (294,324,344) by checking with UV visible spectrophotometer and the functional group of C=O, C-H ,C-O ,CH_3 were observed in IR spectral data. Although there may be many points to clarify further, this research works shall to continue in the year 2024 to find out the actual nature of metabolites and genus and species of all isolates.

Conclusion

It can be concluded that ethyl acetate extract and methanol mixing solution of T5 provided the highest antimicrobial potential on six test organisms than the rest of T2, T3, and T6. All strains from *Vitex trifolia* L. leaves have showed good activity on six test organisms thus they have been abundantly bioactive compound and potentially wide range of medicinal effect. These fungi can be used to produce natural drugs, biopesticides, and biofertilizers that lead to decrease the dangers of synthetic chemicals. This can save the ecosystem and reduce the chemical residue in the environment (Laith, 2020). In the future, the bioactive compound from endophytic fungi of *Vitex trifolia* leaves can be used as natural drugs and endophytic fungi used for pesticides.

Acknowledgement

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ISOLATION OF ENDOPHYTIC FUNGI FROM THE LEAVES OF *MUNTINGIA CALABURA* L. AND THEIR ANTIBACTERIAL ACTIVITIES

Phyo Phyo Soe San[1], Shoon Lae Yee Htwe[2], Nway Nway[3]

Abstract

In this study, the endophytic fungi were isolated from the leaves of *Muntingia calabura* L. in family Muntingiaceae. The plant sources were collected at Yadanabon University Campus, Amarapura Township of Mandalay Region. A total of 7 endophytic fungi were isolated by using surface sterilization method. Morphological characters of endophytic fungi were investigated and were coded MCF-01 to MCF-07. In the study of antibacterial activity of endophytic fungi, six fungi showed the act against test organisms. Among them, the fungus MCF-01 showed the highest activity against on *Salmonella typhi*. This fungus MCF-01 was isolated from the leaves of *M. calabura* L.. In the fermentation studies, maximum antibacterial activity of fungus MCF-01 on *Salmonella typhi* reached at 6 days fermentation with 84 hrs ages (22.25 mm clear zone) and 20% sizes of inoculum (23.49 mm clear zone). Furthermore, distinct characters of selected endophytic fungus MCF-01 was observed with the help of microscope. Endophytic fungus MCF-01 can fight back against typhoid fever disease by *S. typhi.* in humans. Therefore, fungus MCF-01 was selected for the further investigation such as paper chromatography and extraction of antibacterial metabolite.

Keywords: endophytic fungi, morphological character, antibacterial activities

Introduction

Microorganisms are present everywhere on Earth that will support life. These include habitats we are all familiar with-soil, water, animal, and plant-as well as virtually any structures made by humans. (Prescott *et al.,* 2002).

Endophytic fungi live in their host plants and due to this; they must develop certain chemical strategies that favor their existence. By producing metabolites, the endophytic fungi either protect the host from animal or herbivores attack or from other pathogenic microbes infection that will decrease the fungi's colonization (Tan and Zou, 2001).

In this study, the isolation of endophytic fungi was investigated from *Muntingia calabura* L. grown in the Yadanabon University Campus. This plant is belonging to the family Muntingia. It is known as Jamaica cherry in English. Its Myanmar name is Hnget-thagya (Hundley and Chit Ko Ko, 1987). This plant is grown in tropical climate area. It is said to help diabetic patients. A small reduction was recorded in patient's blood sugar levels after consumption. The aim and objectives of this study are to isolate the endophytic fungi of *Muntingia calabura* L., to study the morphology, to investigate the antibacterial activities and to optimize the fermentation conditions for the antibacterial activities.

Materials and Methods

Collection of plant samples

Muntingia calabura L. was collected at Yadanabon University Campus, Amarapura Township of Mandalay Region, during January in 2022. The healthy plant leaves were utilized for the isolation of endophytic fungi. Photograph of the source plant is shown in following (Figure 1).

[1] Department of Botany, Yadanabon University
[2] Department of Botany, Yadanabon University
[3] Department of Botany, Yadanabon University

The identification of the source plant was referred from the Flora of Java Vol.I (Backer, and Brink 1963).

Isolation of Endophytic Fungi

In the isolation of endophytic fungi from the leaves of *Muntingia calabura* L. was carried out by surface sterilization method (Ando, *et.al*, 2004). The leaves of plants samples were washed in running water and then the leaves were cut into pieces. The leaves pieces were sterilized by soaking it in 95% alcohol for 15 seconds and then rinsed several times in sterile distilled water. Then, the piece was dried on sterilized paper and cut into 2 pieces. The pieces were placed on agar plates. After isolation procedures of endophytes from the leaves were done, piece of the cut leaves were placed on petri dishes containing Low Carbon Agar (LCA) medium (Glucose 0.1 g, K_2HPO_4 0.1 g, $MgSO_4$ 0.02 g, Yeast extract 0.02 g, Agar 1.8 g, Distilled water 100 mL, pH 6.5) supplemented with chloramphenicol (250 µg) to suppress bacterial growth and incubated at 27ºC until the outgrowth of endophytic fungi was discerned. The selected endophytic fungi were transferred and cultured in Potato Glucose Agar (PGA) medium (Potato 20.0 g, Glucose 2.0 g, Agar 1.8 g, Distilled water 100 mL, pH 6.5) incubated for 3-7 days. Purified endophytic fungi were cultured in slant agar (Potato Glucose Agar) for keeping availability of microbes as long as possible.

Screening of Antibacterial Activities by Paper Disc Diffusion Assay (Omura, 1985 and Petrini *et.al,* 1986)

The isolated fungi were grown at 25 to 27 °C for 7 days on PGA medium. The isolated fungi were inoculated into seed medium and incubated at 25 to 27 °C for 3 days. The 10 ml of seed culture (Glucose 2.0 g, Sucrose 0.3 g, Yeast extract 0.3 g, KNO_3 0.1 g, K_2HPO_4 0.001g, Distilled water 100mL, pH 6.5) were transferred into the fermentation medium (Glucose 1.0 g, Soluble starch 0.5 g, Yeast extract 0.5 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.01 g, Distilled water 100mL, pH 6.5). The fermentation was carried out for 7 days. After the end of fermentation, was alone the fermented broth (20 µl) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay (Figure 2). Paper disc having eight millimeter diameter (Advantec, Toyo Roshi Kaisha Co., Ltd., Japan) were utilized for antimicrobial assays. The assay medium (Glucose 1 %, Polypepton- 0.3 %, KNO_3 0.1 %, Agar 1.8 %, Distilled water 100 ml, pH 6.5) was used for the antimicrobial activity test. One percent of test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples were applied on the agar plates and the plates were incubated for 24 hours at 28 to 30 °C. clear zones surrounding the test discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms.

The test organisms used in paper disc diffusion assay were *Bacillus pumalis* NITE 47239, *Escherichia coli* AHU 5436, *Micrococcus luteus* NITE 83279, *Salmonella typhi* AHU 9793 and *Staphylococcus aureus* AHU 8465. The test organisms were supported by NITE (National Institute of Technology and Evaluation, Japan) and Faculty of Agriculture, Hokkaido University, Japan (Table 1).

Table 1. Test organisms used in antimicrobial activities (NITE)

NO.	Test organisms	Infections/ Discusses
1.	*Bacillus pumalis* (NITE 47239)	Fever
2.	*Escherichia coli* (AHU 5436)	Diarrhoea
3.	*Micrococcus luteus* (NITE 83279)	Food Spoilage
4.	*Salmonella typhi* (AHU 9793)	Typhoid Fever
5.	*Staphylococcus aureus* (AHU 8465)	Food Poisoning, Skin disease

Fermentation Studies for the production of antibacterial metabolite against *Salmonella typhi* (Omura, 1985)

Study on the effects of ages of inoculums on the fermentation

The stain MCF-01 was inoculated into the medium (Glocuse 0.1g, Yeast extract 0.2g, NZ amine type A 0.3 g, Distilled water 100 mL) and incubated for 72 hours. The culture samples (10mL) were checked in 12 hours intervals for the growth.

Study on the effects of sizes of inoculums on the fermentation

In this study, 5%, 10%, 15%, 20% and 25% of 84 hours seed culture were utilized for the fermentation. The fermentation was carried out 7 days and antibacterial activity was tested by paper disc diffusion assay.

Distinctive characters of Fungus MCF-01

For the study of morphology and microscopical characters, fungus MCF-01was cultured at 25°C on Potato Glucose Agar medium for morphology and Water Agar medium for photomicrograph.

Results

Outstanding characters of *Muntingia calabura* L.

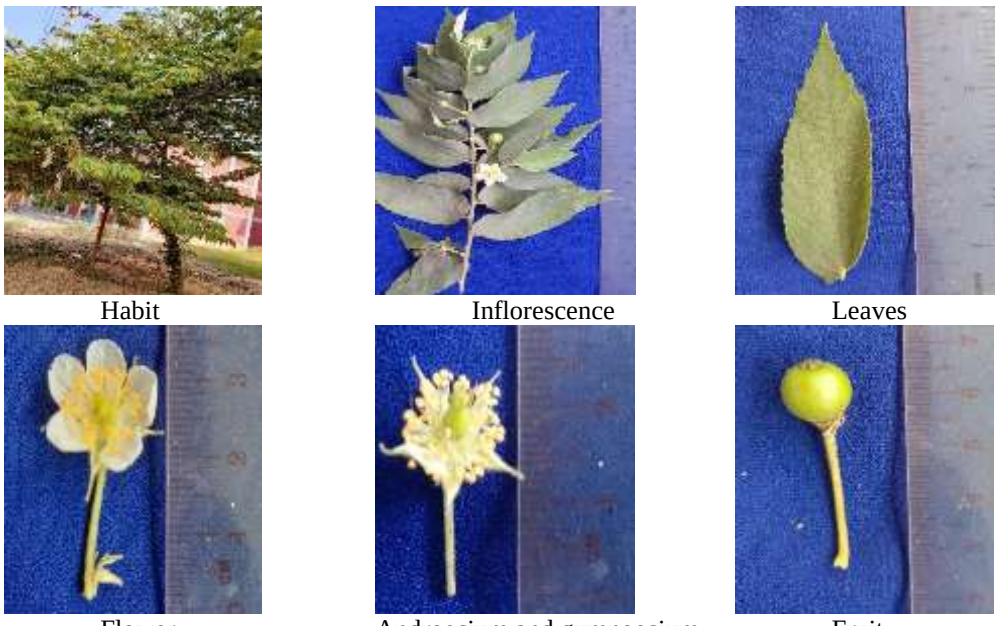


Figure.1. Morphological characters of *Muntingia calabura* L.

Family name : Muntingiaceae

Scientific names : *Muntingia calabura* L.

Myanmar name : Hnget-thagya

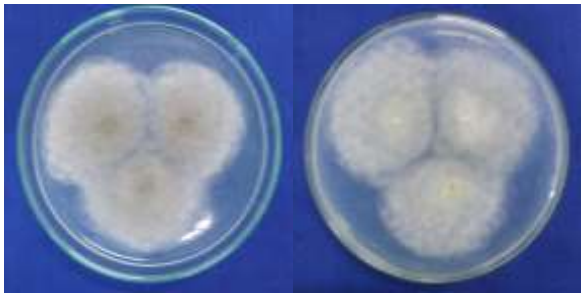
English name : Jamaica Cherry

Flowering period : throughout the year

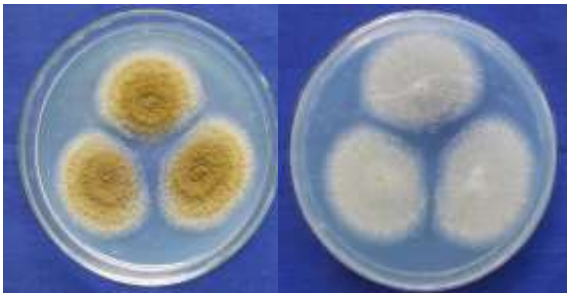
Perennial, shrubs or trees. Leaves simple, alternate; stipulate; petiolate; blades oblong-lanceolate, obliquely subcordate at the base, serrate along the margin**,** acuminate at the apex, softly pubescent beneath. Inflorescences supra-axillary, solitary cymes. Flowers bisexual, actinomorphic, pentamerous, hypogynous, white, pedicellate. Sepals 5, slightly connate at the base, lanceolate, green, densely pubescent. Petals 5, white, suborbicular, shortly clawed. Stamens numerous; filament filiform, connate at the base; anthers dithecous, ovoid, dorsifixed. Ovary superior, the axile placentae; style absent; stigma 5-lobed, glabrous. Fruit berry, sweet. Seeds numerous, minute.

Isolation of endophytic fungi

A total of 7 endophytic fungi were isolated from the leaves *Muntingia calabura* L. plant at Yadanabon University Campus. These endophytic fungi were coded MCF-01, MCF-02, MCF-03, MCF-04, MCF-05, MCF-06 and MCF-07 as shown in Figure 2.

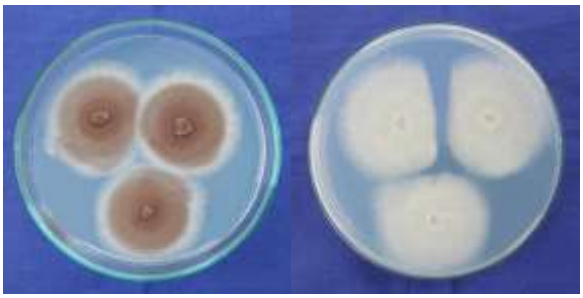


Front view and Reverse view of fungus MCF-01



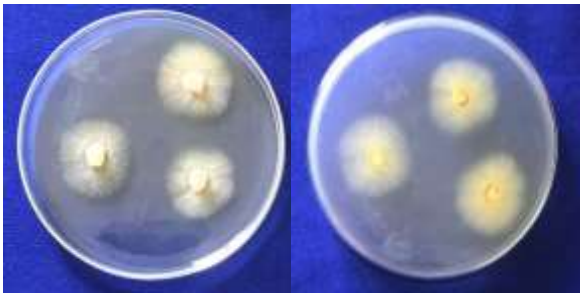
Front view and Reverse view of fungus MCF-02

Front view and Reverse view of fungus MCF-03

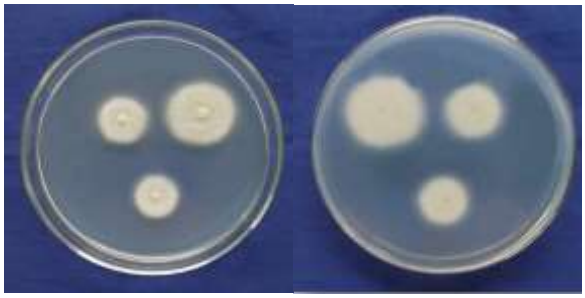


Front view and Reverse view of fungus MCF-04

Front view and Reverse view of fungus MCF-05



Front view and Reverse view of fungus MCF-06



Front view and Reverse view of fungus MCF-07

Figure.2. Morphologies of endophytic fungi on PGA medium

Table 2. The macroscopic characteristics of isolated endophytic fungi from the leaves of *Muntingia calabura* L.

No.	Isolated fungi	Color of Colony Front View	Color of Colony Reverse View	Size of the Colony
1.	MCF - 01	Smokey white	Smokey white	4.5 cm
2.	MCF - 02	Orange amber color	Pale yellow	4.0 cm
3.	MCF - 03	Milky white	Milky white	4.5 cm
4.	MCF - 04	Redish brown	Pale brown	4.0 cm
5.	MCF - 05	Dark slate grey	Greenish black	3.5 cm
6.	MCF - 06	Cream	Cream	2.5 cm
7.	MCF - 07	White	White	2.0 cm

Screening of Antibacterial Activities by Paper Disc Diffusion Assay

The study of 7 fungi were isolated from the leaves of *Muntingia calabura* L. collected at Yadanabon University Campus. During the study of biological properties of these fungi, six stains were exhibited antibacterial activities against *Salmonella typhi* (Figure 3).

Among them, fungus MCF-01 (22.07 mm clear zone) showed more hightly aelectibacterial activity against *Salmonella typhi.* than the other fungi. Therefore, this strain MCF-01 was selected for further investigations such as fermentation for the extraction of antibacterial metabolites.

Table 3. Antibacterial activity of isolated fungal strains

Stain No.	Inhibitory zone (mm) on *Salmonella typhi*
MCF-01	22. 07
MCF-02	17. 61
MCF-03	17. 78
MCF-04	18. 74
MCF-05	15. 02
MCF-06	12. 66
MCF-07	-

(-no activity)

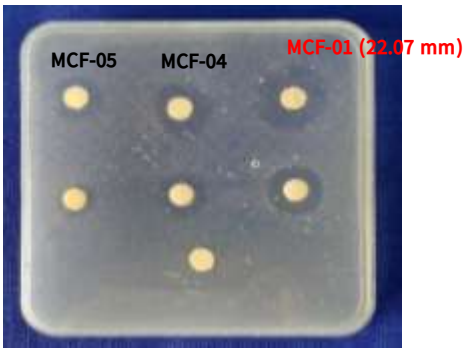


Figure. 3. Antibacterial activity of isolated fungal strains against *Salmonella typhi.*

Fermentation Studies for the Production of Antibacterial metaboliteagainst *Salmonella typhi*

It was observed that seed culture 84 hours age and 20% size were the best for the optimal fermentation as shown in Table 4 and 5, Figure 4.

Table 4. Effects of ages of inoculums on the fermentation

Culture time (hrs)	Inhibitory zone (mm) on *Salmonella typhi*
48	15.08
60	18.30
72	21.77
84	**22.25**
96	19.23

Table 5. Effects of sizes of inoculums on the fermentation

Size (%)	Inhibitory zone (mm) on *Salmonella typhi*
5%	16.54
10%	17.28
15%	20.48
20%	**23.49**
25%	20.76

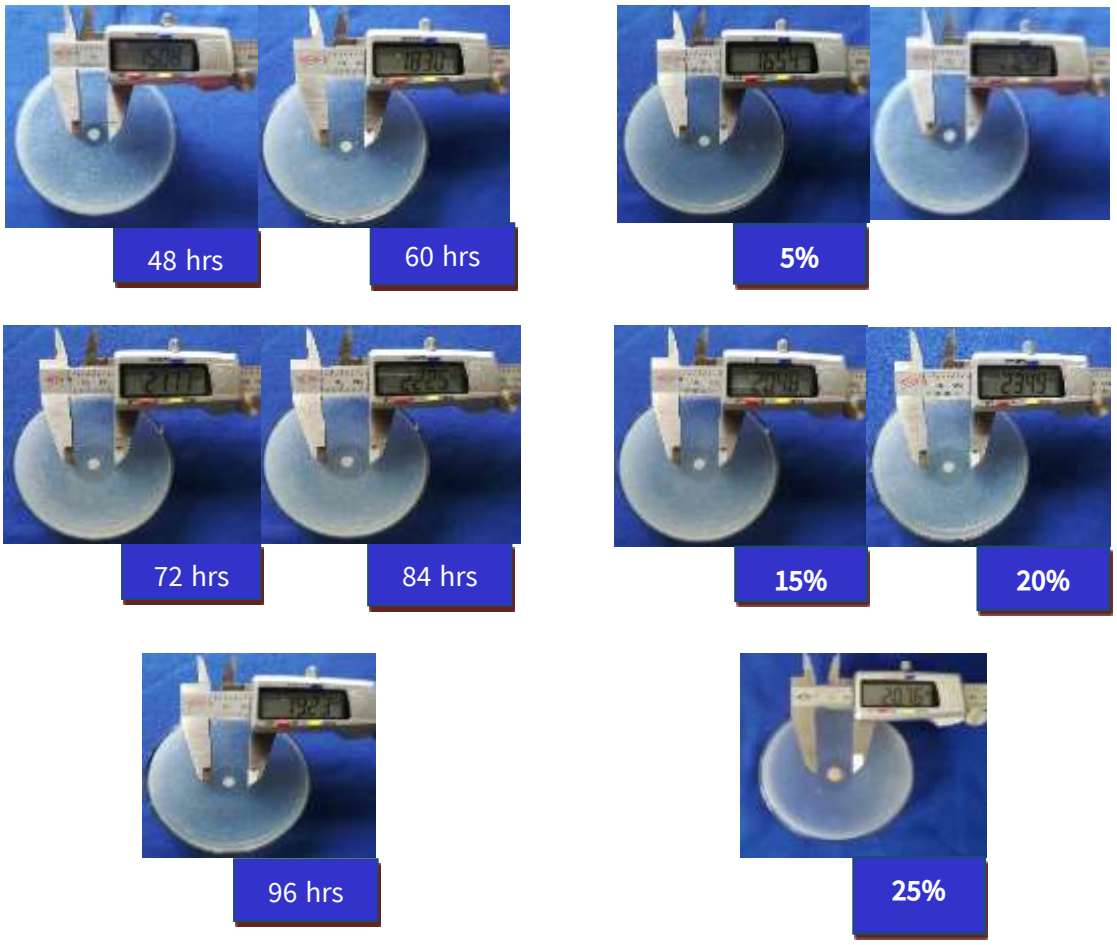


Figure. 4. The Effects of ages and sizes of inoculums on fermentation

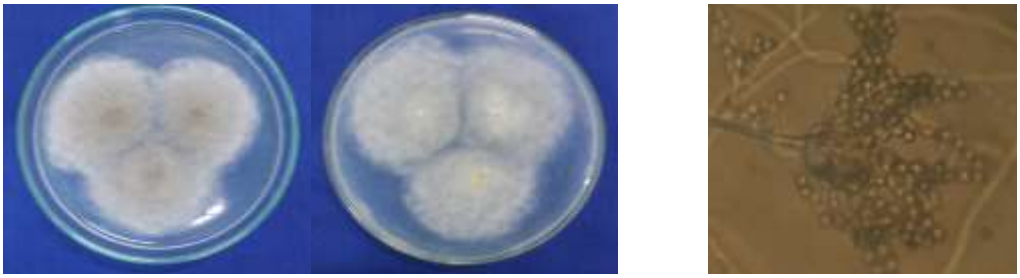
Distinct Microscopical characters of endophytic fungus MCF-01

In the investigation, microscopical study was done by planting the fungus on Water agar medium and incubating for 7 days. The growth appearance was then noted by observing both the reverse and front view of endophytic fungus MCF-01 was observed that the smokey white color.

The spore formation and mycelium of this endophytic fungus was studied with the help of microscope at ×40 magnifications. Distinct characters of fungus MCF-01 was observed and hyphae are long and abundantly branching; conidiophores, simple except at the apex where a cluster of thick, short phialides are produced, dark; conidia single, apical, globose or subglobose, brown, 1-celled (amerospore); some species also produce simple phialides and phialospores in chains, dark as shown in Table 4 and Figure 6. According to the reference of Ando, et al. (2004), this fungus MCF-01 was grouped as the fungi imperfecti.

Table 4. Morphology and Microscopical characters of Endophytic fungus MCF-01

Endophytic Fungus	Morphology	Hyphae Shape	Conidiophore Shape	Conidial Shape
MCF-01	surface and reverse colony color was smokey white color as a whole, the size of the colony reached about 4.0 cm in diameter for 5 days cultivation at 25±30℃	long and abundantly branching, septate	simple except at the apex where a cluster of thick, short phialides are produced, dark,	single, apical, globose or subglobose, brown, 1-celled (amerospore); some species also produce simple phialides and phialospores in chains, dark,



Front view Reverse view Photomicrograph (×40)

Figure. 5. Morphology and Photomicrograph of Endophytic fungus MCF-01

Discussion and Conclusion

A total of seven endophytic fungal stains were isolated from the leaves of the *Muntingia calabura* L. when cultured in the laboratory. These fungi are useful as a source of antibacterial metabolites.

In the investigation, these endophytic fungal stains were tested with five test organisms such as *Bacillus pumalis, Escherichia coli, Micrococcus luteus, Salmonella typhi* and *Staphylococcus aureus*. In this study, it was observed that six fungi (MCF-01, MCF-02, MCF-03, MCF-04, MCF-05 and MCF-06) were exhibited antibacterial against *Salmonella typhi* and remaining four test organisms did not show activity. Among them, fungus MCF-01 showed more activity against *Salmonella typhi* (22.07 mm clear zone) at 6 days fermentation period.

The fermentation studies for the antibacterial metabolite, it was observed that 84 hours ages of seed culture were optmized for fermentation. In the study of sizes of inoculum 20% contration,

it was the best for fermentation. The highest activity reached at 6 days fermentation with 84 hours ages and 20% sizes of inoculumns (23.49 mm clear zone) (Figure. 4).

The study of distinct morphological characters, the spore formation and mycelium of this endophytic fungus MCF-01 was observed with the help of microscope at ×40 magnifications.

Agung Bimantara *et.al* (2022), report that isolated fifteen endophytic fungi from *Muntingia calabura* L. and then investigated antifungal activity with *Candida parapsilosis*. These endophytic fungus (FDK-13) was found in inhibitory yeast growth of *Candida parapsilosis.*

The present studies isolated that endophytic fungus MCF-01 from the leaves of *Muntingia calabura* L. that was highest antibacterial activity against *Salmonella typhi.* Majority of fungi which produced secondary metabolites. That may be beneficial towards pharmaceutical effect and these metabolites are widely used in medicine. In conclusion, this selected endophytic fungus can be regarded as a source of antibiotic for human.

Acknowledgements

We wish to express our sincere gratitude to Dr Tint Moe Thuzar, Rector, Yadanabon University, for her permission to do this research. We are greatefully indebted to Dr Thwe Linn Ko, Dr U Khin Myot, Dr Myint Myint Oo and Dr Khin Maw Maw Soe Pro rectors, Yadanabon University, for their encouragement. Our special thanks go to Professor Dr Khin Pale`, Head of Botany Department, Yadanabon University, for her invaluable support and suggestions. We wish to thank, Dr Khin Myo Win and Dr Win Naing, Professors, Department of Botany, Yadanabon University for their kind help and advice.

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TAXONOMIC STUDY ON FIFTEEN SPECIES FROM MANSI TOWNSHIP BANMAW DISTRICT, KACHIN STATE

Seng Nan[1], Nu Nu Yee[2], Hnin Hnin Yu[3]

Abstract

The floristic study on Angiospermae from Mansi Township, Banmaw District, Kachin State have been undertaken. It lies between 24º 15′ - 23º 45′ N Latitude and 97º 45′ - 96º 45′ E Longitude. The total area is 2932 square kilometers. The plant specimens were collected and identified from October 2020 to February 2022. Among them, 15 species belong to 14 genera of 8 Families were presented. In this study, 7 species of 4 Families were under group of monocots, 6 species belonging 3 Families were under rosids and the other 2 species belonging 1 Family were under asterids. In these species, 4 species were trees, 5 species were herbs and 3 species were shrubs and other species were climber and epiphytic. The leaf types of 2 species were compound and 13 species were simple. *Smilax perfoliata* Lour., *Begonia lipingensis* Irmsch. and *Begonia palmata* D. Don. possessed unisexual flower and the others were bisexual flower. An artificial key to the studied species was also constructed. The economically valuable timber species are *Grewia laevigata* Vahl., *Kydia calycina* Roxb. The most valuable medicinal species are *Curcuma petiolata* Roxb., *Kaempferia galanga* L. These valuable species are needed to be conserved as the long-term programme for natural vegetation of Mansi Township, Banmaw District, Kachin State.

Keywords: Taxonomic, Angiospermae, Trees, Shrubs, Herbs

Introduction

The flowering plants contribute massively to the world's primary productivity and are regularly the most important component of global biodiversity. Not only do they provide the crops that feed us, as well as ornamentals, medicines, poisons, fibers, oils, tannins, beverages and stimulants, and herbs and spices but also constitute the main structure of our terrestrial ecosystem and afford habitats for countless animals (Heywood, 2007).

In the present research, all the species of plants were collected from Mansi Township. The study area is located Banmaw District in the Kachin state of Myanmar. It lies between 24º 15′ - 23º 45′ N Latitude and 97º 45′ - 96º 45′ E Longitude. The elevation of Mansi Township is about 450 m above sea level. The total area is 2932 square kilometer and established by 20 villages tracts in township. It has many mountain ranges running from north to south. Forest vegetation depends on three mains factors such as optimal temperature, good rainfall and fertile soil.

The natural vegetation in Mansi area varies due to climate, topography and kinds of soil. The various types of natural vegetation found in this area are evergreen forest, semievergreen forest, mountain forest, dry and moist mixed deciduous forest and swamp forest. In the lowland and foothills, bamboo and a few scattered trees are found. Most of Mansi Township is covered with forests. Natural vegetation of the township varies according to the elevation and climate of the area.

The aim and objectives of this research work are to study the flowering plants of Mansi Township, to identify and classify the taxonomical characters of wild plants, and to record the floristic information of plant resources.

Materials and Methods

The specimens were collected from Mansi Township from 2020 October to 2022 February. Field observation was made by using GPS (Global Positioning System). Locations of the study area were also noted. The images of inflorescences and flowers were recorded by taking

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photograps. Then these specimens were kept into the plastic bags. The morphological characters of collected specimens were recorded by using a dissecting microscope.

The taxonomic identification of collected plants were carried out by referring to Hooker (1875-1897), Backer and Brirk (1963-1968), Dassanayake (2001-2003) and Hutchinson (1967). The family was systematically arranged according to APG IV (Argiosperm Phylogeny Group) system. The genera and species were also arranged alphabetically. Myanmar names were referred to Hundley and Chit Ko Ko (1987) and Kress *et al*. (2003). The valid names of species were checked in the website of international plant name index. The taxonomic descriptions were presented with their respective figures.

Results

List of collected species of flowering plant in Mansi Township were studied. The collected 15 species belong to 14 genera of 8 families. Arrangements of the Family for collected species are described in Table 1.

Table 1. List of the collected species from Mansi Township

Group	Order	Family	No.	Scientific name
Monocots	Liliales	Smilacaceae	1.	*Smilax perfoliata* Lour.
	Asparagales	Orchidaceae	2.	*Acampe papillosa* (Lindl.) Lindl.
			3.	*Dendrobium chrysotoxum* Lindl.
		Asparagaceae	4.	*Asparagus racemosus* Willd.
	Zingiberales	Zingiberaceae	5.	*Curcuma petiolata* Roxb.
			6.	*Globba orixensis* Roxb.
			7.	*Kaempferia galanga* L.
Rosids	Rosales	Rosaceae	8.	*Prunus cerasoides* D. Don.
			9.	*Rubus ellipticus* Sm.
	Cucurbitales	Bignoniaceae	10.	*Markhamia stipulata* (Wall.) Seem. ex. K. Shcum.
	Malvales	Malvaceae	11.	*Grewia laevigata* Vahl.
			12.	*Helicteres angustifolia* L.
			13.	*Kydia calycina* Roxb**.**
Asterids	Lamiales	Begoniaceae	14.	*Begonia lipingensis* Irmsch.
			15.	*Begonia palmata* D. Don.

1. ***Smilax perfoliata*** Lour., Fl. Coohinch. 2:622. 1790. (Figure 1)

Flowering period : March to May

Perennial monoecious vines; stems and branches terete, stout, restrorsely spines, green, glabrous. Leaves simple, alternate, exstipulate; blades orbicular or ovate-cordate. Inflorescences axillary, umbellate cymes. Flowers unisexual, staminate flowers, greenish-yellow; pedicels terete. Perianth segments 6, in two series. Stamens 6, exserted; anthers dithecous. Pistillate flowers, pale green. Perianth segments 6, in two series. Staminate present. Carpel 3, united; ovary superior; styles filiform, glabrous. Fruits berry.

Specimen examined: Kachin State, Mansi Township, In Ba Pa villages; 24º 02′ N, 97º 36′ E, Elevation 347m; 16 April 2021; Seng Nan, collection no. 7.

2. ***Acampe papillosa*** (Lindl.) Lindl. Fol. Orch. Acampe; 2. 1853. (Figure 2)

Saccolabium papillosum Lindl. Sp. Pl. 222. 1833.

Flowering period : October to January

Monopodial epiphytes. Leaves simple, alternate and distichous, entire margins, tips; leafy at anthesis. Inflorescences axillary corymbose, 10-to 15-flowered. Flowers bisexual, yellow with reddish- brown spots, fragrant; dorsal sepals oblong; the lateral sepals falcately oblong; the labellum not distinctly 3-lobed, white with purple spots, glabrous; anthercaps sub-globose; Pollinia 2. Fruits capsular**.**

Specimen examined: Kachin State, Mansi Township, Madang yang villages; 24º 25′ N, 97º 12′ E, Elevation 210 m; 16 December 2020; Seng Nan, collection no. 3.

3. ***Dendrobium chrysotoxum*** Lindl., Edward's Bot. Reg. 33: ad pl. 19. 1847. (Figure 3)

Flowering period : May to July

Sympodial epiphytes. Pseudobulbs one jointed, fusiform. Leaves simple, alternate, oblong lanceolate. Inflorescences terminal raceme, many-flowered. Flowers bisexual, yellow; floral bracts triangular; dorsal sepal ovate-oblong; lateral sepals oblong-obtuse, brightly yellow; lateral petals bright yellow; labellum orbicular, deeply yellow blotches on the lip; anthercaps oblongoid; Pollinia 4. Fruits capsular.

Specimen examined: Kachin State, Mansi Township, Au Ra Bum; 24º 01′ N, 97º 23′ E, Elevation 420 m; 20 July 2021; Seng Nan, collection no. 8.

4. ***Asparagus racemosus*** Willd., Sp. Pl. ed 4.2: 152. 1799. (Figure 4)

Flowering period : November to January

Perennial rhizomatous scandent shrubs. Leaves simple, spirally arranged, reduced to scales. Flowers bisexual, greenish-yellow or white; ebracteolate. Tepals 6, slightly connate at the base, oblong, deflexed after anthesis. Stamens 6; anthers dithecous. Carpels 3; ovary superior; style short; stigma trifid. Fruits baccate.

Specimen examined: Kachin State, Mansi Township, In Ba Pa villages; 24º 02′ N, 97º 36′ E, Elevation 347m; 5 December 2021; Seng Nan, collection no. 5.

5. ***Curcuma petiolata*** Roxb., Fl. Ind. 1: 36. 1820. (Figure 5)

Flowering period : June to August

Perennial rhizomatous herbs. Leaves simple, alternate and distichous, exstipulate; blades broadly lanceolate. Flowers bisexual, yellow. Calyx infundibuliform, 3-lobed, white. Corolla infundibuliform, 3-lobed, yellow, glabrous. Fertile stamen 1; anthers dithecous; staminodes 2, oblanceolate; labellum ovate, pale yellow with dark yellow blotch at the base. Carpels 3, united; ovary inferior; style terminal; stigma bilobed. Fruits capsular.

Specimen examined: Kachin State, Mansi Township, La Jawng; 24º 08′ N, 97º 23′ 02.53″ E, Elevation 350 m; 12 July 2022; Seng Nan, collection no. 12

6. ***Globba orixensis*** Roxb., Asiat. Res. 11: 3358, pl. 6. 1810. (Figure 6)

Flowering period : June to October

Perennial rhizomatous herbs. Leaves simple, alternate and distichous, exstipulate; blades elliptic-lanceolate. Inflorescences terminal lax panicles, cauline. Flowers bisexual, reddish orange. Calyx infundibuliform, 3-lobed. Corolla infundibuliform, 3- lobed. Fertile stamen 1. Carpels 3, united; ovary inferior; style filiform; stigma turbinate. Fruits capsular.

Specimen examined: Kachin State, Mansi Township, La Jawng; 24º 08′ N, 97º 23′ 02.53″ E, Elevation 350 m; 12 July 2022; Seng Nan, collection no. 13.

7. ***Kaempferia galanga*** L., Sp. Pl. 1: 3. 1753. (Figure 7)

Flowering period : June to November

Prostrate herbs. Leaves simple, opposite and decussate; blades suborbicular to broadly elliptic. Inflorescences radical, terminal dense spike. Flowers bisexual, white with lilac and brownish spot center. Calyx tubular, white. Corolla infundibuliform, white, brownish spot at the tips. Fertile stamen 1; anthers dithecous. Carpels 3, united; ovary inferior; style filiform; stigma subgloboid. Fruits not available.

Specimen examined: Kachin State, Mansi Township, La Jawng; 24º 08′ N, 97º 23′ 02.53″ E, Elevation 350 m; 27 July 2022; Seng Nan, collection no. 14.

8. ***Prunus cerasoides*** Buch-Han. ex D. Don., Prod. Fl. Nepal 239. 1825. (Figure 8)

Flowering period : December to February

Perennial tree. Leaves simple, alternate; blades lanceolate. Inflorescences terminal and axillary, cymes, 3- flowered. Flowers bisexual, dark pink. Calyx campanulate, 5-lobed. Petals 5, obovate. Stamens numerous, in 2 rows, exserted; anthers dithecous. Carpel 1; ovary inferior; styles simple, nearly as long as stamens; stigma simple. Fruits drupe ellipsoid.

Specimen examined: Kachin State, Mansi Township, Gat Rawn villages; 24º 16′ N, 97º 47′ E, Elevation 450 m; 6 January 2021; Seng Nan, collection no. 4.

9. ***Rubus ellipticus*** Sm., Cycl. 30: Rubus no. 16. 1819. (Figure 9)

Flowering period : December to February

Perennial shrubs, prickles, hooked. Leaves pinnately trifoliolate compound, alternate; leaflets 3, orbicular. Inflorescences terminal and axillary corymbose panicles, many-flowered. Flowers bisexual, white. Calyx campanulate, 5-lobed. Petals 5, obovate. Stamens numerous, exserted; anthers dithecous. Carpels numerous; ovary inferior; styles numerous, filiform; stigmas simple. Fruits aggregate.

Specimen examined: Kachin State, Mansi Township, In Ba Pa villages; 24º 02′ N, 97º 36′ E, Elevation 347m; 10 January 2021; Seng Nan, collection no. 5.

10. ***Markhamia stipulata*** (Wall.) Seem. ex. K. Shcum. Nat. Pflanzwnfan. 4.(3b): 242. 1895. (Figure 10)

Spathodea stipulata Wall., Pl. Asiat. Rar. 3: 20. 1832

Flowering period : November to April

Perennial trees. Leaves imparipinnately compound, opposite, exstipulate; leaflets 4- to 8- pairs, elliptic to elliptic ovate. Inflorescences terminal, racemes, 4- to 10- flowered. Flower bisexual, pale yellow; ebracteolate. Calyx closed at anthesis, laterally divided to base, brown-

yellow. Corolla infundibuliform, 5-lobe. Stamens 4, didynamous, inserted; anther dithecous. Carpels 2, united; ovary superior; style curved; stigma 2-lipped. Fruits capsule.

Specimen examined: Kachin State, Mansi Township, Au Ra Bum; 24º 01′ N, 97º 23′ E, Elevation 420 m; 17 January 2021; Seng Nan, collection no. 6.

11. ***Grewia laevigata*** Vahl. Symb. Bot. 1:34. 1791. (Figure 11)

Flowering period : September to November

Perennial trees. Leaves simple, alternate; leaf blades oblong-lanceolate. Inflorescences axillary, racemes. Flower bisexual, yellowish green. Sepals 5, linear - lanceolate, yellowish white. Petals 5, oblong, pale green. Stamens numerous, exserted; anther monothecous. Carpels 2, united; ovary superior; styles cylindrical; stigma peltate. Fruits drupe.

Specimen examined: Kachin State, Mansi Township, Jan Mai villages; 24º 27′ N, 97º 19′ E, Elevation 380 m; 30 October 2020; Seng Nan, collection no. 2.

12. ***Helicteres angustifolia*** L., Sp. Pl. 2:963-964. 1753. (Figure12)

Flowering period : June to August

Perennial shrubs, covered with indumentun of stellate hairs. Leaves simple, alternate, distichous; blades oblong-lanceolate. Inflorescences axillary, cymes, 2- to many-flowered. Flowers bisexual, purple. Calyx bell-shaped, 5-lobes, yellowish green. Petals 5, obovate, pale purple. Stamens 10; anthers dithecous. Carpels 5, united; ovary superior; styles 5-branched; stigma globoid. Fruits follicles.

Specimen examined: Kachin State, Mansi Township, Sa Done Bum; 23º 09′ N, 96º 38′ E, Elevation 316 m; 12 August 2022; Seng Nan, collection no. 15.

13. ***Kydia calycina*** Roxb**.**, Pl. cor 3: 11, pl. 215, 1819. (Figure 13)

Flowering period : September to November

Perennial trees. Leaves simple, alternate; leaf blades orbicular-cordate. Inflorescences axillary or terminal, many flowered. Flowers bisexual, pink with reddish center. Calyx shallowly cup-shaped, 5- lobed, triangular, nearly as long as epicalyx. Petals 5; pink with reddish center. Stamens numerous, monadelphous, exserted; anther monothecous. Carpels 4; ovary superior; styles cylindric; stigma peltate, branches 3. Fruits capsular.

Specimen examined: Kachin State, Mansi Township, Jan Mai villages; 24º 27′ N, 97º 19′ E, Elevation 334 m; 30 October 2020; Seng Nan, collection no. 1.

14. ***Begonia lipingensis*** Irmsch., Mitt. Inst. Allg. Bot. Ham-burg 6: 353. 1927. (Figure 14)

Flowering Period : September to December

Perennial rhizomatous herbs. Leaves simple, alternate; blades broadly ovate. Inflorescences axillary dichotomous cymes, few-flowered. Flowers unisexual, pink. Male flower: tepals 4, pink. Female flowers: tepals 6, pink. Stamens numerous, monadelphous; anthers dithecous. Carpels 2, united; ovary inferior; styles fused at base; stigmas many-branched. Fruits capsular.

Specimen examined: Kachin State, Mansi Township, La Jawng; 24º 08′ N, 97º 23′ 02.53″ E, Elevation 350 m; 16 December 2021; Seng Nan, collection no. 11.

15. ***Begonia palmata*** D. Don, Prodr. Fl. Nepal. 223. 1825. (Figure 15)

Flowering Period : September to December

Perennial rhizomatous herbs. Leaves simple, alternate; blades asymmetrical ovate. Inflorescences axillary dichotomous cymes, few-flowered. Flowers unisexual, pinkish white. Male flowers: perianth 4, white to pink. Female flowers: perianth 5, pink. Stamens numerous, shortly monadelphous; anthers dithecous. Carpels 3, united; ovary inferior; styles fused at base; stigmas 2- branched. Fruits capsular.

Specimen examined: Kachin State, Mansi Township, La Jawng; 24º 08′ N, 97º 23′ 02.53″ E, Elevation 350 m; 13 September 2021; Seng Nan, collection no 9.

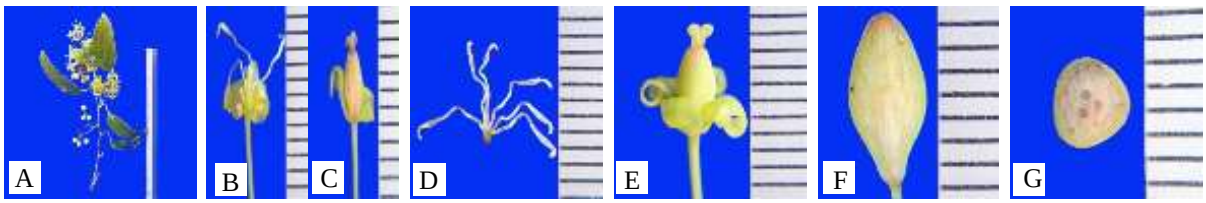

Figure 1. *Smilax perfoliata* Lour.

A. Inflorescence
B. L.S of Male Flower
C. L.S of Female Flower
D. Stamens
E. L.S of ovary
F. L.S of ovary
G. T.S of ovary

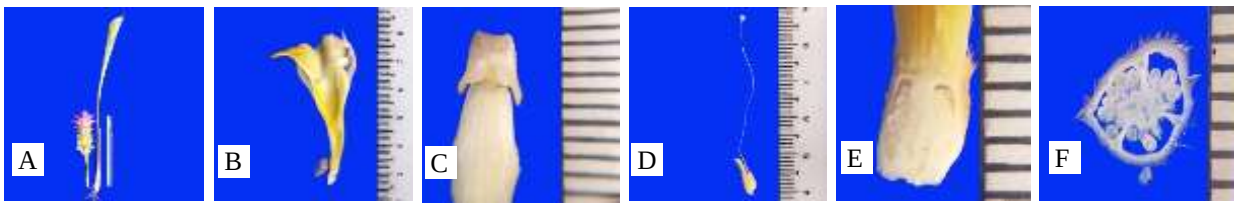

Figure 2. *Acampe papillosa* (Lindl.) Lindl.

A. Inflorescence
B. L.S of Flower
C. Pollinia
D. Pistil
E. L.S of ovary
F. T.S of ovary

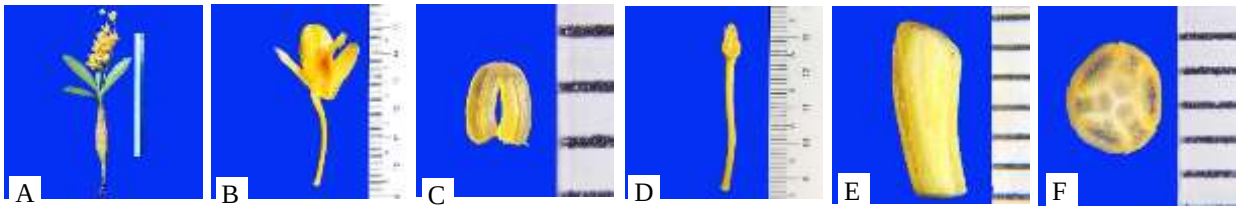

Figure 3. *Dendrobium chrysotoxum* Lindl.

A. Inflorescence
B. L.S of Flower
C. Stamen
D. Pistil
E. L.S of ovary
F. T.S of ovary

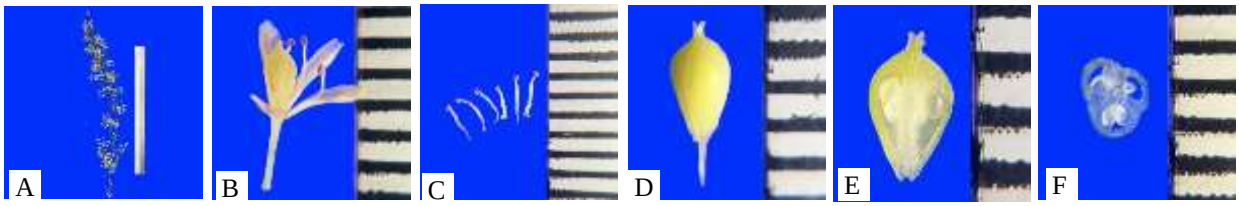

Figure 4. *Asparagus racemosus* Willd.

A. Inflorescence
B. L.S of Flower
C. Stamen
D. Pistil
E. L.S of ovary
F. T.S of ovary

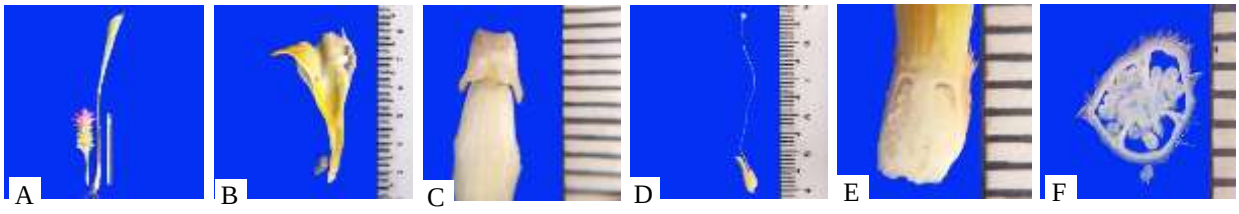

Figure 5. *Curcuma petiolata* Roxb.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

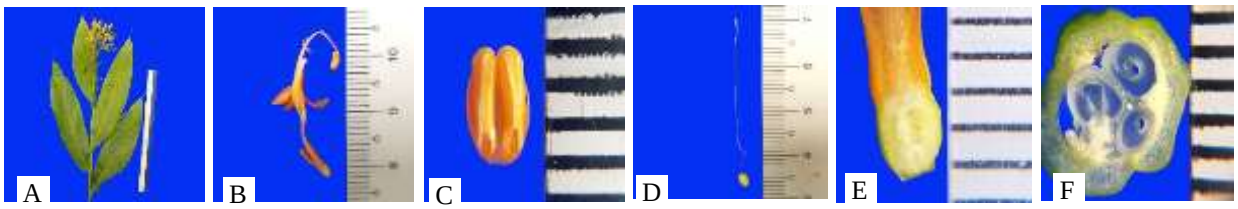

Figure 6. *Globba orixenis* Roxb.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

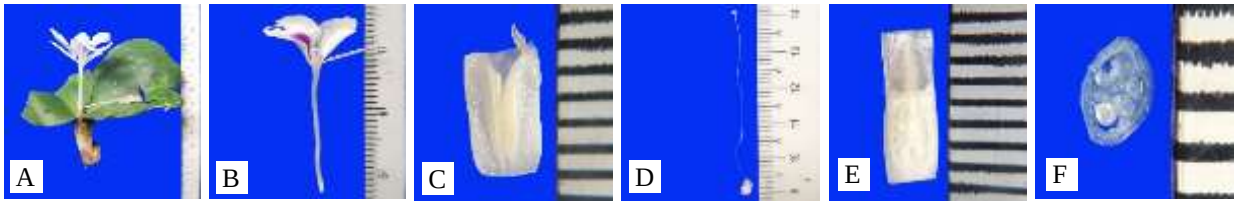

Figure 7. *Kaempferia galanga* L.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

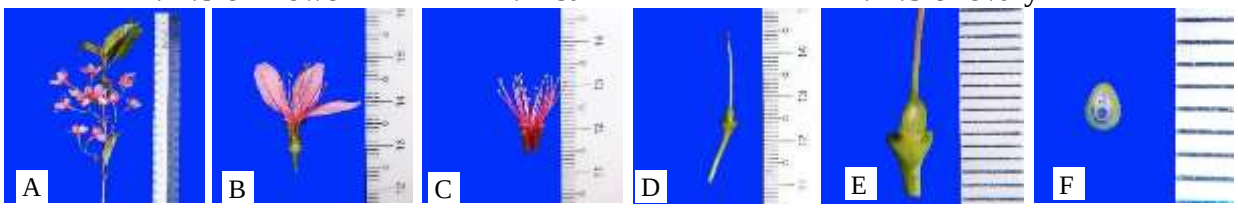

Figure 8. *Prunus cerasoides* D. Don.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

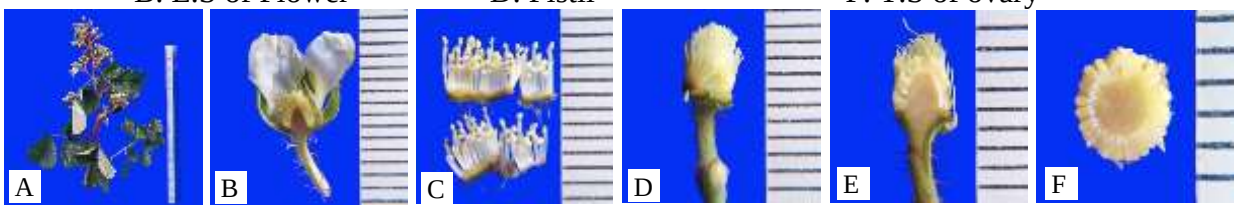

Figure 9. *Rubus ellipticus* Smith.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

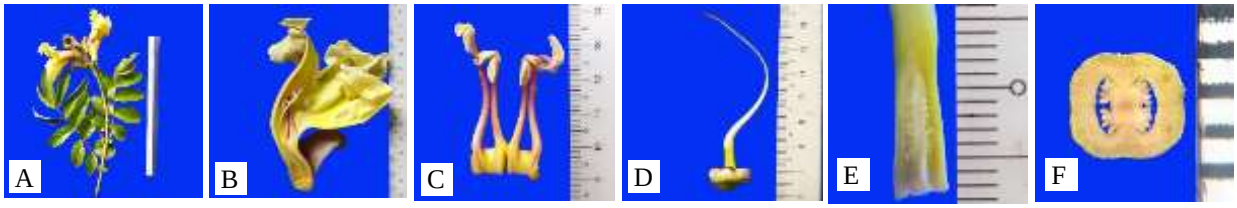

Figure 10. *Markhamia stipulata* (Wall.) Seem.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

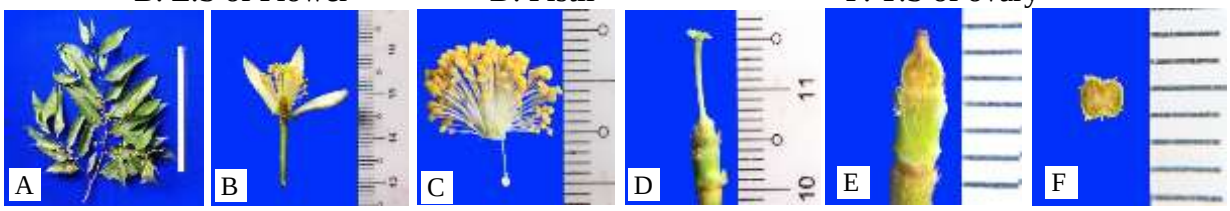

Figure 11. *Grewia laevigata* Vahl.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

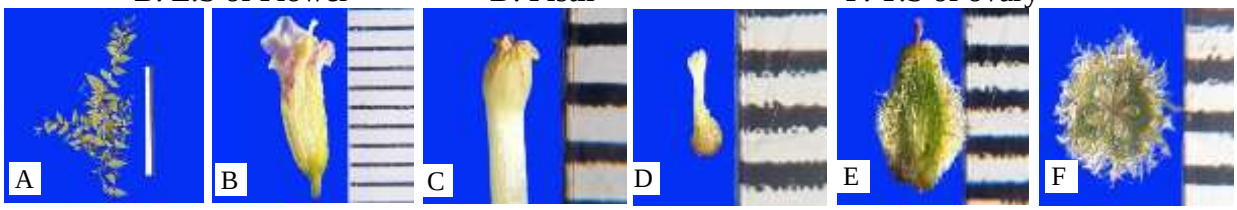

Figure 12. *Helicteres angustifolia* L.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

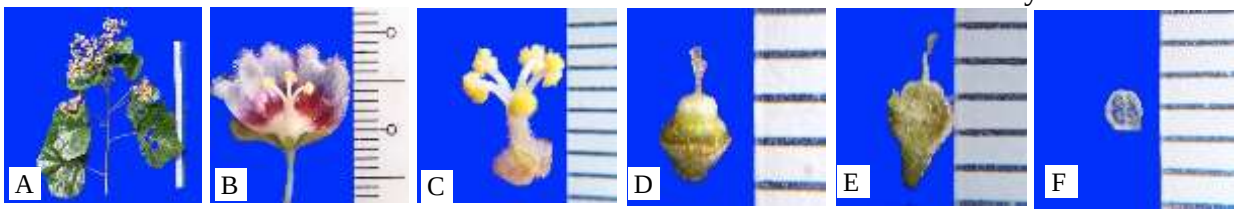

Figure 13. *Kydia calycina* Roxb.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

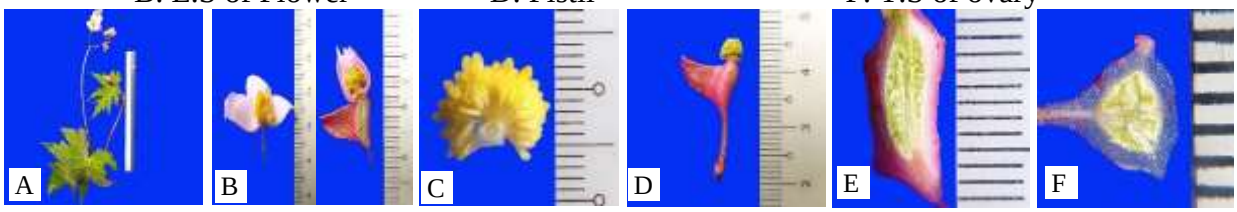

Figure 14. *Begonia lipingensis* Irmscher.

A. Inflorescence C. Stamen E. L.S of ovary
B. L.S of Flower D. Pistil F. T.S of ovary

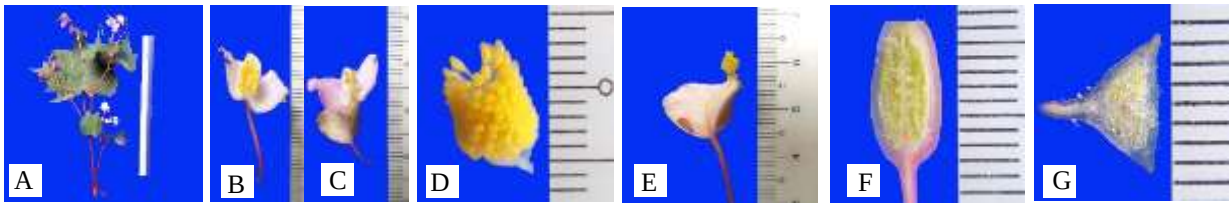

Figure 15. *Begonia palmata* D. Don

A. Inflorescence
B. L.S of Male Flower
C. L.S of Female Flower
D. Stamens
E. Pistil
F. L.S of ovary
G. T.S of ovary

An Artificial Key to the Studied Species

1. Plants monocotyledonous ---------- 2
1. Plants dicotyledonous ---------- 8
   2. Epiphytes or vines ---------- 3
   2. Shrubs or herbs ---------- 5
3. Flower unisexual, pollinia abscent; ovary superior ----------1. *Smilax perfoliata*
3. Flower bisexual, pollinia present; ovary inferior ---------- 4
   4. Plants monopodial; pseudobulbs absent, pollinia 2 ---------- 2. *Acampe papillosa*
   4. Plants sympodial; pseudobulbs present, pollinia 4 ---------- 3. *Dendrobium chrysotoxum*
5. Leaves opposite, stipulate ---------- 7. *Kaempferia galanga*
5. Leaves alternate or spirally arranged ---------- 6
   6. Stems with spines; Flower actinomorphic; ovaries superior ---------- 4. *Asparagus racemosus*
   6. Stems without spines; Flower zygomorphic; ovaries inferior ---------- 7
7. Labellum pale yellow with dark yellow blotch at the base; placentation axile ---------- 5. *Curcuma petiolate*
7. Labellum orange with a central brownish red spot; placentation parietal ---------- 6. *Globba orixensis*
   8. Plants trees ---------- 9
   8. Plants herbs or shrubs ----------12
9. Leaves arranged opposite, exstipulate; flower zygomorphic ---------- 10. *Markhamis stipulata*
9. Leaves arranged alternate, stipulate; flower actinomorphic ----------10
   10. Anther dithecous; ovary inferior; basal placentation ---------- 8. *Prunus cerasoides*
   10. Anther monothecous; ovary superior; axile placentation ---------- 11

11. Epicalyx present, flower pink with reddish center, ovary bicarpellary, anther dorsifixed ---------- 13. *Kydia calycina*

11. Epicalyx absent, flower yellowish white, ovary tetracarpellary, anther basifixed ---------- 11. *Grewia laevigata*

12. Prickles present; leaves compound; flower white ---------- 9. *Rubus ellipticus*

12. Prickles abscent; leaves simple; flower purple or pink ---------- 13

13. Flowers bisexual, zygomorphic; ovaries superior ---------- 12. *Helicteres angustifolia*
13. Flowers unisexual, actinomorphic; ovaries inferior ---------- 14
14. Ovary bicarpellary; stigma more than 4 branches ----------14. *Begonia lipingensis*

14. Ovary tricarpellary; stigma less than 3 branches ---------- 15. *Begonia palmata*

Discussion and Conclusion

The some angiospermae of Mansi Township area were taxonomically studied. As the result 15 species belonging to 14 generas of 8 families have been found in Mansi area.

In the present research, 2 species belonging to 2 genera of the family Orchidaceae are recorded. Orchidaceae differs from other families by its terrestrial or epiphytic, alternate and distichous leaves, lateral or terminal bracteate raceme or spike with zygomorphic flowers and capsule. These findings are in agreement with the characters stated by Heywood (2007), Simpson (2006) and Wu *et al.* (2008). Epiphytic plants were found on the trees and on rocks at the steep sided of valleys such as *Acampe papillosa* (Lindl.) Lindl. and *Dendrobium chrysotoxum* Lindl.

In the present study, totally 3 species belonging to 3 genera from the family Zingiberaceae are recorded. All the studied species are herbaceous with rhizomes. Most of these species are commonly found in moist and shady of the hill sides and used in medicines. *Curcuma petiolata* Roxb. is commonly and abundantly distributed at the elevation of about 1100 meters above sea level. These findings are in agreement with the statements presented by Dassanayake (1980-2001), Heywood (2007), Qi-ming and De-lin (2009) and Singh (2010).

The family Rosaceae is composed of about 100 genera and 3000 species nearly cosmopolitan in distribution but most common in temperate and subtropical parts of the Northern Hemisphere (Cronquist, 1981). 2 species belonging 2 genera were found in the study area. These findings are in agreement with the characters stated by Wu *et al.* (2008) and Qi-ming and De-lin (2009). The species *Prunus cerasoides* Buch-Han. ex D. Don., and *Rubus ellipticus* (Wall.) Seem. ex K. Shcum. have been aboundantly found in the everygreen forest.

The present study, family Malvaceae is composed of 50 genera and over 1000 species in cosmopolitan (Heywood 1978). 3 species belonging to 3 genera are recorded. Among them 2 speceis are woody trees and the rest are shrubs. *Grewia laevigata* Vahl. and *Kydia calycina* Roxb. are grown on the slope of the study area.

The morphological characteristics of all species were observed. Among the studied species, 4 species were trees, 5 species were herbs and 3 species were shrubs and other species were climber and epiphytic. In the studied species, the leaf types of 2 species were compound leaves and other species were simple leaves. The opposite leaves can be observed in the member of the Family of Bignoniaceae and the remaining species possess alternate leaves.

In the present studies, 3 species are unisexual flowers. *Smilax perfoliata* Lour., *Begonia lipingensis* Irmsch., *Begonia palmata* D. Don. possess unisexual flowers and the remaining species

are bisexual flowers. These findings are in agreement with those stated by Hooker (1875-1897), Dassanayake (1980-2001), Wu *et al* (2008) and Heywood (2007).

In Mansi Township, the valuable medicinal species are *Curcuma petiolata* Roxb., *Kaempferia galanga* L. Some orchid species are less commonly found. These species are being gradually lost due to logging, cutting and the impact of local dwellers. Therefore, the valuable medicinal species, economically timber species and orchid species should be conserved as the long-term programme of natural vegetation of Mansi Township, Banmaw Distirct in Kachin State.

Acknowledgements

Firstly, I am greatly indebted to Dr Nay Thwe Kyi, Rector, Banmaw University for her invaluable suggestions, her kind permission to perform this research and providing all the necessary facilities. I would like to mention my thanks to Dr Thu Zar Aung, Professor and Head, Department of Botany, Banmaw University, for their valuable advice. I am especially indebted to supervisor, Dr Nu Nu Yee, Professor, Curriculum Central Unit, Department of Education Research, Yangon, for her valuable advices and overall supervision with the constructive criticisms throughout my research works. I am very grateful to my co-supervisor Dr Hnin Hnin Yu, Associate Professor, Department of Botany, University of Mandalay for her suggestion. Finally, I am also thankful to all my colleagues for their kind assistance in collection of specimens during my field trips.

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STUDY ON THE ISOLATION OF SOIL FUNGI PRODUCING ANTIMICROBIAL METABOLITE FROM TU MYAUNG VILLAGE IN LABUTTA TOWNSHIP

Hnin Yu Hlaing[1], Soe Thuya [2] and Swe Swe Myat[3]

Abstract

In this research paper, fifteen soil fungi were isolated from four different soil samples of Tu Myaung village in Labutta Township of Ayeyarwady Region. A total of fungi were isolated on Low Carbon Agar (LCA) medium and Potato Glucose Agar (PGA) medium by using feeding and chemical treatment dilution methods. These isolated fungi were tested on seven test organisms by using paper disc diffusion assay method for the screening of antimicrobial activities. In the biological properties of isolated fungi, HY-08 showed highest antibacterial activity of clear zone (27.03 mm) against *Bacillus pumilus.* Therefore, this fungus (HY-08) was selected for further investigation. This fungus was studied for the age of culture and size of inoculum. According to the result, 60 hrs of ages and 20 % of sizes of inoculum were suitable for the best bioactive metabolite production of selected fungus.

Keywords: Feeding method, Chemical Treatment Dilution method, Paper disc diffusion assay method

Introduction

Soil microbiology is the study of organism in soil, their function and how they affected soil properties. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups has characteristics that define them and their function in soil (Omura, 1985).

Continuous use of chemical fertilizers over a long period may cause imbalance in soil mycoflora and thereby indirectly affect biological properties of soil leading to soil degradation. Fungi are an important component of the soil micro biota. Micro fungi play a focal role in nutrient cycling by regulating soil biological activity (Vinay *et al.*, 2015).

Distribution of soil fungi depend upon the nature of the organic content, climatic condition, surface vegetation and soil texture. Direct relationship is observed between the soil texture and moisture content. Silt and clay soil holds the highest moisture content that's why there is increased population of fungi is observed (Marchner *et al.*, 2003).

Antibiotics (metabolites) may be more useful than synthetic chemicals in the treatment and control of diseases. These metabolites are produced from microorganisms such as fungi, bacteria and actinomycetes (Kurtzman, 1992).

Microorganisms are the important sources of bioactive compounds with enormous potential to be developed as new molecules for drug discovery. Microorganisms grow in unique and extreme habitats that provide them the capability to produce unique and unusual metabolites. The antimicrobial properties of secondary metabolites from various groups of fungi are widely reported. Several fungal species produce bioactive compounds, secondary metabolites and

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chemical models having pharmaceutical importance. Antibiotics can be classified according to their mode of actions (Lambert, 1977).

Microbial growth kinetics is necessary to understand before fermentation to produce the metabolites. Proper cultivation ages and sizes are also crucial for the production of primary and secondary metabolites (Omura, 1985).

The composition of the fermentation medium must include the nutrient essential to support the growth of the microbial strain and the formation of the desired product. Production of secondary metabolites from microbial sources is greatly influenced by cultural and nutritional conditions used in the fermentation process (Sanchez *et al*., 2010).

Therefore, the aim and objectives of this research are to find out the various fungi in different soil samples, to observe the morphological characters, to study the antimicrobial activity and to know the proper cultivation ages and sizes of these isolated fungi.

Materials and methods

I. Area of Study

The soil sample was collected at Tu Myaung Village of Labutta Township in Ayeyarwady Region, July 2020. Tu Myaung village located coordinates in degrees, minutes and seconds (DMS) of 16° 21′ 00.9″N and 94° 41′ 38.9″E.

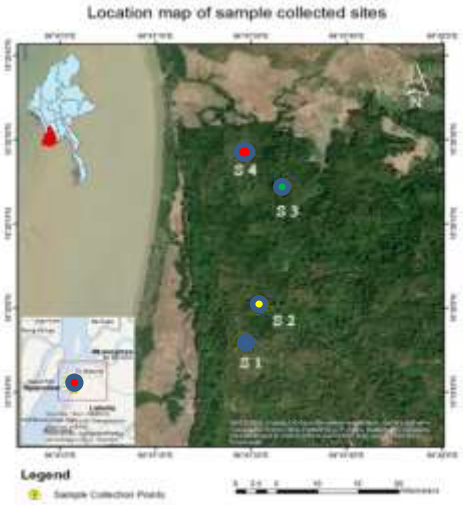


Figure.1. Tu Myaung village in Labutta township

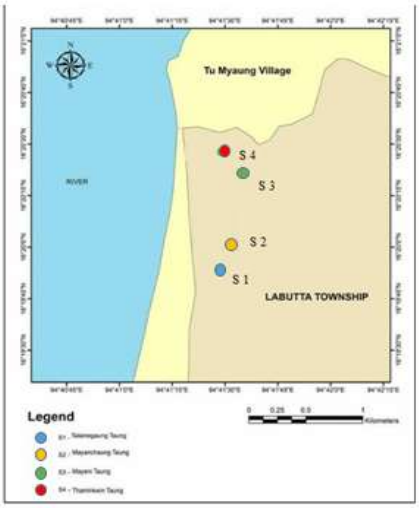


Figure.2. Tu Myaung village sites of soil samples

II. Collection of Soil Samples

Soil samples were collected from different forest fields (15 cm depth) with the help of spatula, in sterile plastic bags. The samples were properly labeled and brought to the Microbiology Laboratory, Department of Botany, Pathein University for further studies. The collected soil samples were transported to Department of Agriculture (Land Use) Yangon Township for tested the analytical texture and pH.

Table 1. Four different samples collected from four different places

Soil sample No.	Collected place	Soil type	pH	Location	Collected date
1.	Talainegaung taung	Clay	5.16	N 16˚19′ 43.897 ″ E 94˚41′ 19.478 ″	17.7.2020
2.	Mayanchaung taung	Clay	5.37	N 16˚20′ 52.012 ″ E 94˚41′ 40.527 ″	17.7.2020
3.	Mayeni taung	Clay loam	5.59	N 16˚20 ′ 34.362 ″ E 94˚41 ′ 30.584 ″	18.7.2020
4.	Thaminkwin taung	Clay	5.09	N 16˚20 ′ 26.463 ″ E 94˚41 ′ 32.486 ″	18.7.2020

III. Feeding Method (NITE, 2004)

Soil sample (1.0g) was poured into 100 mL SLN culture medium (Glucose 0.2g, sucrose 0.2g, K_2HPO_4 0.1g, $MgSO_4.7H_2O$ 0.05g, KNO_3 0.1g, KCl 0.05g, DW 100mL) and it was incubated overnight. Next day, 0.1mL soil suspension was transferred into 5mL of sterile water tube and shaked for minutes. And then, 0.5mL suspension was transferred into 4.5mL sterile water tube. Then 1.0 mL suspension was also transferred into 4.0mL sterile water tube. After shaking for minutes, 30 µL samples were cultured on low carbon agar medium (LCA medium) plates (Glucose 0.2g, Sucrose 0.2g, K_2HPO_4 0.1, KNO_3 0.1g, KCL 0.05g, Agar 1.8g, DW 100mL) and incubated for 5 - 10 days.

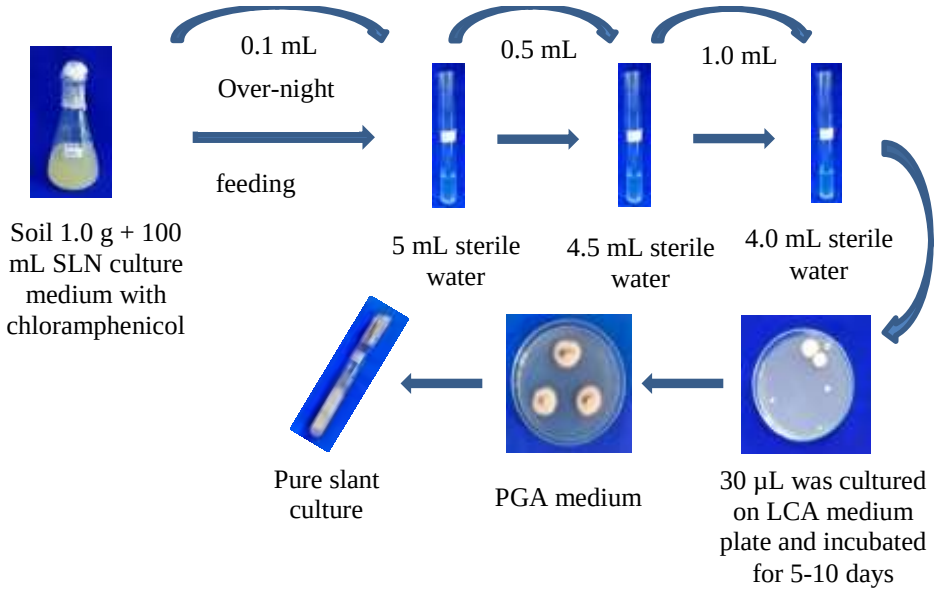


Figure.3. Procedure of feeding method for isolation soil fungi

IV. Chemical Treatment Dilution Method (Harayama and Kobayashi, 2005)

The collected soil samples were air-dried at room temperature, grounded and sieved. And then 2 g of soil samples was added into the 4 mL of sterile water tube. After 6 hours, 14 mL of 70% ethanol were added into this tube and then settle for 1 minute. 0.1 mL soil suspension from this tube was transferred into the 5 mL of sterile water tube, and then 0.5 mL soil suspension was transferred into the 4.5 mL of sterile water tube. After that, 1.0 mL soil suspension was transferred

into the 4.0 mL of sterile water tube and 30 µL soil suspension of this tube was placed on LCA medium plate. The plate was incubated for 3-5 days. After the colonies of fungi were observed on medium surface, isolated on PGA medium and stored as the slant cultures.

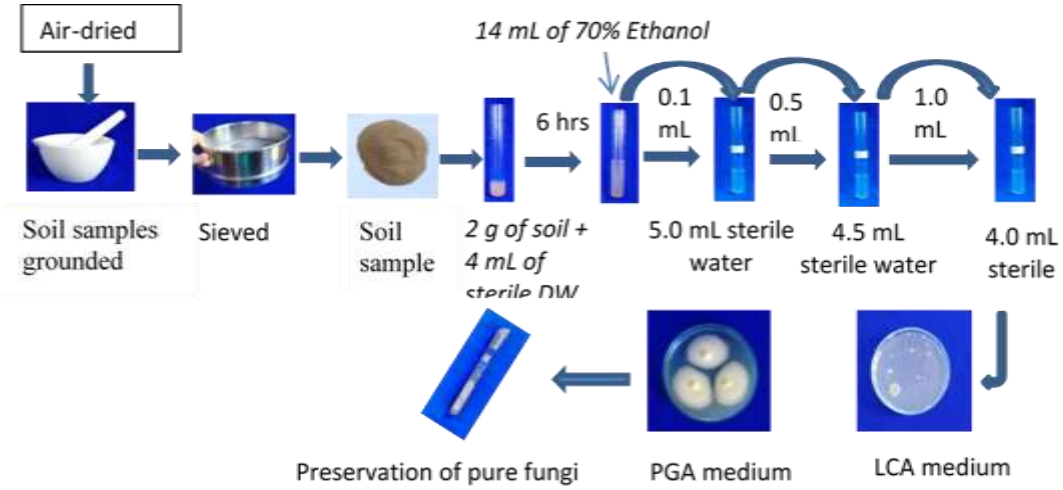


Figure.4. Procedure of chemical treatment dilution method for soil fungi

V. Preliminary Antimicrobial Activity by Paper Disc Diffusion Assay Method (Tomita, 1988)

The isolated fungi were incubated on PGA medium at room temperature for 3 days. Twenty mL of seed culture was transferred into the fermentation medium and incubated at room temperature for 5 days. 20 µL of fermented broth was put on the paper disc and placed on assay plate containing test organisms.

Table 2. Test organisms used in antimicrobial activities

No.	Test organisms	Diseases
1	*Agrobacterium tumefaciens*	Plant disease
2	*Bacillus pumilus*	Fever
3	*Candida albicans*	Candidosis
4	*Escherichia coli*	Diarrhoea
5	*Micrococcus luteus*	Skin disease
6	*Pseudomonas fluorescens*	Rice disease
7	*Staphylococcus aureus*	Boils and Food poisoning

Study on the Microbial Growth Kinetics of Fungus HY-08 (Omura, 1985; Crueger and Crueger, 1989)

The fungus HY-08 was inoculated into 100 mL medium (Glucose 1.0 g, Yeast extract 0.2 g, NZ amine type 0.3 g, pH 6.5) and incubated for 108 hrs at 100 rpm rotary shaker. The culture sample (5 mL) was checked in 12 hrs intervals for the growth. The sample (5 mL) was centrifuged at 2000 rpm for 30 mins and PCV% (Packed Cell Volume) was calculated.

Study on the Effects of Ages of Inoculum for the Fermentation (Omura, 1985; Crueger and Crueger, 1989)

A pure culture of selected fungus was transferred into the seed medium. According to the growth kinetic results, seed cultures were transferred into the fermentation medium at the incubation time (36 hrs, 48 hrs, 60 hrs, 72 hrs and 84 hrs), and tested antibacterial activities by using paper disc diffusion assay method for 7 days.

Study on the Effects of Sizes of Inoculum for the Fermentation (Omura, 1985; Crueger and Crueger, 1989)

A pure culture of selected fungus was transferred into the seed medium. 5%, 10%, 15%, 20%, 25%, 30% and 35% of seed culture (60 hrs) were transferred into the fermentation medium, and tested antibacterial activities by using paper disc diffusion assay method for 7 days.

Seed medium		**Fermentation medium**	
Glucose	2.0 g	Glucose	2.0 g
Peptone	0.3 g	Yeast extract	0.8 g
KNO_3	0.1 g	K_2HPO_4	0.01 g
K_2HPO_4	0.1 g	$MgSO_4$	0.01 g
DW	100 mL	$CaCO_3$	0.1 g
pH	6.5	DW	100 mL

Results

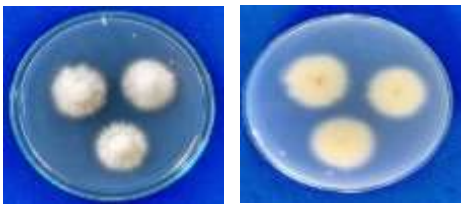
The results revealed the colony morphological characters of fungi from soil samples of Tu Myaung village. A total number of 15 fungi were isolated by chemical treatment dilution and feeding methods.

Table 3. Isolation of soil fungi from four different soil places

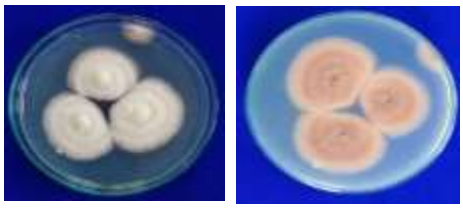
Soil samples	**Soil types**	**Isolated fungi by two methods**	
		Chemical treatment dilution method	**Feeding method**
S-1	Clay	HY-01 to HY-02	HY-03 to HY-04
S-2	Clay	HY-05 to HY-07	HY-08 to HY-09
S-3	Clay loam	HY-10	HY-11 to HY-12
S-4	Clay	HY-13 to HY-14	HY-15
	Total	**8 fungi**	**7 fungi**

Table 4. Morphological colours of isolated fungi

No.	Isolated Fungi	Morphological Color	
		Front Color	**Reverse Color**
1	HY - 01	White	White
2	HY - 02	White	Brown
3	HY - 03	Whitening pink	Reddish
4	HY - 04	Yellow	Yellowish
5	HY - 05	Yellow	Golden yellow
6	HY - 06	Centre yellow, white at margin	Yellow
7	HY - 07	Centre green, white at margin	Greenish yellow
8	HY - 08	White	Cream
9	HY - 09	Purple	Yellowish
10	HY - 10	Dark brown	Yellowish brown
11	HY - 11	Yellow	Yellowish
12	HY - 12	Cream	Yellowish
13	HY - 13	White	White
14	HY - 14	Gray	Grayish
15	HY - 15	Center green, white at margin	Pale green



(Front and reverse view of HY-01)



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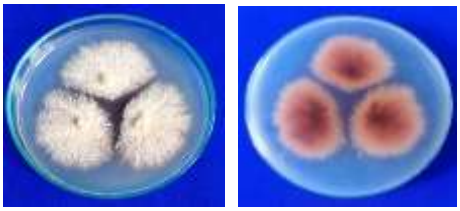

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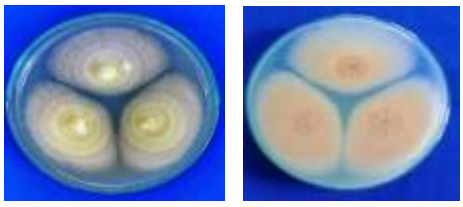

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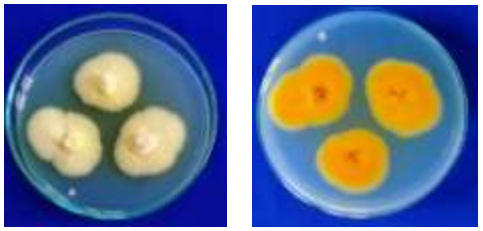

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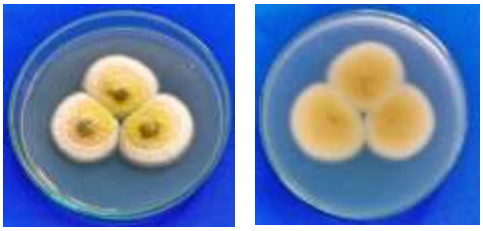

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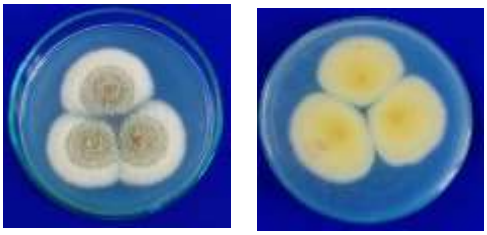

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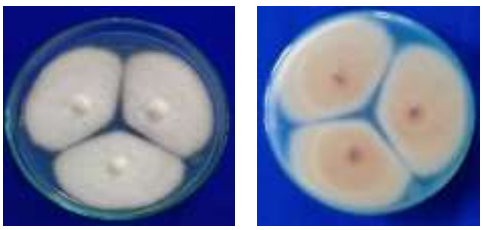

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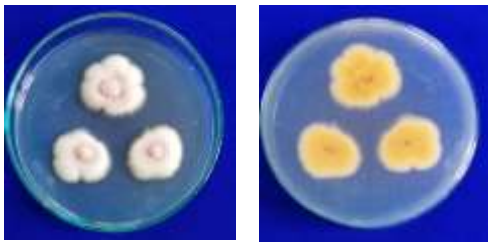

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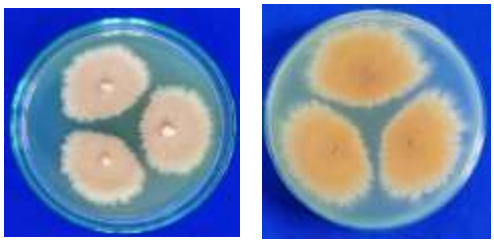

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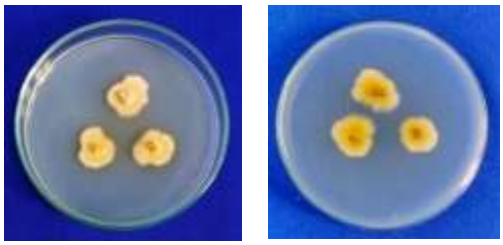

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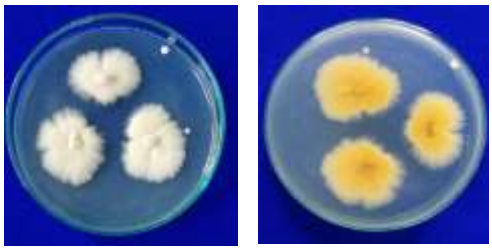

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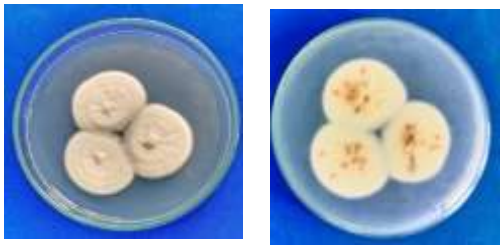

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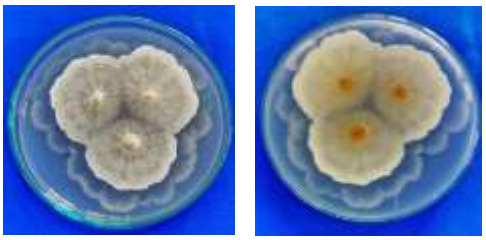

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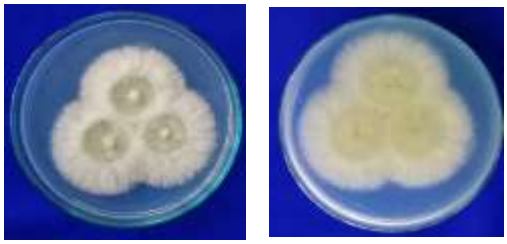

(Front and reverse view of HY-15)

Figure 5. Morphological characters of isolated fungi (7 days old culture)

Table 5. Preliminary of antimicrobial activities on seven test organisms

Fungi No.	Antimicrobial activities on test organisms (inhibitory zone, mm)						
	A. tumefaciens	***B. pumilus***	***C. albicans***	***E. coli***	***M. luteus***	***P. fluorescens***	***S. aureus***
HY-01	-	20.48	20.16	20.07	-	14.76	-
HY-02	19.00	21.53	21.53	-	18.32	15.77	10.00
HY-03	-	20.85	21.34	19.95	-	14.56	17.80
HY-04	-	22.13	24.28	-	14.74	19.34	-
HY-05	-	24.19	21.04	-	-	12.32	-
HY-06	12.56	25.22	24.13	22.50	14.32	14.62	-
HY-07	-	25.40	19.40	-	15.86	14.96	18.12
HY-08	12.24	27.03	26.79	26.22	23.25	-	-
HY-09	-	25.82	25.21	-	18.97	13.20	-
HY-10	-	23.85	25.00	-	22.75	10.76	15.70
HY-11	-	20.77	25.35	21.71	16.46	-	12.28
HY-12	-	21.00	-	24.42	14.34	12.43	-
HY-13	-	18.31	20.02	-	-	-	-
HY-14	-	24.17	-	-	-	-	14.36
HY-15	-	17.34	21.22	-	15.02	-	-

(-) = No activity Paper disc size = 8 mm

Bacillus pumilus *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas fluorescens*

Figure 6. Antimicrobial activities of isolated fungi on test organisms

The Effect of Ages of Inoculum on Selected Fungus HY-08 against *Bacillus pumilus*

According to the growth kinetic results, the selected fungus HY-08 was studied at the incubation times (36 hrs, 48 hrs, 60 hrs, 72 hrs and 84 hrs) for the age of seed culture.

Table 6. The effects of ages of inoculum HY-08 antibacterial activity against *Bacillus pumilus*

No.	Culture Times (Ages of culture)	Antibacterial Activity (Clear zone, mm)
1.	36 hrs	24.15
2.	48 hrs	25.92
3.	60 hrs	30.21
4.	72 hrs	26.58
5.	84 hrs	25.46

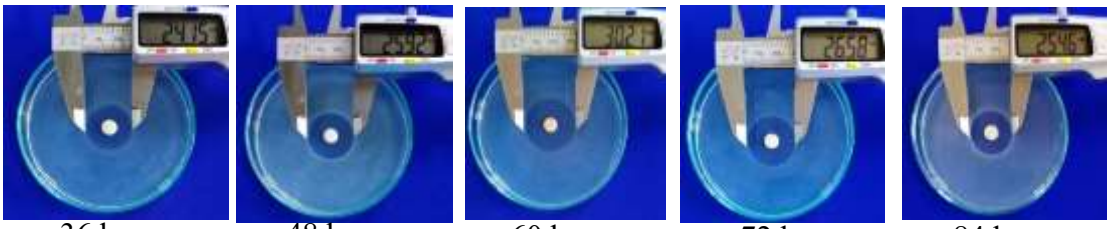

Fig.7. Antibacterial activity of ages of inoculum on *B. pumilus*

The Effect of Sizes of Inoculum on Selected Fungus HY-08 against *Bacillus pumilus*

In this study, 5%, 10%, 15%, 20%, 25%, 30% and 35% of seed cultures were utilized for the antibacterial activity. Incubated seed cultures were transferred as the above sizes into the fermentation conical flasks. Antibacterial activities were tested for seven days. The highest activity showed at 20% sizes of inoculum.

Table 7. The effects of sizes of inoculum HY-08 antibacterial activity against *Bacillus pumilus*

No.	Culture Times (Sizes of culture)	Antibacterial Activity (Clear zone, mm)
1.	5 %	21.00
2.	10 %	22.18
3.	15 %	23.12
4.	20 %	32.19
5.	25 %	26.72
6.	30 %	28.03
7.	35 %	25.35

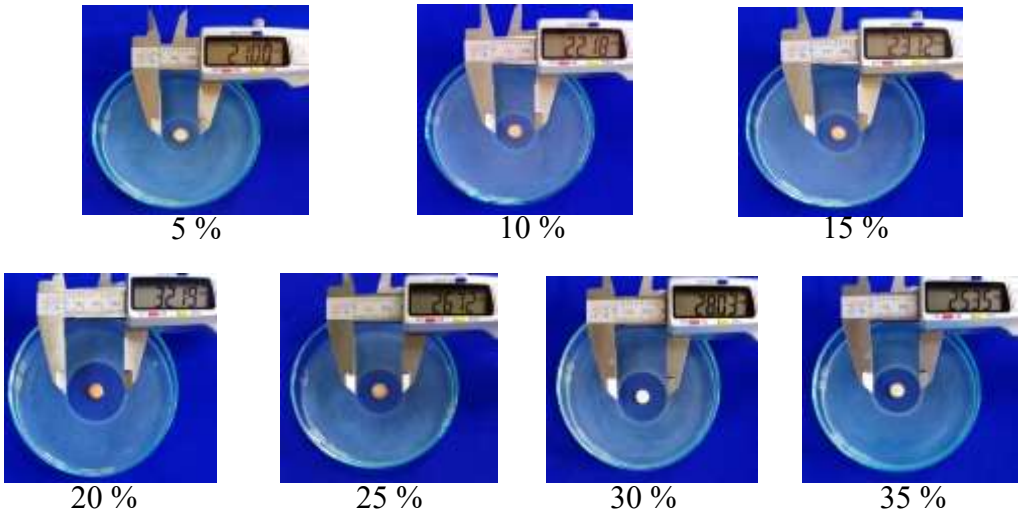

Figure.8. Antibacterial activity of sizes of inoculum on *B. pumilus*

Discussion and conclusion

Soil microorganisms have continually been screened for their useful biological active metabolites, such as antibiotics since long ago. Antibiotics have an important role in human health. Their necessity emerged from the spread of various diseases. As a result, scientists are trying to produce and discover more antibiotics. To help this purpose in some way, this research study was firstly started to discover antibiotics from four different soil samples from Tu Myaung village. The purification of the culture was done either by single spore isolation or by culturing of the hyphal tips on the LCA medium and then was transferred to fresh agar plates of PGA medium. Gaddeyya *et al.* (2012) reported that PGA or PDA medium is the most commonly used culture media and it was stated to be the best media for mycelia growth due to its simple formulation and potential to support wide range of fungal growth.

Two fungi from soil 1, two fungi from soil 2, two fungi from soil 3 and one fungus from soil 4 were also cultured by feeding method. In addition, two fungi from soil 1, three fungi from soil 2, one fungus from soil 3 and two fungi from soil 4 were isolated respectively by using chemical treatment dilution method. The pure isolated fungi were preserved by slants culturing.

In the report of Roberts (1998), antimicrobial agents play the most important role in the treatment of microbial infections and wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic producing microbes. The emergence of new diseases and reemergence of multiple-antibiotic resistance pathogens that render the effectiveness of existence clinically used antibiotics have spurred the needs for the discovery of new antibiotics. According to this aim, the isolated fungi were tested with seven test organisms; *Agrobacterium tumefaciens, Bacillus pumilus, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens and Staphylococcus aureus,* by using paper disc diffusion assay method. Exceptively *Agrobacterium tumefaciens* and *Staphylococcus aureus,* nearly all isolated fungi showed more antimicrobial activities on *B. pumilus* and *C. albicans*. Among these fungi, HY-08 was against more on *B. pumilus* than the other isolated fungi. So, this soil fungus (HY-08) was selected for further studies.

Borsa reported that *Bacillus pumilus* is a bacterium, though rarely, as the causative agent of various infections such as sepsis, endocarditis, skin infections and food poisoning in human. So, the study was continued against on *B. pumilus*.

Growth media and incubation conditions have a very strong influence of secondary metabolite production. Therefore, the growth kinetic was studied for the optimization of inoculum age. The selected fungus HY-08 was cultured at the incubation times (36 hrs, 48 hrs, 60 hrs, 72 hrs and 84 hrs) and tested the antibacterial activities. The highest antibacterial activity was found at 60 hrs (30.21 mm). For the proper size of inoculum, 5%, 10%, 15%, 20%, 25%, 30% and 35% of seed culture were inoculated and tested the antibacterial activities. The highest activity showed at 20% (32.19 mm) size of inoculum.

Variations in the fermentation environment often result in an alteration of antibiotic production. The alteration involves changes both in yields and in the composition of the substances. Therefore, the selected fungus HY-08 will be cultured base on these above results for further studies such as identification, extraction and purification of antibacterial compounds. This study may also contribute in providing information on the antibiotic producing microorganisms in soil.

Acknowledgements

We would like to express heartfelt gratitude to Dr Than Tun, Rector, Pathein University, Dr Yin Yin Aye Pro-Rector, Pathein University and Dr Nilar Kyuu, Pro-Rector, Pathein University, for permitting us to do this research. We are deeply indebted to Dr Moe Moe Aye, Professor and Head, Department of Botany, Pathein University, Dr Khin Soe Soe, Professor, Department of Botany, Pathein University, and Dr Naw Blute Tser, Professor, Pathein University for their warm encouragement and providing necessaries at the department. Finally, a special thanks to Dr Swe Swe Myat, Associate Professor, Pyay University for her invaluable encouragement, supervision and support from an early stage of this research and providing me extraordinary experiences throughout the work.

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MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF *ARTOCARPUS LAMELLOSUS* BLANCO (LEAVES)

Tin Lae Win[1], Khin Aye Aye San[2], Cho Cho Thin[3], Padauk Wah[4]

Abstract

The plant ***Artocarpus lamellosus*** Blanco belongs to the family Moraceae. It is collected from University of Yangon Campus, Kamayut Township during flowering and fruiting period in 2022. This plant is an unrecorded species in Myanmar. It possesses high medicinal value especially in the treatment of inflammation, malarial fever, ulcers, diarrhea, obesity and diabetes. Thus, this plant was selected for further investigation. The collected plants were classified and identified according to standard procedures. In this paper, morphological and histological characters of leaves were investigated. In morphological study, this plant was a monoecious tree with simple leaves, stipulate. Inflorescences (also called syncarp) were axillary and terminal, capitate. Flowers were yellow, unisexual. The fruits were syncarp (aggregate fruit). In histological study, epidermal cells of both surfaces were polygonal in shape with slightly wavy anticlinal walls. Stomata were found only the lower surface and anomocytic type. Cystoliths were present in epidermal cells of lamina and laticiferous were present on the surface view and transverse sections of midrib, petiole and stems. Unicellular and glandular trichomes were present on the surface of petiole and stem but midrib was present only one type unicellular trichomes. In addition, the powdered sample of leaves were investigated and presented as diagnostic characters for medicinal purposes.

Keyword: ***Artocarpus lamellosus*** Blanco morphological and histological characters

Introduction

The plant ***Artocarpus lamellosus*** Blanco belongs to the family Moraceae. It is widely distributed in Philippines. This family consists of 60 genera comprising 14,000 species distributed in the tropical and subtropical regions of Asia with all parts contain white latex. The plant is occouring in Southern China, Myanmar, Thailand, Indo-China, Malaysia. (Cronquist. 1981)

The plant ***Artocarpus lamellosus*** Blanco has also been used as traditional folk medicine in South-East Asia for the treatment of inflammation, malarial fever and treat the ulcers, abscess and diarrhea. The leaves and stem barks have been used to treat anemia, asthma, dermatitis, diarrhea, cough and as an expectorant. The latex mixed with vinegar promotes healing of abscesses, snakebite and glandular swellings. ***Artocarpus*** species are rich in phenolic compounds including flavonoids, stilbenoids, arylbenzofurons present in all plant parts and jacalin, a lectin present in seeds of certain ***Artocarpus*** species (Somashekhar, *et al*. 2013). Several pharmacological studies of the natural products from ***Artocarpus*** have conclusively established their mode of action in the treatment of various diseases. ***Atrocarpus lamellosus*** Blanco extract is as a pancreatic lipase inhibitor, and has antiobesity action, can be used for diseases such as prevention or treatment of obesity. (CN,2009)

The objectives of this paper are to botanical identification and histological characters of fresh specimen of ***Artocarpus lamellosus*** Blanco (leaves).

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Materials and Methods

Collection and Identification of Plant

Artocarpus lamellosus Blanco was collected from University of Yangon campus, Kamayut Township. The collection was done during the flowering period (January to March). Botanical identification of ***Artocarpus lamellosus*** Blanco was performed by verifying the references of Conquist (1981), Gardner, et al (2020) and Dassanayake (1981) in Department of Botany, University of Yangon.

Preparation of *Artocarpus lamellosus* Blanco leaf powder

The collected leaves were washed and dried in shade for one week. The dried leaves were ground to get powder and stored in air tight containers. The sensory and diagnostic characters of powdered leaves were studied.

Histological Study of *Artocarpus lamellosus* Blanco

The microscopical characters of fresh specimens were examined by freehand sections and under microscope. The microchemical tests for the presence of lignin, tannin were made according to the methods and reagents given in Metclafe and Chalk (1965), Esau (1953), Trease and Evans (2002) at the department of botany, University of Yangon. The following reagents were used to examine the free hands sections and powdered samples.

1. Chloral hydrate solution as the clearing reagent.
2. Phloroglucinol solution for testing the lignin.
3. Iodine solution for testing starch.
4. Ferric chloride testing secretory cells.
5. Sudan III for testing oil cells.
6. Acetic acid and 80% sulphuric acid for testing calcium carbonate crystals.

Diagnostic Characters of Powdered Leaves of *Artocarpus lamellosus* Blanco

Microscopical characters and sensory characters of powdered leaves were studied.

Results

Key to the taxa comprising *Artocarpus lamellosus* Blanco

1. Adjacent pistillate flowers proximally free 2

1. Adjacent pistillate flowers completely fused 6

2. Syncarp subglabrous; nigrescent when dry ..... *A. griffithii*

2. Syncarp pubescent; not nigrescent when dry 3

3. Syncarp pubescence dark red-brown .....*A. borneensis*

3. Syncarp pubescence otherwise 4

4. Syncarps up to 5 cm across, velutinous, flesh bright pink ripening orange- pink; leaf apices rather blunt ..... *A. parvus*

4. Syncarps seldom exceeding 3 cm, short-pubescent, flesh otherwise; leaves acuminate 5

5. Syncarps seldom deeply lobed; leaf acumen up to 3 cm, lateral veins markedly ascending, usually drying brown, attachment cuneate..... …… *A. humilis*

5. Syncarps usually deeply lobed; leaves shortly acuminate, without markedly ascending lateral veins, often drying blue-grey above, attachment often rounded to subcordate.....…… *A. lamellosus*

Scientific Classification

Family	:	Moraceae
Tribe	:	Artocarpeae
Genus	:	Artocarpus
Sub-genus	:	*A.* subg. *Pseudojaca*
Species	:	***Artocarpus lamellosus*** Blanco
Synonyms	:	*Artocarpus lamellosus* Trecul
		Artocarpus nitidus Trecul
		Saccus lanceolatus (Trecul) Kuntze
		Saccus nitidus (Trecul) Kuntze

Morphological Characters

Scientific Name	-	***Artocarpus lamellosus*** Blanco
Myanmar Name	-	Unknown
Common Name	-	Butong (Tag)
Family	-	Moraceae

Trees up to 17m tall, evergreen, with latex; monoecious, straight. Bark black to brown, longitudinally fissured. Branchlets cylindric, wrinkled, 2-3 mm thick. Leave simple, alternate, stipules lanceolate, caduceus. Petiole 0.5-2 cm; leaf blade oblong to orbicular or ovate, 7-15 x 3-7 cm, lathery to thinly lathery, glabrous, abaxilly pale green, adaxially dark green. Young leaves with both surface black, when dry base cuneate, margin entire or irregularly shallowly veins 6-10 on each side of midvein, adaxially prominent. Male inflorecsences capitate, obovoid or oblong 2.5-12 x 2.7-7 mm. Male flowers calyx lobes 2-4, basally connate for 0.5-0.7 mm. Female inflorescences capitate or globose; peduncle 1.5-8 mm. Female flowers calyx tubular, style exserted. Fruiting syncarp red, reddish orange or yellow, brown when dry, globose, 1.5-5 cm in diam, fleshy, glabrous or sparsely covered with coarse pubescence, peduncle to 5 mm; bracts persistent. The results were shown in figure 1 to 7.

Flowering and fruiting period – January to March.

Figure 1. Habit

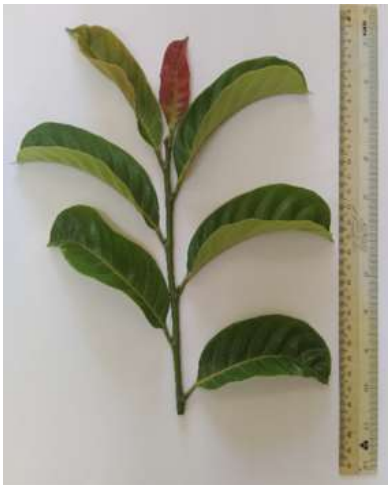

Figure 2. Arrangement of leaves

Figure 3. Lower and upper view of leaf

Figure 4. Staminate inflorescence

Figure 5. Pistillate inflorescence

Figure 6. Syncarp (aggregate fruit)

Figure 7. Longitudinal Section of Syncarp (aggregate fruit)

Histological Study of *Artocarpus lamellosus* Blanco

Lamina

In surface view, the cuticle is striated on upper surface. The epidermal cells were thin-walled, polygonal in shaped. Stomata were occurred only on lower surface. Anomocytic types stomata with wavy anticlinal walls and two reniform-shaped guard cells. Cystolith of calcium carbonate crystals were present in the epidermal cells. Trichomes were absent on both surfaces.

In transverse section, the epidermis was covered with thin cutical on upper surface. Upper epidermis and lower epidermis were one-layered thick and bulliform- shaped cells. Mesophyll was differentiated into 1-2 layered palisade and 3-4 layered spongy region. The palisade mesophyll was composed of elongated and compactly arranged parenchyma cells. The spongy cells were arranged with air spaces and mostly rounded to oval- shaped parenchyma cells.

The vascular bundles embedded in mesophyll region. Bundles were collateral types. Xylem lies toward the upper epidermis and composed of reticulate and pitted vessels, tracheids, fibers and xylem parenchyma cells. Phloem lies toward the lower epidermis consists of sieve tubes, companion cells and phloem parenchyma cells. The results were shown in figures 8 to 10.

Figure 8. Upper surface view of lamina showing epidermal cells (x400)

Figure 9. Lower surface view of lamina showing epidermal cells with anomocytic stomata and cystolith (x400)

Figure 10.T.S of lamina showing epidermis and mesophyll cells (x100)

Midrib

In surface view, the epidermal cells were thin-walled, rectangular to polygonal in shaped and elongated along the axis. Trichomes were mainly of unicellular type and glandular trichomes were very rare. In transverse section, a single layered epidermis was covered with unicellular trichome and followed by cortex. Cortex region consisted of 3 to 4 layered collenchyma cells and 7 to 10 layered of parenchyma cells. Latex tubes and crystal glands were more on the apical region

than the middle and basal regions of midrib. Collenchyma was occurred in ground tissue of upper midrib.The vascular bundle was semicircular shaped and closed collateral type. Xylem was endarch and surrounded by phloem. The xylem cells were hexagonal and arranged in radial rows, composed of vessel, tracheids, fibers and xylem parenchyma cells. The phloem cells were thin-walled and occurred outside of the xylem tissue, mainly composed of sieve tubes, companion cells and phloem parenchyma cells. The vascular bundle was surrounded by pericycle fiber. The results were shown in figure 11 to 16.

Figure 11. Upper surface view of midrib showing epidermal cells with unicellular and glandular trichomes (x400)

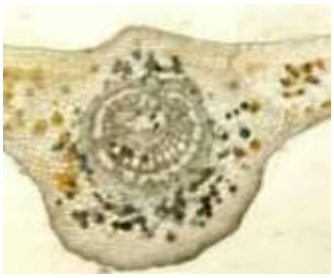

Figure 12. T.S of apical region of midrib (x100)

Figure 13. T.S of middle region of midrib (x100)

Figure 14. T.S of basal region of midrib (x100)

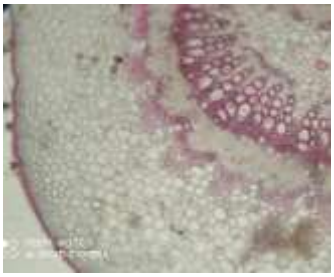

Figure 15. T.S of Midrib showing the parenchymatous cells (x400)

Figure 16. T.S of midrib showing the vascular bundles (x400)

Petiole

In surface view, the epidermal cells were thin-walled, rectangular to polygonal-shaped parenchymatous cells. Crystal glands were present in parenchymatous cells. Trichomes were present unicellular and glandular type.

In transverse section, the petiole was oval in outline. The epidermal cells were barrel-shaped, compactly arranged and one-layered thick. There were 2 to 3 layered collenchymatous cells under the epidermis. The parenchymatous cells were 8 to 10 layered thick. The parenchymatous cells were thin-walled and oval to rounded in shape.

The vascular bundles were circular in outline, collateral and closed type. Xylem vessels were endarch. Xylem lies toward the inside and composed of vessels, trachids, fibers and xylem parenchyma cells and phloem lie toward the outside and composed of sieve tubes, campanion cells and phloem parenchyma cells. There were 2 to 5 layered phloem cells. Both xylem and phloem were surrounded by pericycle fiber as patches and composed of sclerenchymatous cells. Pith occupied at the center. The results were shown in figure 17 to 20.

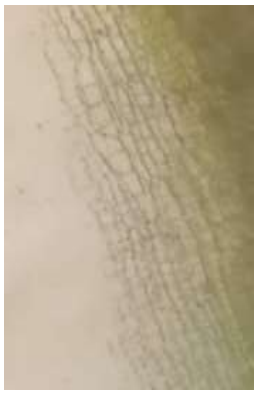

Figure 17. Upper surface view of petiole showing epidermal cells (x400)

Figure 18. Upper surface view of petiole showing epidermal cells with unicellular and glandular trichomes (x400)

Figure 19.T.S of petiole showing parenchymatous cells and trichomes (x100)

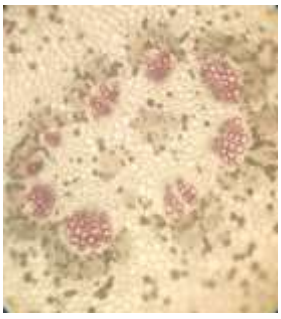

Figure 20. T.S of petiole showing the close-up view of vascular bundles(x400)

Stem

In surface view, the epidermal cells were thin-walled parenchymatous cells and polygonal-shaped. Trichomes were present unicellular and glandular type.

In trasverse section, the stem was semi-circular in outline. The epidermals cell was one-layered thick and covered with unicellular and glandular trichomes. Cork layer was present under the epidermis. There were 3 to 4 layered collenchymatous cells under the epidermis. The parenchymatous cells were 15 to 18 layered thick. The collenchymatous cells were isodiametric in shaped and parenchymatous cells were thin-walled and oval to polygonal in shaped. Latex tubes, unicellular and glandular trichomes were present. Pericycle present as patches and compose of sclerenchymatous cells.

The vascular bundles were collateral and closed type. Xylem vessels were endarch. Xylem lies toward the inside and composed of vessels, trachids, fibers and xylem parenchyma cells and phloem lie toward the outside and composed of sieve tubes, campanion cells and phloem parenchyma cells. There were 5 to 7 layered phloem cells. Pith was present. The results were shown in figure 21 to 23.

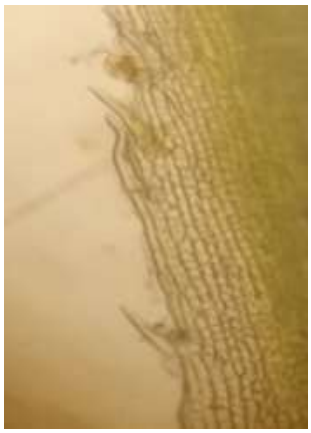

Figure 21. Upper surface view of stem showing epidermal cells with unicellular and glandular trichomes (x400)

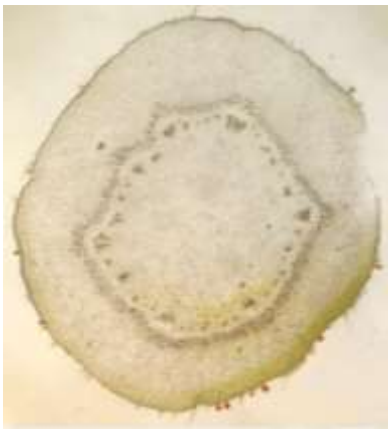

Figure 22. T.S of stem showing parenchymatous cells(x100)

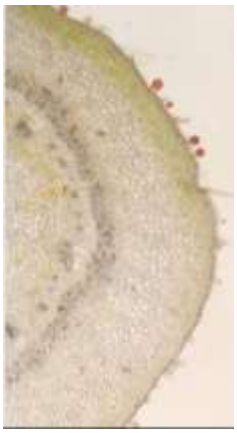

Figure 23. T.S of stem showing the close-up view of vascular bundles (x400)

Diagnostic Characters of Powdered Leaves of *Artocarpus lamellosus* Blanco

Microscopical Characters of Powered Leaves of *Artocarpus lamellosus* Blanco

The microscopical characters of powered leaves contained fragment of epidermal cells, tracheid, fibre, pitted and annual vessels, anomocytic stomata, cystolith, cystal and unicellular trichome. The color of powdered leaves was brown. The results were shown in figure 24 to 38.

Sensory Characters of Powered Leaves of *Artocarpus lamellosus* Blanco

The powder of ***Artocarpus lamellosus* Blanco** was brown color and non-odour. The taste was astringency and granular. The results were shown in table 1 to 34.

Table 1. Sensory Characters of Powered Leaves *Artocarpus lamellosus* Blanco

Characters	Colour	Odour	Taste	Texture
Leaves	Brown	Non	stringency	Granular

Figure 24. Powder of Leaf

Figure 25. Fragment of Epidermal Cells

Figure 26. Showing Stomata

Figure 27. Unicellular trichomes

Figure 28. Phloem Fiber

Figure 29. Fiber

Figure 30. Tracheids

Figure 31. AnnualVessel

Figure 32. Pitted Vessel

Figure 33. Starch grains

Figure 34. Crystals

Discussion and Conclusion

In the present research, the morphological characters on the vegetative and reproductive parts of the plant and histological study of leaves have been undertaken. Trees are evergreen, with latex; monoecious, straight. Leaves simple, alternate, stipules lanceolate, caduceus; petiolate; leaf blade oblong or ovate. Inflorecsences capitate, obovoid or oblong or globose. Fruiting syncarp red, reddish orange or yellow, brown when dry. These characters were in agreement with those given by Gardner, *et al* (2020), Cronquist (1981).

According to the results of histological characters of ***Artocarpus lamellosus*** Blanco**,** in the surface view of lamina, the epidermal cells were thin-walled, polygonal in shaped. Anomotic types of stomata were occurred only on lower surface. In transverse sections of midrib, petiole and stem, the epidermal cells were thin-walled, rectangular to polygonal in shaped. The vascular bundle was semicircular in shaped, closed and collateral type. Xylem vessels were endarch. The vascular bundles were surrounded by pericycle fiber as composed of sclerenchymatous cells. In midrib, trichomes were mainly of unicellular type and glandular trichomes were very rare. In transverse sections of midrib, petiole and stem, latex tubes, unicellular and glandular trichomes were present

and containing chambered crystals. These finding were in agreement with those of Metcalfe and Chalk (1950), Esau (1965) and Wallis (1967).

In powdered sample of leaves of ***Artocarpus lamellosus*** Blanco fragment of epidermal cells, tracheid, fiber, pitted and annual vessels, anomocytic stomata, cystals and unicellular trichome were found. These characters are similar to these mentioned by Treas and Evans, (2002).

In surface view of lamina, trichomes were absent on both surfaces, Cystolith of calcium carbonate crystals were present in the epidermal cells. In transverse section of midrib, latex tubes were more on the apical region than the middle and basal regions of midrib. These finding were carried out from this research.

In conclusion, morphological and histological characters of ***Artocarpus lamellosus*** Blanco were carried out. This genus, ***Artocarpus*** is a tree in the family Moraceae and a wild species of the breadfruit/ jackfruit genus. Detailed morphological identification of ***Artocarpus lamellosus* Blanco** collected in the University of Yangon campus revealed that was an unrecorded species in Myanmar. According to the above data, it is proved that the both morphological and histological characters of the specimen studied are useful for identification standardization of drugs. For further study, the other pharmacological effects of ***Artocarpus lamellosus*** Blanco such as antidiabetes activity, antimalarial activity and antioxidant activity should be carried out.

Acknowledgement

We would like to express our gratitude to Dr. Tin Maung Tun, Rector of the University of Yangon and we also with to express our gratitude to Dr. Thidar Oo, professor and Head, Department of Botany, the University of Yangon for giving us the opportunity to do research and providing invaluable guidance throughout this research.

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GENESIS OF SN-W MINERALIZATION AT MOSAKHEE, MAWCHI SN-W REGION, MYANMAR: CONSTRAINTS FROM MINERALOGY AND GEOCHEMISTRY*

Aung Zaw Myint[1]

Abstract

Tin-tungsten mineralization in the Mawchi Sn-W region, Myanmar, is predominantly confined to an Eocene granite and Carboniferous to Early Permian sedimentary and metasedimentary rocks. Apart from the other deposits of the region, stannite-kesterite is the major tin mineral of the Mosakhee, where Sn-W bearing quartz veins cut the metasediments sub-vertically. Stannite-kesterite and wolframite, the major ore minerals of the Mosakhee, are associated with galena, sphalerite, hematite, raspite, cassiterite, and pyrite. The homogenization temperature (*Th*) of vein quartz ranges between 215 and 300 ˚C, corresponding to salinities of less than 10 wt% NaCl equiv. The calculated temperature from the stannite-kesterite and sphalerite pair also coincides with the vein-filling temperatures resulting from fluid inclusion microthermometry. The $\delta^{34}S$ values of stannite (3.6 - 4‰) and galena (4.6 - 6‰) indicate the homogeneous sulfur source. The $\delta^{34}S$ values of galena are heavier than those of the Mawchi deposit, implying a likely different sulfur source derived from the country rocks. A brief account of oxygen isotope reveals that $\delta^{18}O_{H2O}$ calculation of ore fluid at 280 ˚C is 2.9‰ to 4.8‰, suggesting the ore fluid is mainly composed of meteoric water.

Keywords: Sn-W mineralization, Mosakhee, stannite-kesterite, fluid inclusions, stable isotope

Introduction

In Myanmar, tin-tungsten occurrences are spatially associated with the Cretaceous to Eocene granites and metasediments of the Mawchi-Mergui Belt (Gardiner et al., 2016; Aung Zaw Myint et al., 2017, 2018, 2019, 2021; Li et al., 2018a, 2018b, 2019; Mitchell, 2018) (Fig. 1a). Although the common tin minerals in the Southeast Asia tin belt are cassiterite, stannite, and malayaite (Hosking, 1970), the last two tin mineral species are not common in tin-tungsten deposits of Myanmar. Cassiterite is the major tin mineral of Sn-W deposits of Myanmar and is associated with the tungsten minerals of wolframite and scheelite: the latter tungsten species is found only in a few deposits (e.g., Mawchi and Kanbauk). Mawchi Sn-W region is famous for its historic world-class Sn-W deposit, Mawchi, with other prospects such as Khetaung Galay and Hteelakhee (Aung Zaw Myint et al., 2017, 2018) (Fig. 1b). Among the Sn-W occurrences within the Mawchi Sn-W region, Mawchi and Mosakhee are represented by the Sn-W ores with sulfide assemblage, while other prospects have no common sulfide minerals. The steeply dipping Mosakhee quartz veins strike N-S and contain stannite and wolframite as major constituents that are associated with a subordinate amount of sulfides. There is no record of stannite-kesterite occurrence in Myanmar, as the author explained before and, thus, he focuses on the mineralogy, fluid inclusion, and stable isotope to determine the ore genesis of the Mosakhee Sn-W deposit.

Geological background

The Western Granite Province (Cobbing et al., 1986, 1992) of SE Asia contains Jurassic to Miocene I- and S-type granites (Khin Zaw, 1990; Cobbing et al., 1992; Barley et al., 2003; Searle et al., 2007; Mitchell et al., 2012; Gardiner et al., 2016, 2018; Aung Zaw Myint et al., 2017, 2018,

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* First Prize (2023)

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2021; Crow and Khin Zaw, 2017; Li et al., 2018a, 2018b, 2019) hosted by the metamorphic rocks of the Mogok Metamorphic Belt (MMB; Searle and Haq, 1964; Mitchell et al., 2007) and the sedimentary rocks of the Mergui-Mawchi Belt (Mitchell et al., 2004; Aung Zaw Myint et al., 2021) (Fig. 1a). The MMB occurs as a western margin of the Sibumasu terrane, comprising a metasedimentary and metaigneous sequence of marbles, calc-silicate rocks, schists, quartzites, gneisses, and migmatites (Searle and Haq, 1964; Mitchell et al., 2007; Searle et al., 2007). The Mergui-Mawchi Belt consists of Carboniferous to Early Permian glacio-marine diamictites, including sedimentary rocks and their metasedimentary equivalents defined as Mawchi, Lebyin, Taungnyo, and Mergui Groups in Myanmar, as Kaeng Krachan and Phuket Groups in Thailand, and probably as Kongshuhe Formation in western Yunnan.

The Sn-W mineralization is spatially and genetically related to the Mawchi-Mergui Belt-hosted peraluminous granitic rocks in the Western Granite Province.

Mosakhee prospect is located in the linear metasedimentary rocks of the Mawchi Group (Aung Zaw Myint et al., 2017, 2018), a part of the Mawchi-Mergui Belt, that lay between the high-grade metamorphic rocks of the Mogok Metamorphic Belt (Searle and Haq, 1964: Mitchell et al., 2007) and granitic rocks of Western Granite Province (Cobbing et al., 1992) on the west, and Cambrian to Cretaceous rocks of Shan Plateau on the east (Fig. 1b). Small granite bodies are exposed along the NNW-SSE striking fracture zone, parallel to the regional strike of the Mawchi Group. These granites are mostly biotite granite that is partly altered to tourmaline granite, especially in the Mawchi mine area. The granites are highly evolved, high-K calcalkaline rocks with transitional magmatic-hydrothermal characters (Aung Zaw Myint et al., 2017). Previously reported LA-ICP-MS U-Pb zircon concordia ages of 42.72 ± 0.94 Ma (MSWD = 2) and 43.71 ± 0.39 Ma (MSWD = 1.02) are assigned as magmatic age of biotite granite and tourmaline granite, respectively (Aung Zaw Myint et al., 2017). The prominent rocks of the Mawchi Group are argillites, mudstones, fine-grained sandstone, metagreywacke, slate, and grit. LA-ICP-MS U-Pb geochronological data of detrital zircons from siltstone reveals the depositional age of the Mawchi Group is Carboniferous to Early Permian (Aung Zaw Myint et al., 2017).

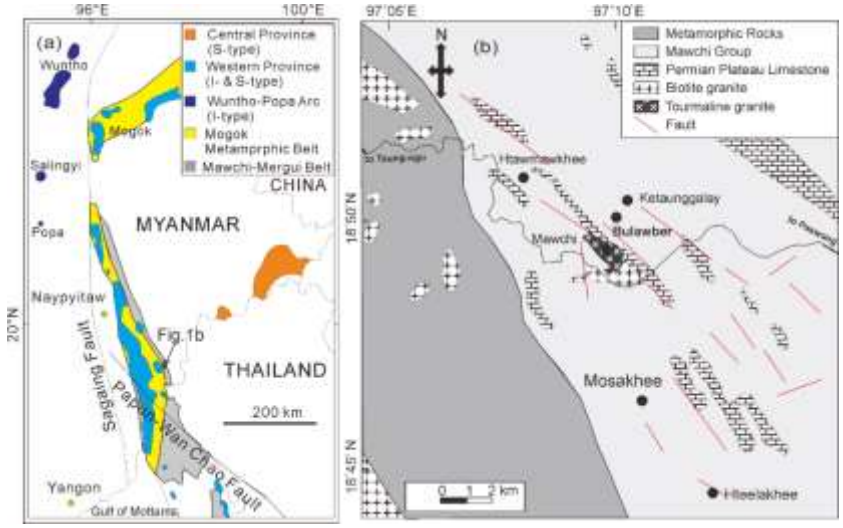

Figure 1: (a) Simplified geological map of Myanmar showing the granitoid belts together with Mogok Metamorphic Belt and Mawchi-Mergui Belt (Aung Zaw Myint, 2015; modified after Cobbing et al., 1992; Mitchell et al., 2007; DGSE, 2008), (b) Geological map of the Mawchi Sn-W district (Aung Zaw Myint et al., 2017, 2018; modified after Mawchi mine project map).

The principal mineralization in Mawchi is represented by N-S striking quartz veins that are confined to the tourmaline granite and metasedimentary rocks. Cassiterite, wolframite, scheelite, pyrite, arsenopyrite, galena, sphalerite, chalcopyrite, cosalite, quartz, tourmaline, fluorite, danalite, micas, and clay minerals occur as major constituents of the vein. Mawchi Sn-W mineralization is confined to both tourmaline granite and metasediments whereas other deposits are mostly hosted by metasediments. A molybdenite Re-Os model age of 42.4 ± 1.2 Ma indicates that the Sn-W mineralization at Mawchi coevals with the timing of granite emplacement (Aung Zaw Myint et al., 2018). Moreover, $^{40}Ar/^{39}Ar$ hydrothermal muscovite plateau ages of (40.14 ± 0.14 Ma; MSWD = 1.48) and (40.80 ± 0.12 Ma; MSWD = 0.47) define the timing of hydrothermal alteration and simultaneous veining that accompanied the late stage of ore-forming at Mawchi (Aung Zaw Myint et al., 2018). The Htawmawkhee prospect is composed of parallel and inclined small quartz veins that strike NW-SE. These 2–14 cm thick veins are hosted by argillite and comprise cassiterite, wolframite, and tourmaline. Nearly N-S trending sheeted veins are also exposed at Htawmawkhee. In the Ketaunggalay prospect, wolframite-bearing quartz and pegmatite veins are hosted by the metasandstone of the Mergui Group and they strike nearly N-S. Hteelakhee, located 12 km south of the Mawchi, represents a tabular and sheeted vein system containing cassiterite and wolframite confined to the argillite and sandstone.

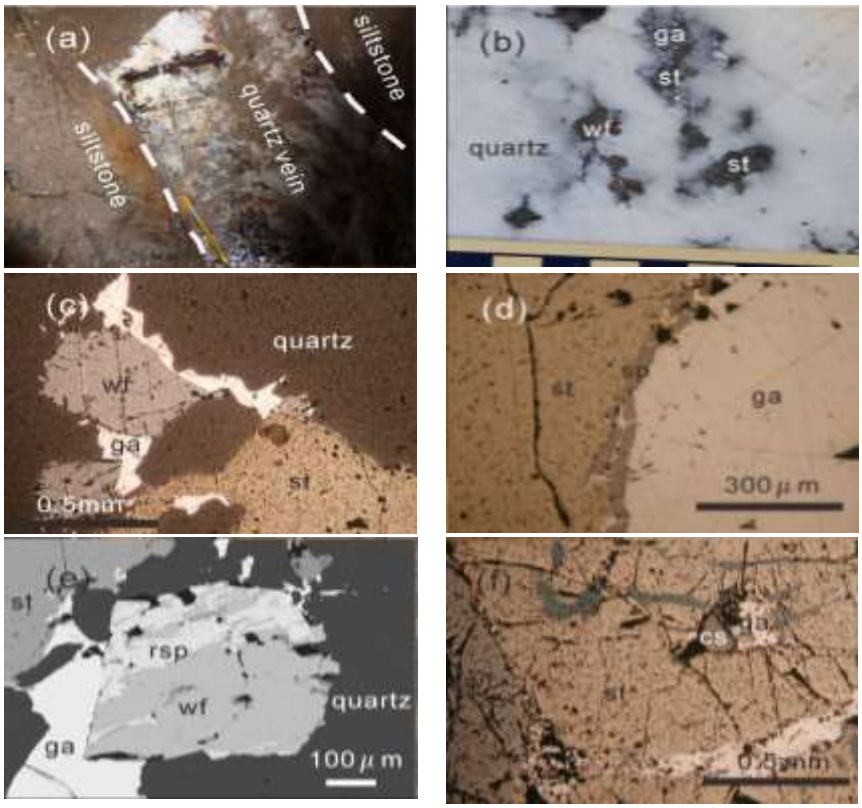

Figure 2: Outcrops (a-b), reflected light photomicrographs (c-d, f), and BSE image (e) displaying the mineralization style and ore mineral assemblages of Mosakhee: (a) sub-vertical quartz vein hosted by siltstone, (b) sulfide and oxide mineral association in the vein quartz, (c) wolframite and stannite marginally replaced by galena, (d) galena and sphalerite replace along the margin of stannite, (e) raspite replacing along the cleavages of wolframite grain, and (f) small cassiterite grain locating in the microfractures of stannite (cs: cassiterite, ga: galena, rsp: raspite, sp: sphalerite, st: stannite, wf: wolframite).

The Mosakhee Sn-W mineralization occurs in the southern part of the Mawchi Sn-W region. At Mosakhee, fine-grained sandstone hosts tabular quartz veins in which wolframite, stannite, and galena are major constituents (Figs. 2a, b). The N-S trending quartz vein comprises individual patches of oxide and sulfide minerals.

Analytical methods

Elemental analyses were performed by a Shimadzu Superscan SSX-550 scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) and a JEOL JXA8530F field emission electron probe microanalyzer at Kyushu University. A calibrated Linkam-THMS600 heating-freezing stage housed in the Economic Geology Lab, Kyushu University, is used for the final ice melting point (salinity calculations) and homogenization temperature measurement of the fluid inclusion samples. Sulfur and oxygen isotopic analysis was conducted at the Scottish Environmental Research Centre (SUERC), Scotland, United Kingdom, and the analytical procedure is explained in Aung Zaw Myint et al., 2018.

Results

Mineralogy and Elemental Determination

The most common vein minerals are stannite-kesterite, wolframite, sphalerite, chalcopyrite, galena, hematite, raspite, and pyrite. Wolframite, the most common tungsten mineral of the deposit, appears as euhedral to subhedral crystals and is veined and marginally replaced by galena (Fig. 2c) and other sulfides. Its composition is generally hubneritic and contains FeO (3.19 to 3.55 wt%) and MnO (20.91 to 21.24 wt%). Stannite-kesterite is the common tin mineral of the deposit, and it contains a range of Fe (4.47 to 7.49 wt%) and Zn (5.53 to 9.21 wt%), showing a small variation of composition. It is spatially associated with galena and fills in the voids of wolframite.

Apart from Mawchi sphalerite, Mosakhee sphalerite rarely hosts chalcopyrite and stannite blebs. Sphalerite contains 1 to 2.38 wt% Fe and 1.41 to 1.97 wt% Cd; it is relatively lower than those of Mawchi sphalerite (2.4 to 11.6 wt% Fe and 2.89 to 3.58 wt% Cd, respectively; Aung Zaw Myint, 2015). Sphalerite replaces the margin of stannite and wolframite, and occurs as the void fills in the stannite (Fig. 2d). Minute pyrite grains can be found as inclusions in stannite. Chalcopyrite occurs as a minor sulfide that represents small crystals associated with sphalerite and galena and replaces stannite. Galena, likely the last forming sulfide mineral in the vein system following sphalerite and chalcopyrite, is the most common sulfide mineral after stannite and sphalerite. Galena veins and replaces along the fractures and margins of wolframite and stannite (Fig. 2c). Raspite (PbWO4), containing 49.28−51.35 wt% WO_3 and 47.88−48.71 wt% PbO, replaces along with the fractures (cleavages) and grain boundaries of wolframite (Fig. 2e). Cassiterite, the most common tin mineral in other deposits within the Mawchi Sn-W district, occurs as small crystals associated with pyrite, galena, and stannite in Mosakhee (Fig. 2f). Hematite occurs as the secondary product of pyrite, containing the relict of the latter.

Fluid Inclusion Studies

Vein quartz from the Mosakhee prospect hosts liquid-rich two-phase fluid inclusions (Fig. 3a) with forty-one fluid inclusions heated to determine the homogenization temperature (*Th*). The fluid inclusions show a size variation from 5 to 25 μm characterized by irregular to tubular shape. The *Th* ranges from 231 to 300 ˚C with a mode of 280 ˚C. Final melting temperatures (*Tm*) range

from -1 to -5 ˚C and calculated salinity is less than 10 wt% NaCl equiv. The highest salinity samples correspond to the samples of low homogenization temperature (Fig. 3b).

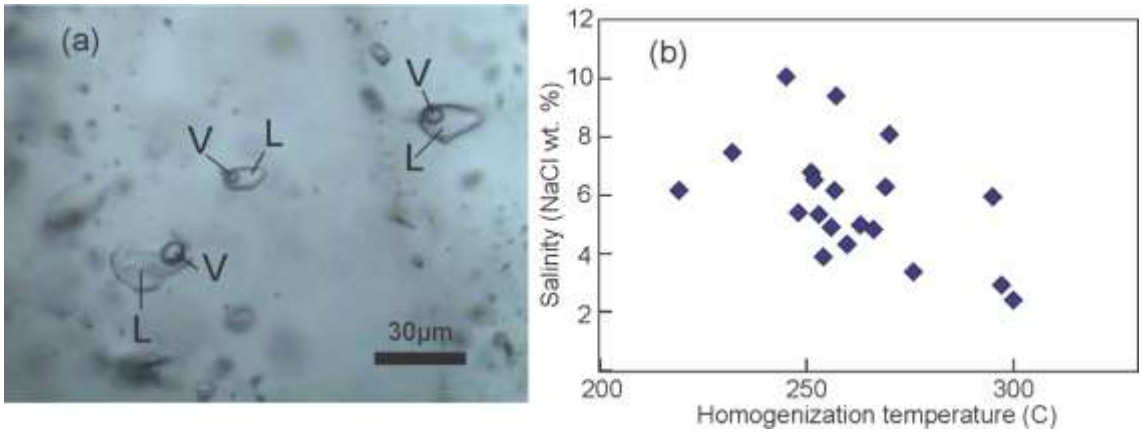

Figure 3: (a) Photomicrograph of liquid-rich two-phase fluid inclusions in the vein quartz of Mosakhee, (b) demonstrating the negative correlation between homogenization temperature and salinity

Stable Isotope

Stannite and galena from the Mosakhee Sn-W prospect gave $\delta^{34}S$ of 3.6-4‰ in stannite and 4.5-6‰ in galena, revealing the homogeneous sulfur source (Fig. 4a). Oxygen isotopes in the wolframite and quartz were analyzed to determine the source of ore fluid, and the $\delta^{18}O_{H2O}$ calculation of ore fluid is based on the mean homogenization temperature of quartz (i.e., 280 ˚C). The $\delta^{18}O_{mineral}$ values of wolframite and quartz were 1.4‰ and 13.2 to 13.3‰, indicating the $\delta^{18}O_{H2O}$ values of 2.9‰ and 4.7 to 4.8‰, respectively (Fig. 4b).

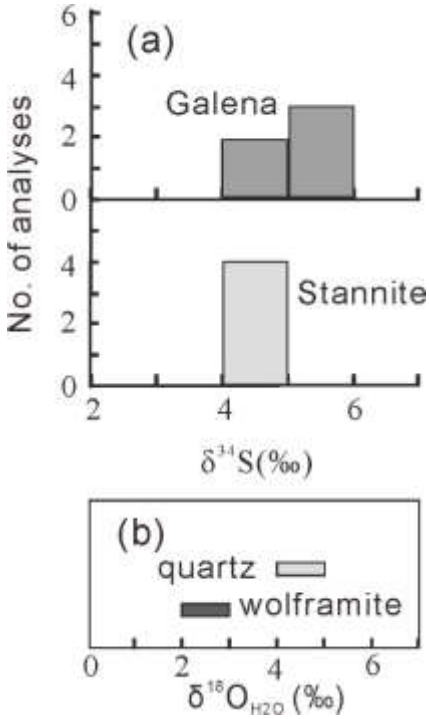

Figure 4: (a) Sulfur isotope values of sulfide minerals and (b) calculated $\delta^{18}O_{H2O}$ values of ore fluid from the Mosakhee

Discussion

Nature of Ore Fluid

Fluid inclusions microthermometric data reveals that the minimum vein filling temperature at Mosakhee is ~ 280 ˚C. It is coherent with the stannite-sphalerite thermometric data that indicates ca. 275 ˚C (Fig. 5). The negative correlation between the *Th* and salinity of the fluid inclusions also suggests a mixing of moderate temperature and low salinity magmatic descent fluid with a cooler and more saline meteoric water percolated in the sedimentary sequence of the Mawchi Group. In addition, the $\delta^{18}O$ values of quartz samples from various veins range from 11.5 to 13.4‰ and they are quite similar to those of Mawchi (11.8 to 13.4‰), Khetaungkalay (13.4‰), and Htawmawkhee (12.9‰) (Aung Zaw Myint, 2015; Aung Zaw Myint et al., 2018). Calculated $\delta^{18}O_{H_2O}$ values of Mosakhee vein quartz range from 3 to 4.9‰, indicating the deficiency of magmatic water component (Taylor, 1974, 1979). Thus, it can be suggested that the magmatic descent water may be involved in the source of ore fluid, but meteoric water is the principal component of ore fluid.

Source of Sulfur

The general mean of $\delta^{34}S$ data (4.6 ± 0.8‰; 1σ, n = 9) is heavier than that of the Mawchi deposit (2.9 ± 2.9‰; Aung Zaw Myint et al., 2018), indicating a relative enrichment in the ^{34}S content of the sulfur source. The $\delta^{34}S$ values of galena (4.5 to 6‰) from Mosakhee are significantly heavier than those of Mawchi (-1.3 to 1.8‰). The source of sulfur for the Mawchi deposit was mainly derived from the granitic magma (Aung Zaw Myint et al., 2018), but the heavier sulfur in Mosakhee was probably derived from country rocks. In addition, relatively lower Cd content in Mosakhee sphalerite than that of Mawchi sphalerite indicates that the metal source from crustal derived magmatic source diminished in the ore deposition at Mosakhee. The ancient crust that produced the magma for Mawchi granite is likely enriched in cadmium that has a similar ionic radius with zinc and can readily be deposited in the places of Zn in sphalerite isomorphically (e.g., Ye et al., 2012).

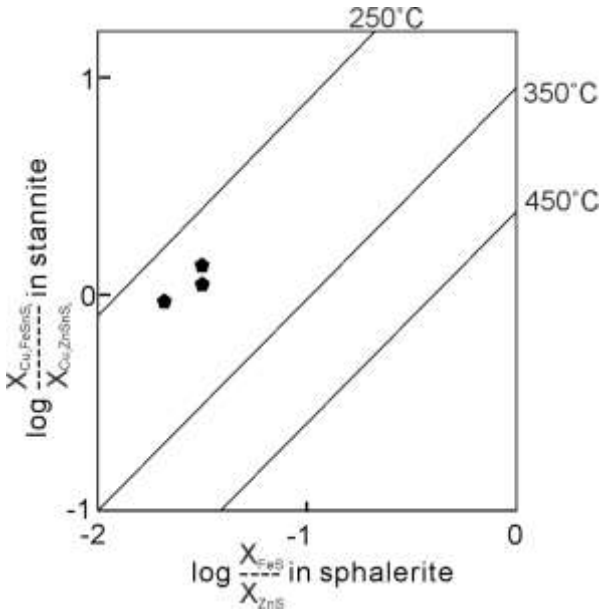

Figure 5: Stannite-sphalerite geothermometric diagram showing the ore-forming temperature of Mosakhee

Implications for Sn-W Exploration

Combined with the previous data (Aung Zaw Myint et al., 2017, 2018), exploration targeting for Sn-W mineral deposits in the Mawchi Sn-W region should meet the following parameters:

(i) Only tourmaline is the indicator mineral of the Sn-W mineralization, although fluorite is found at the Mawchi deposit.

(ii) The N-S fracture system is the most favorable site to host the mineralization.

Conclusions

Mosakhee prospect of the Mawchi Sn-W region is the first stannite-kesterite occurrence in Myanmar. The source of sulfur was slightly contaminated in the formation of sulfides by adding sedimentary sulfur; it is hard to discriminate the contaminated source of sulfur. Ore fluid is mainly derived from the mixing of moderate temperature and low salinity magmatic descent fluid with cooler and more saline meteoric water.

Acknowledgements

The author is indebted to the ASEAN University Network/Southeast Asia Engineering Education Development Network (AUN/SEED-Net) Japan International Cooperation Agency (JICA) for the PhD scholarship for the author. Associate Professor Kotaro Yonezu is thanked for his kind support of lab facilities at Kyushu University. The author acknowledges Professor Adrian Boyce for his support in stable isotope analysis.

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GEOLOGICAL PROSPECTING AND ORE MINEALIZATION OF EPITHERMAL DEPOSIT IN THE SHWEBONTHA AREA, MONYWA DISTRICT, CENTRAL MYANMAR

Toe Naing Oo[1]

Abstract

The Shwebontha area is located about one-kilometer ENE of the Letpadaung Cu-Au deposit. This study is aimed to investigate and characterize the geological condition, rock, alteration and ore mineralization. The geology of the Shwebontha area consists of volcanic and volcaniclastic rocks of the Late Oligocene to Middle Miocene Magyigon Formation that served as the host rocks of ore mineralization. Geochemically, the volcanic rocks having calc-alkaline nature and they are classified as volcanic field (rhyolite). Three types of alteration are developed in vicinity of mineralized quartz veins including silicic, argillic, and propylitic alteration zones. Mineralization is characterized by gold-bearing silicified massive ore and chalcedonic quartz vein in rhyolite host rocks. Optical microscopy and X-ray diffractometer (XRD) analysis indicate that these veins typically contain several ore mineral assemblage such as pyrite, sphalerite, galena, chalcopyrite, gold, covellite, goethite and hematite, associated with gangue mineral characterized by quartz, calcite, chlorite/epidote and clay minerals. Based on the current available data from hydrothermal alteration, mineralization types and ore mineral assemblages from the Shwebontha area develops forming under an epithermal environment.

Keywords*:* Hydrothermal alteration, Ore mineralization, Geochemisty, Epithermal deposit, Shwebontha

Introduction

The Shwebontha area is located approximately 6.5 km NW of Monywa, across the Chindwin River in Salingyi Township, Central Myanmar (Figure 1). The study area is located between 22º 4' N and 22º 9' N and between 95º 0' E and 95º 6' E, covering approximately a 30-square-mile area. The terrain is a fairly rugged and moderately to densely vegetated. The study area can be reached by vehicles from Nyaungbingyi through Nyaungbingyi Yinmabin road and therefore, it is accessible throughout the year. The location of the study area is shown in Figure 1.

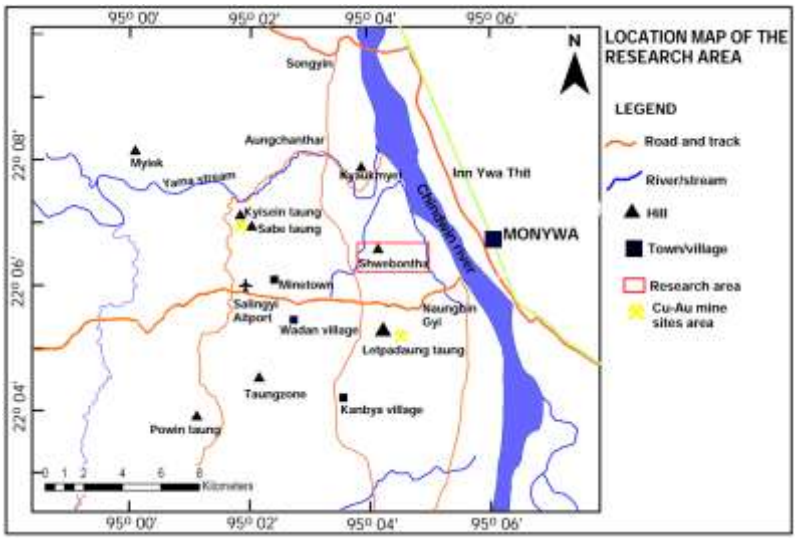

Figure 1. Location map of the study area

The Shwebontha area was discovered by Ivanhole Copper Company Limited in 1995 during the extensive exploration in the search for Cu-Au deposit, Au-Ag deposit and base metals

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mineralization in the Monywa district. An exploration program discovering of copper-gold and gold-base metal mineralization in the research area had been searched by based on the detailed geological, geochemical and geophysical investigations. This paper presents new data on hydrothermal alteration, mineralized quartz veins, sulfides mineralogy, ore chemistry at the Shwebontha area. The results of this study confirm that ore mineralization at the Shwebontha area is an epithermal deposit.

Regional Geologic Setting

Myanmar is divided into two tectonic provinces by the 1500 km long N-S trending Sagaing Fault (Khin Zaw, 2017) Figure 2. The eastern part of the country is the Sibumasu terrane; it encompasses the Shan Plateau, the Mogok-Mandalay-Mergui Belt (MMMB), and the Shan Scarps. The western part is the West Burma terrane comprising the Indo-Myanmar Ranges and the Central Volcanic Belt (also called the Inner Volcanic Magmatic Arc) (Mitchell et al., 2012) Figure 2. The Central Volcanic Belt is a N-S trending magmatic and metallogenic belt and located between the Western Inner Myanmar Tertiary Basin and the Eastern Inner Myanmar Tertiary Basin. It extends from Mt. Loamy and the Jade mines in the far north of Myanmar to the Gulf of Martaban in the south and beyond to the islands of west of Sumatra, Indonesia Figure 2. The Central Volcanic Belt is composed of Late Cretaceous to Tertiary granodioritic intrusive rocks with a subordinate sequence of Late Cretaceous to Quaternary volcanic rocks (Khin Zaw, 2017).

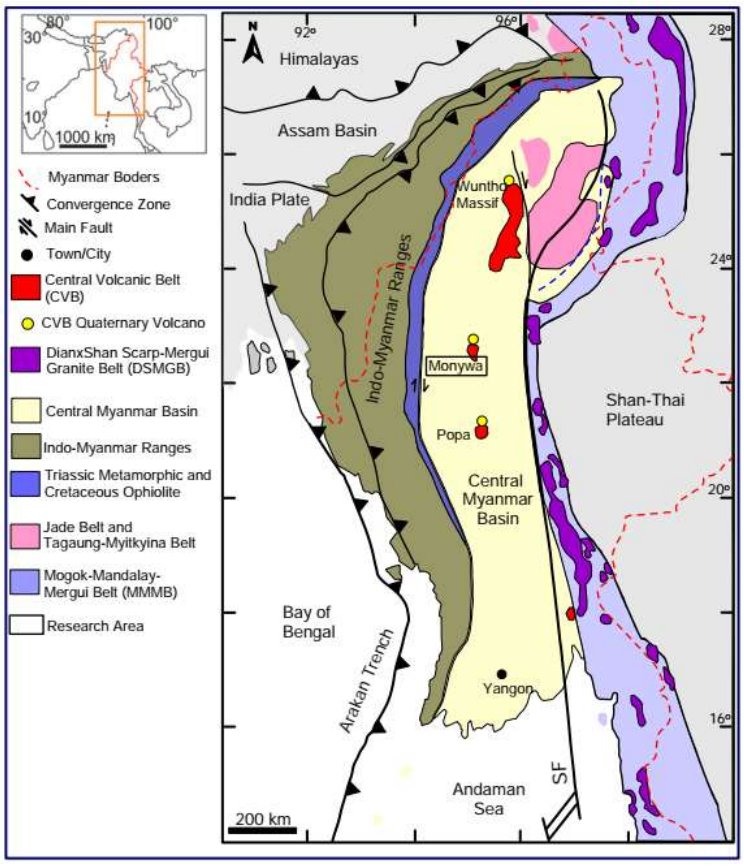


Figure 2. Simplified geological map showing structural features, Tertiary volcanoes, and Central Volcanic Belt in Myanmar and related regions (modified from Searle et al., 2007).

The Monywa District is located in the Central Volcanic Belt within the Central Volcanic Belt (Mitchell et al., 2011; Khin Zaw, 2017). The district has attracted the attention of geologists because of its great potential for economic Cu and Au deposits. In this district, metallic ores are found as both high-sulfidation epithermal-type Cu and low-sulfidation epithermal-type Au

deposits. Before mining, the district's resources amounted to approximately 2 × 109 tons of ore containing more than 7 × 107 tons of Cu metal making Monywa the second largest Cu deposit in SE Asia (Mitchell et al. 2011; Zaw et al. 2017). The Monywa copper deposits and nearby gold-silver prospects (including Kyaukmyet) lie at a few kilometers east of a cluster of small inliers of basement rocks and granitic intrusions that coincide with a regional aeromagnetic anomaly on a geanticlinal axis (Kirwin, 1994; Maung Maung Naing, 2003). The basement rocks are overlain locally by volcanic rocks and both are overlain unconformably by eastward-dipping, probably Eocene, quartzofeldspathic sandstones with a prominent west-facing scarp slope at Powintaung. Most of the metallic deposits between Powintaung and the Chindwin River are hosted by the Magyigon Formation and porphyritic intrusions.

Research Methods

Methods employed in this research were petrography, X-ray diffractometry (XRD), X-ray Fluorescence (XRF) and Atomic absorption spectrometry (AAS) method. A total of thirty-four (34) samples were collected from the surface outcrops in the Shwebontha area, Monywa district: hydrothermally altered rock (15 samples) and mineralized quartz vein (10 samples). Fifteen samples were prepared for thin sections, doubly-polished thin sections, or polished sections. Thin sections and polished sections were determined petrographically to identify the primary and secondary (alteration) mineral assemblages. A detailed study on ore microscopy of polished thin sections using both transmitted and reflected light were done to observe ore mineral assemblages and textural relationships. Clay minerals were identified by XRD. Subsequently, a total of 12 representative rock samples were selected for whole-rock geochemistry. The concentrations of major and minor elements of 12 rhyolite rocks were analyzed by X-ray Fluorescence (XRF). In a subsequent study, a total of eight (8) representative ore samples were selected for ore geochemistry. Ore chemistry particularly for Au, Ag, Cu, Pb and Zn was determined by Atomic absorption spectrometry (AAS) method. All laboratory analyses were carried out at Geological Engineering Department, Faculty of Engineering, Gadjah Mada University, Yogyakarta, Indonesia.

Results and Discussion

Geology of the Shwebontha Area

The Shwebontha area is divided into two parts including Shwebontha Hill and Shwebontha East. Both of these two parts are similar lithologic units, mineralization and alteration styles. The Shwebontha area is predominantly volcanic, volcaniclastic and sedimentary rocks including rhyolite, hydrothermal breccia, tuff breccia, tuff, tuffaceous sandstone as well as alluvium deposit (Figure 3). Stratigraphically, the hydrothermal breccia is the oldest rock unit in the research area, where it is distributed in the central part. The hydrothermal breccia unit is unconformably underlain by rhyolite rock unit which is the most dominant rock unit in the research area. Gold and base metals mineralization is mainly hosted by in rhyolite unit as well as hydrothermal breccias unit, as a member of Upper Oligocene-Middle Miocene Magyigon Formation. Most of mineralization veins are observed in the silicic alteration zone where gold (electrum) is associated with pyrite, galena, sphalerite and chalcopyrite. Their vein trends are generally followed the regional structural trend, probably related to be NE-ENE trending in direction that might be responsible for the formation of gold and base metals mineralization in the Shwebontha area.

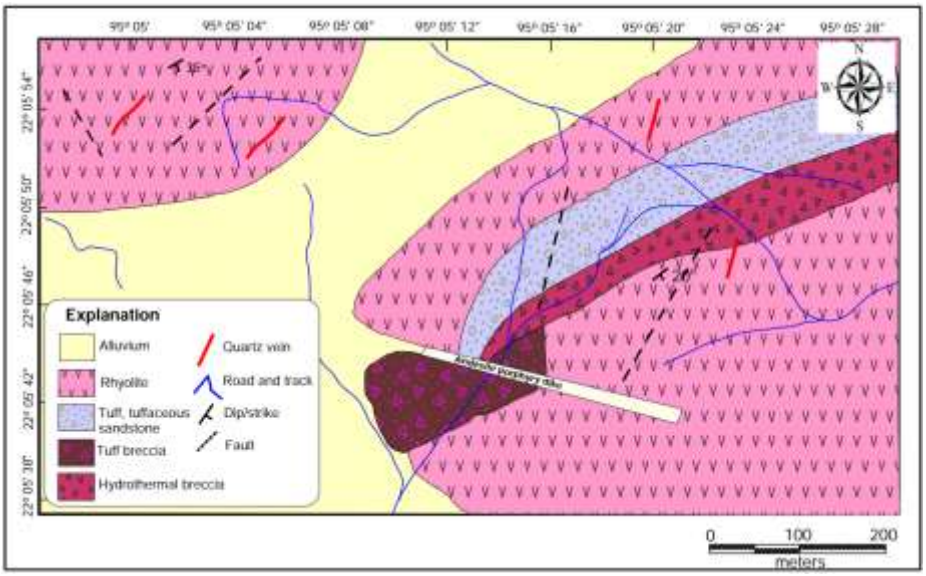


Figure 3. Simplified geological map of the Shwebontha area, Monywa district, central Myanmar (Win Min Htet, 2008).

Petrographic Characteristics

In the Shwebontha prospect, this unit is the most widespread and extensively covered rock unit. Based on the field investigation, variety of rhyolite is observed such as ash-white colour of rhyolite flow, pinkish colour of rhyolite flow and white/buff colour of rhyolite flow. Rhyolites are hard, compact, and moderately jointed. In some place, it shows faint flow structure and minerals are unidentifiable by naked eye in hand specimen. Rhyolite outcrops are generally grey colored on weathered surface (Figure 4).

Figure 4. (a) Field photographs of light to reddish brown color of weathered surface on flow banded rhyolite and (b) ash-white colour of faint flow structure of rhyolite, (c) an outcrop of planar flow banding in the rhyolite outcrop and (d) an exposure of strongly rhyolite flow fold in a rhyolite dyke.

Stratigraphically, hydrothermal breccia is the oldest rock unit. Ore mineralization mainly occurs in the rhyolite belonging to the Central Volcanic Belt in the study area. Mineralization is recognized by gold-bearing silicified massive ore and chalcedonic quartz veins in which sulfides are clustered and disseminated in the rhyolite host rocks. This mineralization vein is intimately

associated with a silicic alteration zone characterized by the presence of pyrite, galena, sphalerite, chalcopyrite and gold. Their vein trends generally followed the regional structural trend, which might be related to NE-ENE trending in a direction that is probably considered to be responsible for the formation of ore mineralization in the Shwebontha prospect.

Petrography: Rhyolite is typically showing hypocrystalline and rhyotaxitic texture and composed of quartz, alkaline feldspar, minor plagioclase as well as trace amount of biotite and opaque minerals. It is grey to white and porphyritic with flow structures in a groundmass with a glassy or microcrystalline texture (Figure 5a,b). The main phenocrysts (~15% of the rock mass) are quartz, alkali feldspar and minor plagioclase. Biotite and opaque mineral occur in a lesser amount in this rock unit. The groundmass is dominated by aphanitic felsic minerals and glass. The size of the phenocrysts generally ranges from 0.2 mm to up to 2 mm and groundmass minerals are generally <0.1mm.

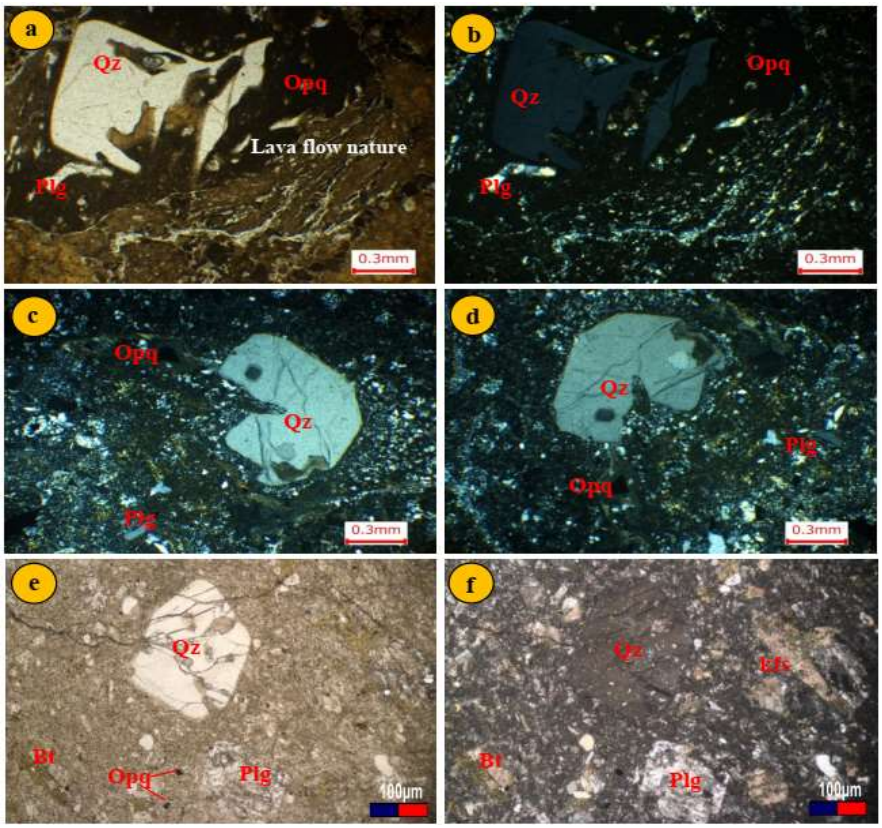


Figure 5. Photomicrograph of rhyolite. Abbr: Plg-Plagioclase; Qz-quartz; Kfs-K-feldspar; Bt-biotite; Opq-opaque.

The content of quartz is about 35 percent of total volume of the constituent minerals. It's size ranges from 0.5 mm to 2 mm. Most of quartz observes as phenocrysts as well as a groundmass. But, some quartz grains are deeply corroded by mesostasis and show a rhyolitic quartz aspect (Figure 5a,b). Quartz is deeply corroded by mesostasis. Some quartz phenocrysts display corroded outline and hexagonal outline and wavy extension as a result of strain (Figure 5c-f). Quartz grains observe as coarse-grained, euhedral to subhedral form where some coarse quartz grains occur as phenocrysts. Their characters of colorless, lack cleavage and wavy extinction are assisted to recognize well. It shows rounded to subrounded crystal shape with low relief.

Geochemistry of Host Rock

The volcanic rocks from the Shwebontha area mainly constitute rhyolite. These rhyolite rocks have been moderately affected by hydrothermal alteration processes and usually include

secondary minerals of quartz, chlorite, sericite, and calcite. However, the analyzed samples were carefully selected and weathered surfaces were removed. Most of the rhyolite samples present a SiO_2 content higher than 75%, being classified as "high silica rhyolitic systems" according to Mahood and Hildreth (1983) and Metz and Mahood (1991). In general, rhyolite rocks with these characteristics have small ranges of SiO_2 variation, difficulting the classification based on geochemical parameters. The concentration of major (wt%), trace and rare earth element (ppm) of the rhyolite rocks from the Shwebontha prospect are displayed in Table 1. The rhyolite rocks show the SiO_2 contents ranged between (75.1-79.98%), Al_2O_3 (9.11-12.75%), FeO*(tot) (0.08-1.22%), TiO_2 (0.8-0.10%), MnO (0-0.1%), MgO (0.44-0.7%), CaO (0.14-0.21%), Na_2O (0.50-0.88%), and K_2O (6.43-9.73%) (Table 1). Based on the plotting result in the TAS (Total Alkali versus Silica) diagram of (Middlemost, 1994), (Figure 6a) can be confirmed that this unit is consisted of rhyolite. Volcanic rock compositions were also confirmed by immobile trace elemental plot (Figure 6b) by applying Zr/Ti and Nb/Y diagram (Pearce, 1996).

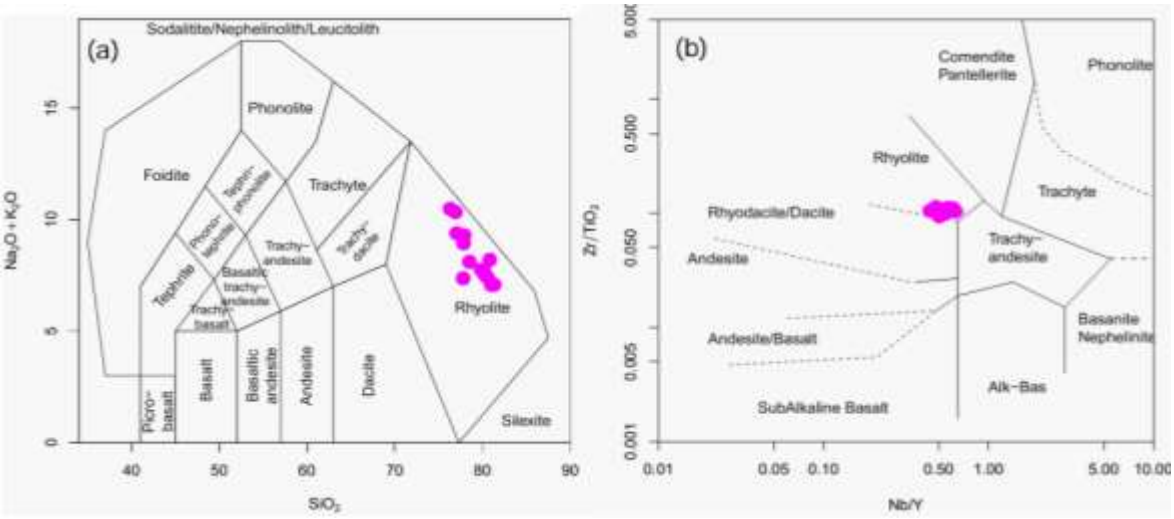


Figure 6. (a) TAS (total alkalis versus silica) classification diagram for volcanic rocks (Middlemost, 1994), (b) Nb/Y vs Zr/TiO_2 plot of volcanic rocks from the Shwebontha area (Pearce, 1996).

On the basis of binary plot diagram of SiO_2 versus Na_2O+K_2O Irvine and Baragar, 1971) volcanic rocks (rhyolites) of the Shwebontha area are displayed the nature of subalkaline to alkaline affinity (Figure 7a). AFM diagram is classified between tholeiitic and calc alkaline differentiation trends in the sub-alkaline magma series. Volcanic rocks (rhyolite) from the Shwebontha area are plotted on the AFM diagrams Irvine and Baragar, 1971). Triangular AFM plot shows that the rhyolite rocks are located in the field of the calc-alkaline series (Figure 7b). The SiO_2 and some of major oxide elements cannot be applied because of alteration product in the magmatic evolution processes. For this reason, incompatible element 'Zr' is used as a replacement for SiO_2. Trace element variation diagram in this study exhibits that Rb, Nb, Ba, Sr and Y versus Zr display positive correlation (Figure 8) which are recognized to be mobile with altered volcanic (rhyolite) rock during hydrothermal alteration.

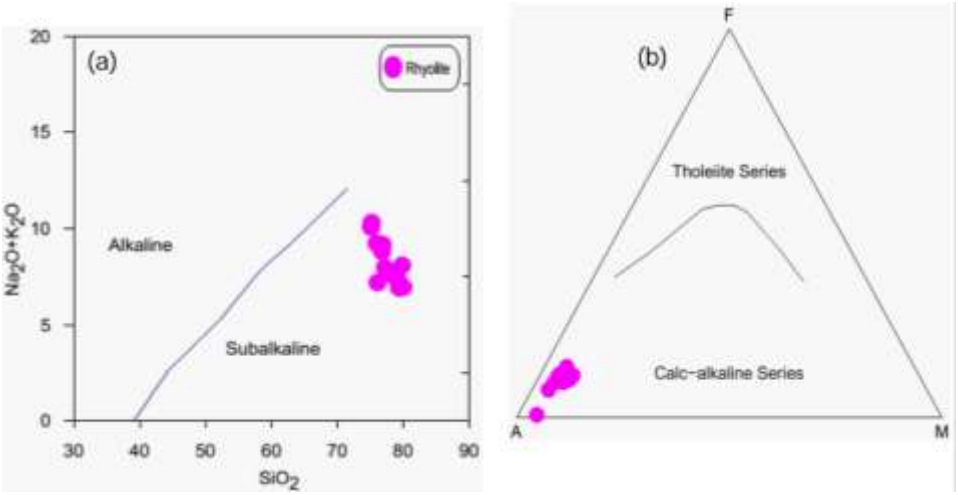


Figure 7. Subalkaline and alkaline classification plot diagram (SiO_2 vs Na_2O+K_2O) (Irvine and Baragar,197) and AFM classification diagram Irvine and Baragar, 1971) for rhyolite rocks of the Shwebontha area.

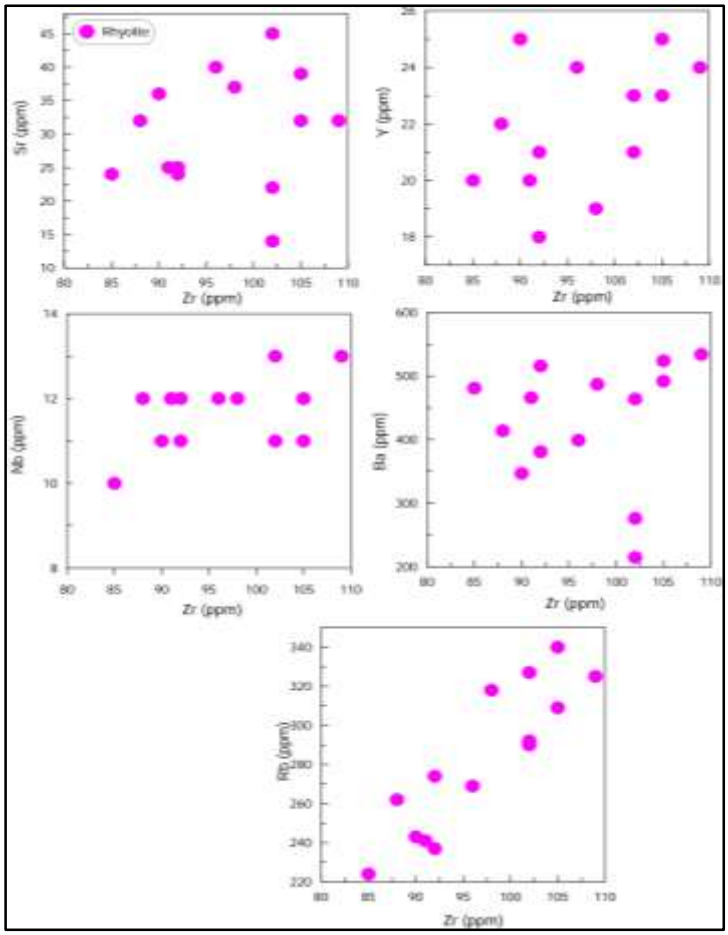


Figure 8. Binary plot diagrams of Rb, Nb, Sr, Ba, Y, and Zr (all in ppm) for rhyolite rocks at Shwebontha area.

Table 1. Whole-rock major- and trace-element concentrations of rhyolite rocks from the Shwebontha area.

Sample ID	S11	S9	S4	S1	S7	S2	S10	S3	S5	S12	S6	S14	S12	S8
Major elements (in wt%)														
SiO_2	76.1	77.1	75.2	79.2	78.9	78.4	79.9	78.9	75.9	76.8	79.8	79.4	75.1	76.8
TiO_2	0.10	0.10	0.10	0.09	0.09	0.10	0.09	0.08	0.09	0.09	0.09	0.08	0.10	0.09
Al_2O_3	12.8	11.3	11.5	10.2	10.4	10.3	9.56	10.0	11.5	10.9	9.11	10.0	11.7	11.08
FeO	0.99	1.12	0.84	0.86	0.80	0.92	0.93	1.22	1.16	0.96	1.07	0.82	0.08	1.19
MnO	0.01	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
MgO	0.54	0.53	0.45	0.55	0.57	0.70	0.67	0.49	0.48	0.46	0.44	0.58	0.47	0.50
CaO	0.14	0.17	0.14	0.17	0.17	0.17	0.16	0.16	0.19	0.21	0.16	0.18	0.14	0.19
Na_2O	0.52	0.56	0.60	0.51	0.52	0.52	0.51	0.64	0.80	0.88	0.62	0.50	0.58	0.77
K_2O	6.69	7.42	9.73	6.75	6.90	7.09	6.46	6.98	8.45	8.30	7.48	6.43	9.51	8.05
P_2O_5	n.d	n.d	n.d	n.d	n.d	0.01	0.01	n.d	0.01	0.01	n.d	n.d	0.01	0.01
H_2O	2.10	1.55	1.27	1.61	1.57	1.62	1.54	1.37	1.31	1.22	1.07	1.88	1.46	1.25
Total	**99.9**	**99.8**	**99.8**	**99.9**	**99.9**	**99.9**	**99.9**	**99.9**	**99.9**	**99.9**	**99.85**	**99.9**	**99.1**	**99.9**
Trace elements (in ppm)														
V	17	18	14	3	10	14	7	5	13	10	6	0	5	6
Cr	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Co	30	36	42	45	50	27	30	55	50	40	34	36	23	25
Ni	11	13	8	8	8	7	12	9	8	9	13	7	6	11
Cu	2.01	23	31	22	41	24	30	78	49	25	19	31	5	3
Zn	46	50	n.d	26	9	3	n.d	47	18	33	7	12	10	20
Pb	5	9	8	11	22	17	6	n.d	6	23	31	41	23	45
As	13	7	8	6	8	31	43	9	10	12	19	9	7	11
Mo	13	11	11	11	9	11	7	7	10	10	9	8	9	10
Rb	292	290	340	237	241	269	243	274	318	327	262	224	325	309
Sr	14	22	32	24	25	40	36	25	37	45	32	24	32	39
Ba	215	276	524	516	466	399	347	381	487	464	414	481	534	492
Y	21	23	25	21	20	24	25	18	19	23	22	20	24	23
Zr	102	102	105	92	91	96	90	92	98	102	88	85	109	105
Ta	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Nb	11	11	12	12	12	12	11	11	12	13	12	10	13	11

Hydrothermal Alteration

In the Shwebontha area, mineralization and hydrothermal alteration are observed in the rhyolite host rock unit. Alteration developed around open space mineralized vein at breccia zones. In the research area, there are three principal kinds of hydrothermal alteration zones that have evolved including silicic, argillic and propylitic alteration types. They are examined by optical petrographic observations (Figure 9). Silicification is also a common type of hydrothermal alteration in the Shwebontha area, and is closely related to ore mineralization. Silicified rock is

characterized by equigranular microcrystalline quartz, hematite and sulfide minerals (Figure 9). This alteration is represented by chalcedony, disseminated pyrite with medium to coarse-grained quartz and quartz veinlets in the brecciated sulfide quartz vein and chalcedonic quartz vein (Figure 9). And, it also occurs as mineralized veins and is associated with breccias cements, vein-veinlet and stockwork (up to 2-3 cm width) quartz veins (Figure 9).

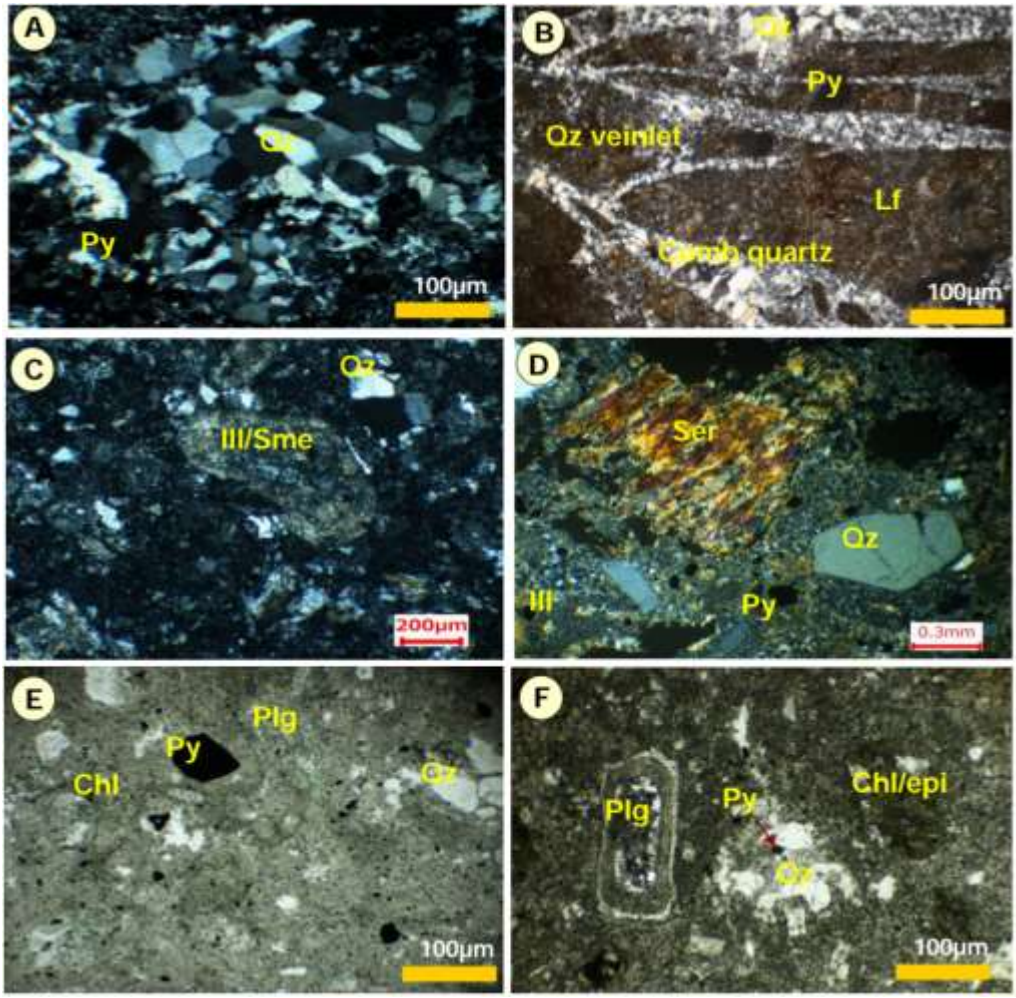


Figure 9. Photomicrographs showing hydrothermal alteration minerals at the Shwebontha area. (Qz-quartz, Ill-illite, Sme-smectite, Chl-Chlorite, Epi-Epidote, Py-Pyrite, Plg-Plagioclase, Lf-Lithic fragment).

Argillic alteration is characterized by variable amount of quartz, plagioclase, opaque mineral and clay minerals (sericite, illite, illite/smeciite, and kaolinite) (Figure 9 and 10a,b). Anhedral to subhedral quartz found as a phenocryst and fine-grained groundmass (Figure 9). Opaque minerals (pyrite) are occurred dissemination (Figure 9) which is associated with clay minerals (illite, smectite and quartz). Altered plagioclase replaced by yellowish brown colour of sercicite and kaolinite (Figure 9). In addition, plagioclase phenocrysts and groundmass partially replaced by illite, illite/smectite mixed layer mineral, pyrite and quartz minerals. Secondary quartz mainly replaced the groundmass or matrix of the rhyolites (Figure 10). According to the microscopic study and XRD analysis, the common propylitic alteration minerals are quartz, chlorite, epidote and pyrite (Figure 9 and 10c). The presence of chlorite and epidote can record the alteration type as propylitic alteration. Altered plagioclase is replaced by quartz, chlorite, epidote, and some clay minerals (Figure 9).

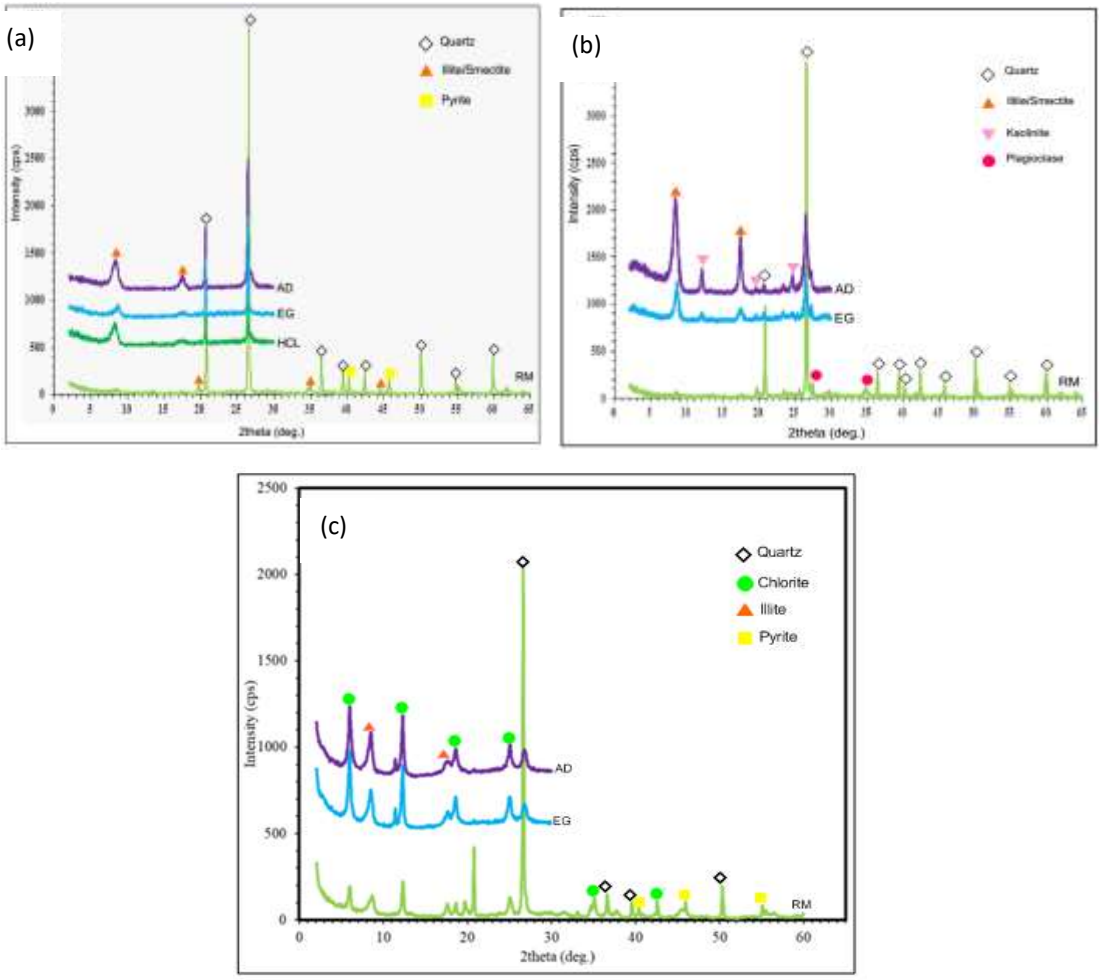


Figure 10. X-ray diffractograms (XRD) of argillic and propylitic alteration (AD-air-dried, RM-random mount, EG-ethylene glycolated).

Ore Mineralization

Ore mineralization is mainly hosted by rhyolite rock unit in the Shwebontha area, Monywa district. Massive orebody of gold-bearing silicified massive ore and chalcedonic quartz vein are concentrated on the foremost veins and in zones of argillic altered wall-rocks and oxidized zones (Figure 11). The veins belong to open-space filling and occasionally disseminated nature. Sulfide minerals are also occurred as in the chalcedonic quartz veins alternating with strongly silicified zones cut by cherty or sugary quartz vein in the rhyolite host rock as dissemination (Figure 11). Pyrite is the most common sulfide mineral. It is observed either as fine-grained disseminations and aggregates in quartz or as infillings in vugs. Primary (hypogene) ore minerals are pyrite, sphalerite, galena, chalcopyrite, and gold. Secondary (supergene) ore minerals include covellite whereas gangue minerals are mainly composed of quartz.

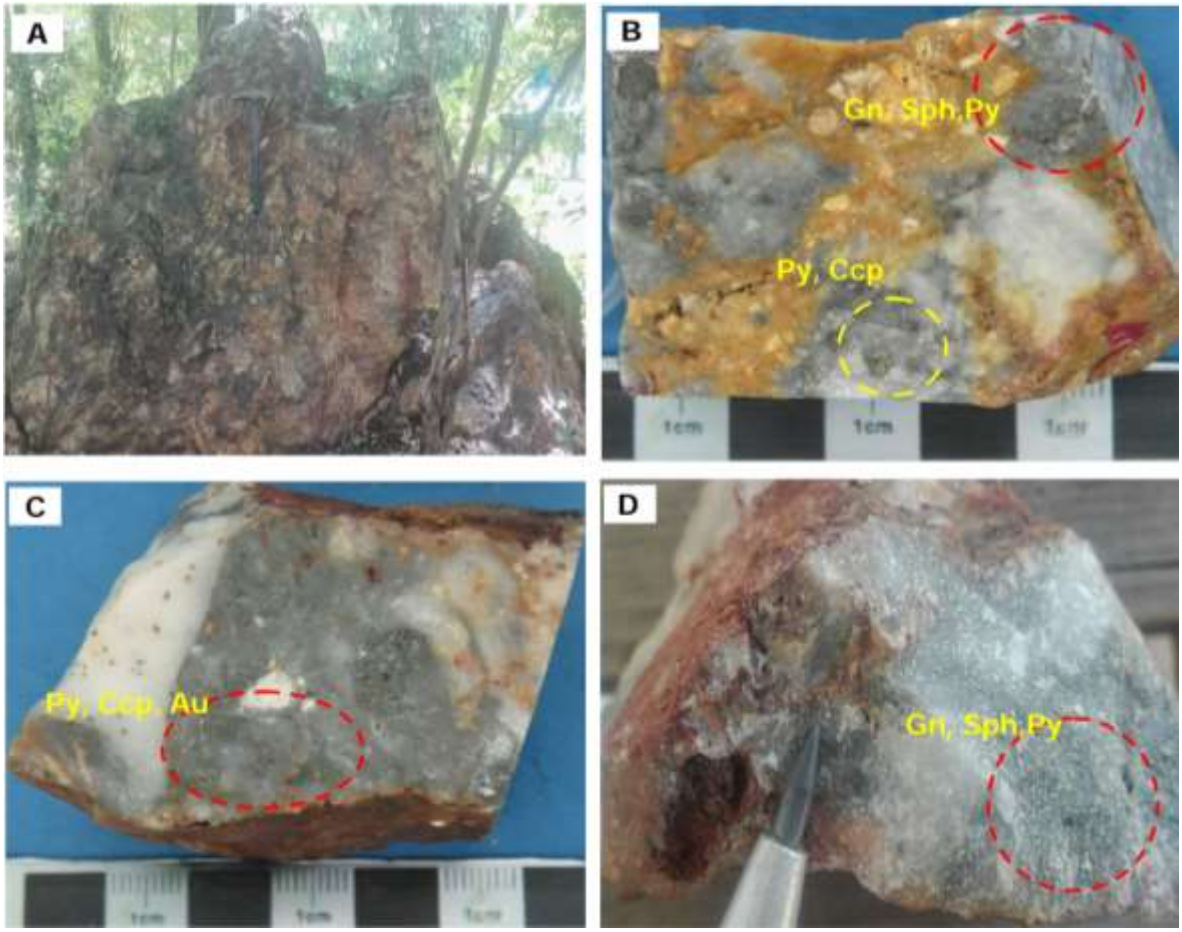


Figure 11. Outcrops and hand specimens showing (a) gold- base metal bearing silicified massive ore, (b,c) gold-bearing brecciated quartz veins and (d) chalcedonic quartz vein in the rhyolite host rock.

Pyrite is distributed and is the most abundant sulfides in the mineralized veins and host rocks. It shows anhedral to euhedral (Figure 12), pale yellow to yellowish white. On the other hand, irregular cracks and cataclastic deformation of pyrite are observed in gangue matrix. Most of pyrite were replaced by anhedral grains of galena, sphalerite, and chalcopyrite (Figure 12). Sphalerite is observed as anhedral grains. It is grey and displays internal reflection. Sphalerite appears to have replaced pyrite (Figure 12b). Galena exhibits light grey color and anhedral form. It occurs as a mineral that replaced pyrite (Figure 12c). Chalcopyrite displays yellow to brassy yellow in color and fairly high reflectance and weak anisotropism. It occurs as anhedral and irregular grain as well as enclosed in euhedral pyrite crystal (Figure 12d). Gold which is significant occurs as native gold or electrum granular grains in euhedral pyrite crystal (Figure 12e). It is very fine-grained (1–2μm), occasionally up to 200 μm. Covellite occurs as a secondary mineral and is generally found as fine-grained disseminated crystals replacing in pyrite (Figure 12f).

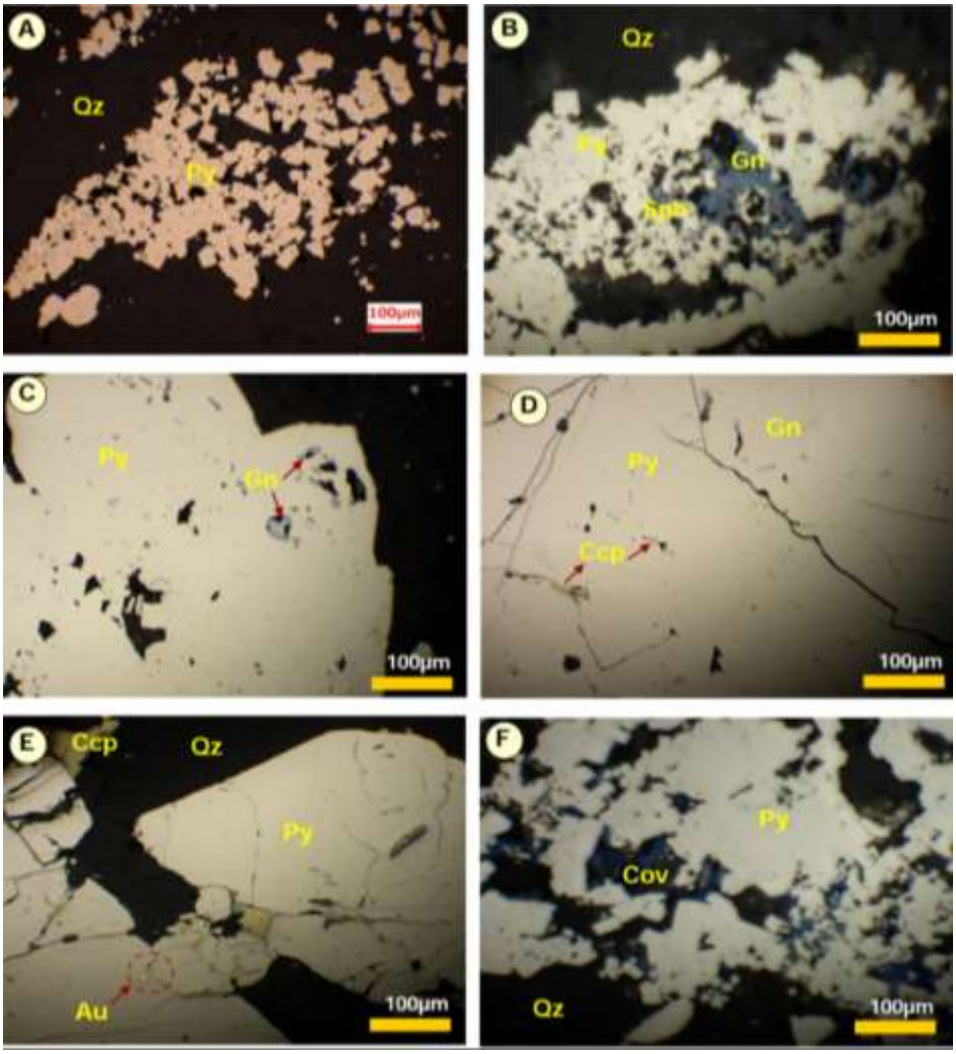


Figure 12. Reflected light photomicrographs of ore mineral assemblages from the Shwebontha prospect. (Py-Pyrite, Gn-Galena, Sph-Sphalerite, Au-Gold, Ccp-Chalcopyrite, Cov-Covellite, Qz-quartz).

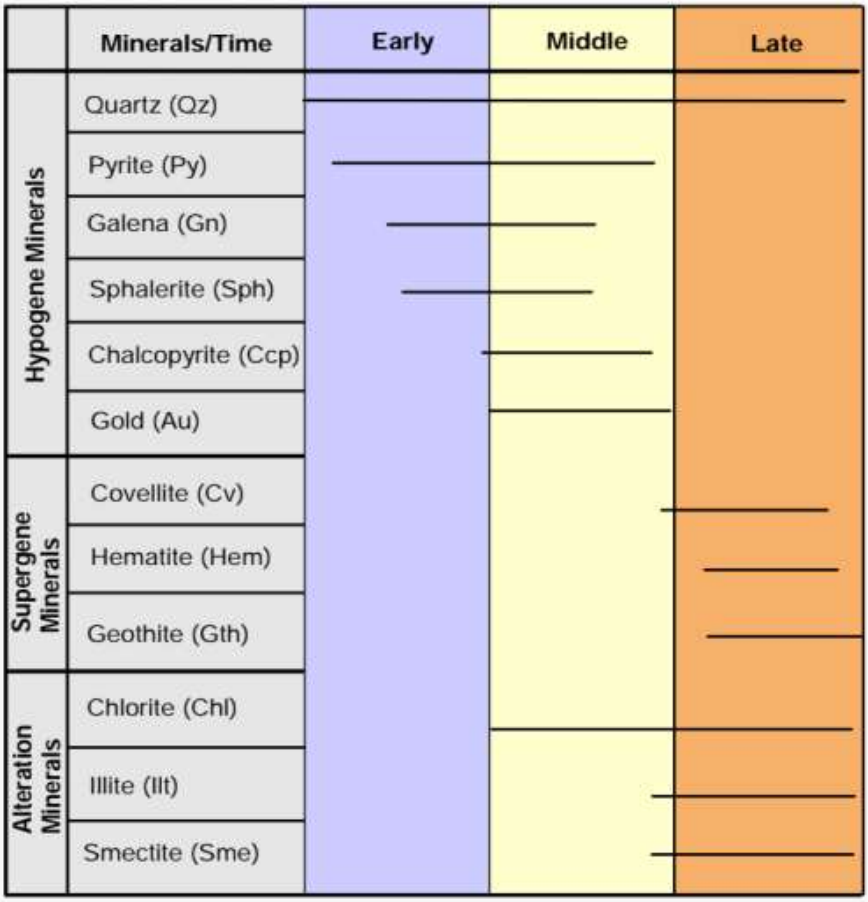
Ore-Gangue Minerals Paragenesis

Textures of minerals such as banded, exsolution and replacement, distribution have been interpreted to correspond to the order of deposition. According to ore textures, the early formed quartz and pyrite, minerals are found while late phase sulfide and quartz are seen in the center of vein. In fact, early formed pyrite and quartz minerals showed their euhedral crystal forms that indicated anhedral sphalerite is younger than quartz and pyrite in order of deposition. Generally, crystals of quartz and pyrite were formed during the entire period of mineralization. Some anhedral sphalerites are replaced by galena along the boundary. Some small grain of chalcopyrite occurs as exsolution in sphalerite. It means that sphalerite is earlier in deposition than chalcopyrite and galena. And chalcopyrite crystal also occurred in euhedral pyrite crystal. In addition, covellite replaces along the margin of pyite crystals.

Based on the textural characteristics, order of deposition can be divided into two main stages (stage I and stage II) and oxidation stage. Quartz, pyrite, galena, sphalerite, chalcopyrite and gold/electrum were formed in early stage of deposition as primary hypogene ore minerals whereas covellite, hematite, and goethite were formed as secondary supergene minerals. Supergene minerals occurrences reflect the oxidation of primary sulfides such as pyrite and chalcopyrite by

surficial water. The generalized paragenesis of the ore and gangue minerals of the research area are shown in Table 2.

Table 2. Generalized paragenesis sequence of Shwebontha area.



Ore Geochemistry

In general, ore mineralogy and metal occurrences in hydrothermal deposits are commonly controlled by the composition of the ore fluids. The chloride (Cl^-) and sulphide (specifically, HS^-) are regarded as being essential in order to solubilize gold and base metals in aqueous solutions (Seward and Barnes, 1997). Moreover, the redox states of the fluid and sulfur activity affect the mineralogy (Einaudi et al., 2003). Selected ore samples from mineralization veins were conducted in chemical analyses. Based on the AAS measurement of eight (8) mineralized quartz vein samples, the concentration of Au and Ag, Cu, As, Pb, Zn and Sb were performed by AAS technique. The composition of selected ore samples is shown in (Table 3). Chemical analyses of ore samples from mineralization veins are shown as the significant concentration of Cu, Zn, Pb, Ag, Sb, As and Au constructed to know the variation among these elements (Figure 13). Accordingly, plots of gold (Au) versus silver (Ag), copper (Cu), lead (Pb), zinc (Zn), tin (Sn) and antimony (Sb) were constructed to determine their correlation. The relation of gold (Au) versus silver (Ag), and copper (Cu) showed a positive correlation with Au. The Au versus zinc (Zn), lead (Pb), and antimony (Sb), arsenic (As) are shown negative in correlations (Figure 15). The analyzed mineralized samples concentration of gold ranges from 0.19 ppm to 7.25 ppm, silver 15 ppm to 75.5 ppm, copper 33 ppm to 170 ppm, lead 14 ppm to 163 ppm, zinc 6 ppm to 82 ppm, arsenic 126 ppm to 1030 ppm and stibnite 27 ppm to 2013 ppm is respectively shown in (Table 3).

Table 3. Ore chemistry of some selected ore samples by AAS analyzed at Shwebontha area

Sample	Au (ppm)	Ag (ppm)	Cu (ppm)	Pb (ppm)	Zn (ppm)	Sn (ppm)	Sb (ppm)
SBV-4	0.81	20	93	19	18	252	68
SBV-7	7.25	70	122	105	6	517	72
SBV-5	4.21	60	165	14	33	126	27
SBV-6	0.89	30	52	53	11	174	49
SBV-3	0.23	19	49	71	30	354	51
SBV-1	0.32	21	43	77	10	647	50
SBV-8	0.37	34	170	163	82	1020	203
SBV-2	0.19	18	33	28	13	1030	55

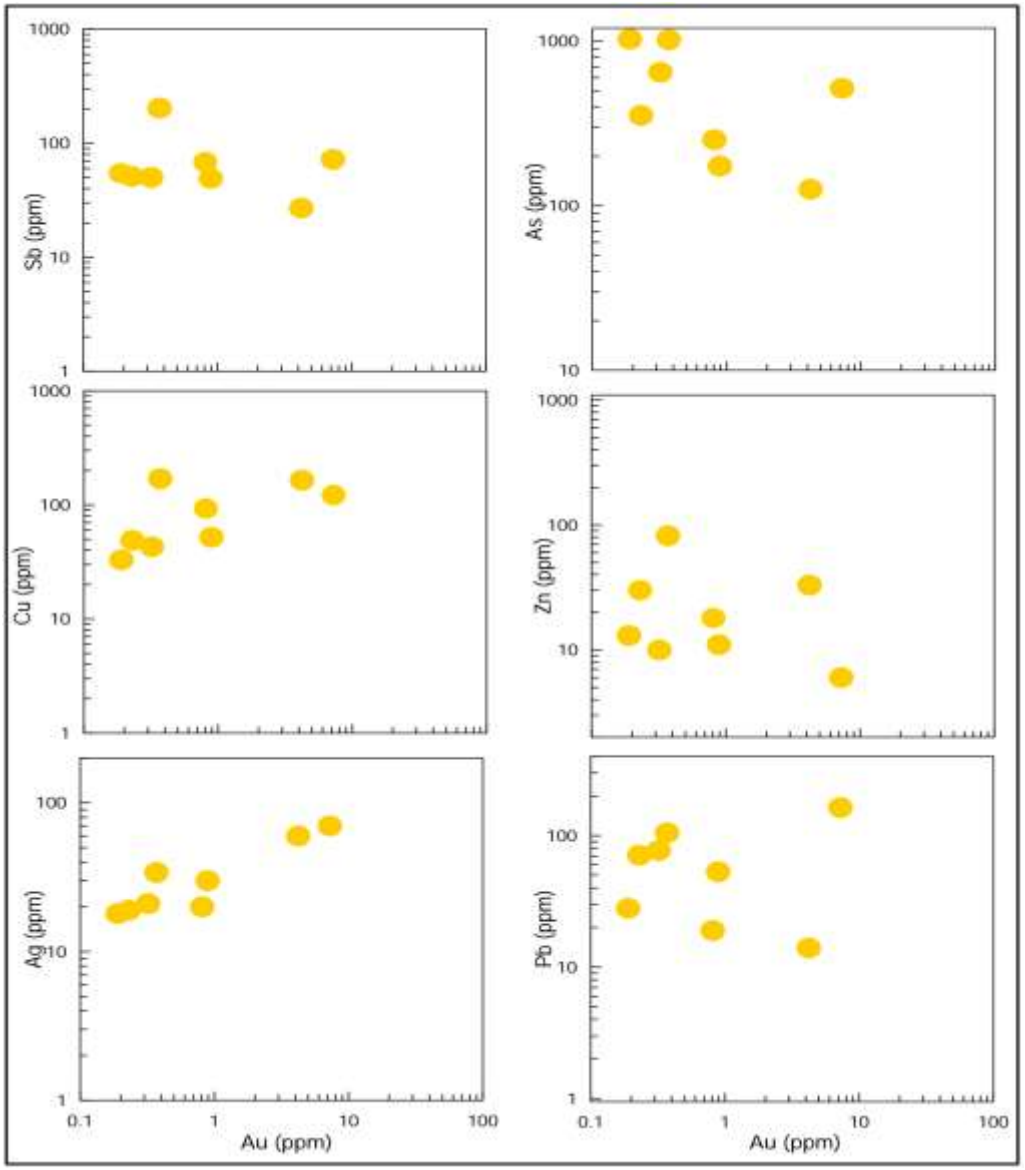


Figure 13. Multi-element plots of gold versus Ag, Cu, As, Sb, Sn, Zn and Pb showing their relation at Shwebontha area.

Conclusions

The Shwebontha area is located west of the Chindwin river and Monywa City in Central Myanmar. Geologically, the Shwebontha prospect area is characterized by magmatic extrusion that occurred during the Upper Oligocene to Middle Miocene of Magyigon Formation which led to the outcrops of rhyolite rocks. According to the geochemical data, rhyolite rocks from the Shwebontha area are plotted in the rhyolite/dacite field and calc-alkaline area. Rhyolite rocks are also classified using major element (TiO_2) and trace elements (Zr, Nb, and Y), showing that all host rocks fall in the fields of rhyolite. In addition, SiO_2 vs Na_2O+K_2O plot diagram as well as AFM diagram indicate that most of rock samples are classified in the field of the calc-alkaline series. The rhyolite rocks surrounding the mineralized veins are commonly extensively altered. The mineral assemblages show that the types of alteration include silicification as well as argillic and propylitic alteration. The alteration mineral assemblages include quartz, adularia, calcite, chlorite, epidote, kaolinite, sericite, illite and illite/smectite. Most of the ore mineralization is in open-space filling veins with lesser amounts in replacement and disseminated ore minerals in the volcanic and volcaniclastic host rock. The mineralized rocks contain gold-bearing brecciated quartz vein and chalcedonic quartz vein from the Shwebontha prospect. The most common primary ore minerals in the mineralized veins at the Shwebontha area include pyrite, galena, sphalerite, chalcopyrite, and gold/electrum. Covellite, goethite, and hematite occur as late supergene minerals in the shallow portions of the veins. The ore minerals occur as replacements, disseminations, and massive accumulations in the mineralized veins. On the basis of precious data and current understanding from the all available data including hydrothermal alteration, mineralized quartz veins and ore mineral assemblages, the Shwebontha area is considered to be epithermal type deposit.

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PETROGENESIS AND GEOCHEMISTRY OF GRANITIC ROCKS IN ZAW PHO KYIN MINE, DAWEI TOWNSHIP, TANINTHARYI REGION

Ye Zaw[1], Aung Zaw Myint[2]

Abstract

The present research area was conducted at Zaw Pho Kyin Mine situating about 70.84 km east of Dawei, Tanintharyi Region. The total area coverage is about 32 km^2. Two major rock units, the metasedimentary rocks of Mergui Group and biotite granite are widely exposed. Granite is well exposed in the central part of the study area. Granite is highly weathered at Zaw Pho Kyin prospect in which the wolframite bearing quartz veins are intruded. The texture of the biotite granite in the Dawei district varies from coarse to medium-grained, consists of quartz, microcline, partly sericitized, plagioclase, biotite and minor muscovite. Geochemically, granitic rocks show high - K calc-alkaline series and are predominantly peraluminous. In Harker's variation diagram, Al_2O_3, TiO_2, FeO, CaO, Na_2O, K_2O and MnO are negatively correlated with SiO_2 whereas MgO and P_2O_5 are positively correlated with SiO_2. Plots of Harker's variation diagrams, selected trace elements of W, Sn, Mo, Ba, Rb, Y, Ni and Zn are negatively correlated with SiO_2 and Zr is positively correlated with SiO_2. Standard CIPW normative corundum ranges from 1.826 to 6.809 and A/CNK ratio is > 1.1, indicated S-type granite and peraluminous granite from Zaw Pho Kyin mine area.

Keywords*:* *S-type granite; paraluminous; Zaw Pho Kyin Mine, Dawei Township*

Introduction

Myanmar is located at the south of the East Himalayan Syntaxis. The major topographic and tectonic features of Myanmar are generally NNW-SSE direction. As a result of the study of plate tectonic evolution, the geotectonic provinces of Myanmar, from east to west are divided into four main provinces, such as the Eastern Highlands Province (EHP) or Sino-Burma Ranges**,** the Central Myanmar Belt (CMB) or Inner-Burma Ranges**,** the Western Ranges (WR) or Indo-Burma Ranges**,** the Rakhine Costal Belt (RCB) or Arakan Coastal Area (Win Swe *et.al,* 2012). Tanintharyi Ranges are composed various deformation and weakly metamorphosed clastic sedimentary rocks that referred to as the Mergui Group in Late Carboniferous to Early Permain. The Mergui Group of rocks is exposed along the Tanintharyi Region, including both the mainland and Myeik Archipelago (Win Swe,1975 and 1976). The region is part of the tin-tungstern metallogenic province. This province is about 1500 km (932 miles) long, which extend from Yunan in the north to Malaysia and Indonisia in the sourth. This tin- tungstern belt passes through the Shan-Tanintharyi Region. This belt is a middle segment of a great tin-tungstern province of SE-Asia. The tin-tungsten deposits constitute one of the most important minerals resource of Myanmar. The study area is located southern part of tin-tungsten belt in Myanmar. The tin-tungsten mineralization is spatially related to granitic intrusion. Dawei is famous for its Sn-W deposits and occurrences situating in the Eastern Highland Province (EHP). The EHP is composed Kachin State from the north, Shan plateau in the middle and Tanintharyi ranges together with Myeik Archipelago to the south. The oldest rocks in Myanmar are exposed only in EHP. The regional geology of the Tanintharyi area, Dawei Township, according to the regional geological map of Dawei area (Aung Zaw Myint, 2016), two main rock types occurred metasedimentary rock of Mergui Group and granitic rocks. Mergui Group is the oldest rock unit in this area. It is widely distributed in the Dawei area. The main purpose of the present study was to know the origin of

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granitic rock in Zaw Pho Kyin mine area, Dawei Township, Taninthayi Region (Figure 1). The objectives were to construct seveal discrimination and variation diagrams, to distinguish the rocks types, to identify their geochemical characters and to constrain the tectonic setting of the granitic magmatism.

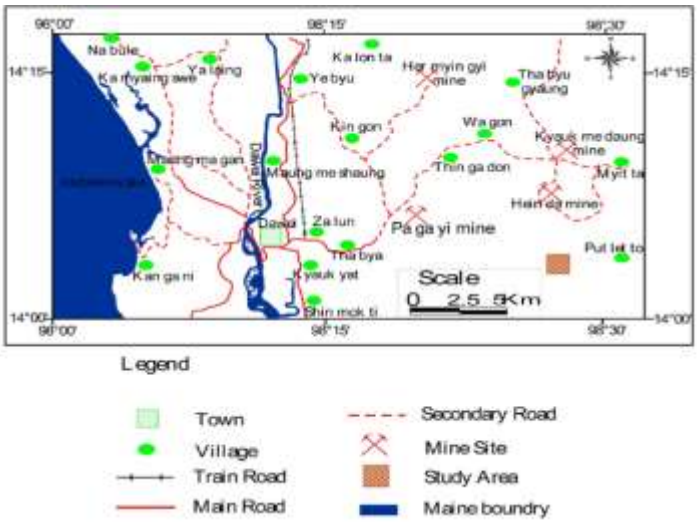

Figure 1. Location map of the study area

Materials and Methods

Study area and study period

Dawei is located 639.17 km southeast from Yangon. The study area is situated about 70.84 km east from Dawei city (Figure 1). The study area is between Latitudes 14°03´00´´-14°05´00´´N and Longitude 98°25´45´´- 98°29´45´´ E in one-inch topographic map of 95-J/8. It is situated between vertical grid No.380 to 460 and horizontal grid No. 530 to 570 in UTM map of 1498/08. It covers length about 8 km in East-West direction and width about 4 km in North-South direction. The total area coverage is about 32 km^2.This paper presents mineralization as well as ore mineralogy, wall rock alteration, and geochemical characteristics of tungsten-tin mineralization.

The present research area was conducted at Zaw Pho Kyin Mine situating about 70.84 km east of Dawei Township, Tanintharyi Region. Two major rock units, the metasedimentary rocks of Mergui Group and biotite granite are widely exprosed in the research area. They are Mergui Group (pebbely mudstone and metagraywacke) and granitic rocks (biotite granite and greisenized granite) are exposed in the research area. The Mergui Group is widely distributed around the Putletto area and the oldest unit in the study area. Generally, they trend NNW-SSE direction and dipping to the east. The mainly prominent rocks are bluish grey color and highly jointed metagraywacke. In the study area, the plutonic (intrusive) igneous rocks can be divided into two types. They are Biotite granite and greisenized granite. This granite is well exposed in the central part of the study area and it hosts tin-tungsten mineralized quartz vein. Geological map of the study area is shown in Fig (2). The study was lasted in 2018.

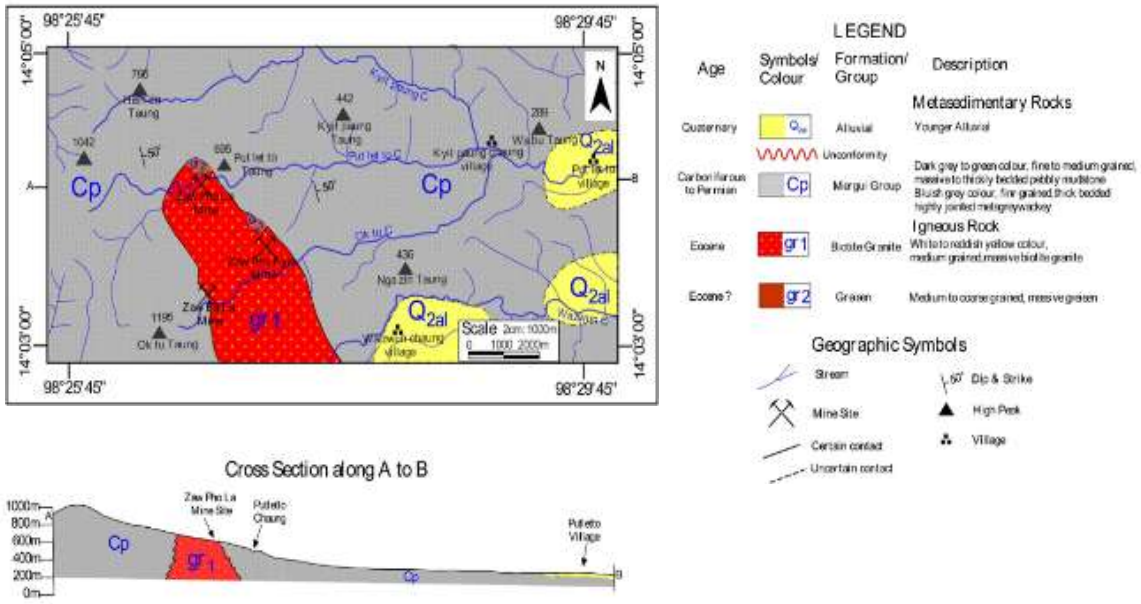

Figure 2. Geological Map and Cross-Section of the Study area

Study of petrographical analysis

Thin-sections of the rock samples were prepared for detail petrographic studies using transmitted light polarizing microscope. These samples were described megascopically and microscopically with representative hand specimen photographs and photomicrographs.

The representative igneous rock samples including 7 biotite granites and 2 greisen from the study area were selected for analysis. The major oxide and trace elements abundance were determined by X-ray fluorescence spectrometry. All igneous rock samples from the study area were analyzed at Economic Geology Laboratory, Department of Earth Resources Engineering, Kyushu University.

Results

Petrogenesis of granitic rocks

Granite is well exposed in the central part of the study area. Granite is highly weathered at Zaw Pho Kyin prospect in the study area. The wolframite bearing quartz vein intrudes the granite of the study area. The texture of the biotite granite in the Dawei district varies from coarse to medium-grained. In study area, biotite granites are coarse-grained, hypidiomorphic granular texture. It is essentially composed of quartz, plagioclase, alkali feldspar (orthoclase, microcline, perthite and albite), biotite and minor amount of muscovite (Figure 3a &b)

Quartz grains are elongate, euhedral to subhedral. Plagioclase occurs as subhedral grains with polysynthetic twins and normal zoning some are altered to saussuritization (Figure 4a)

Alkali feldspar consists of perthite, microcline and orthoclase. The most common alkali feldspar is orthoclase that has subhedral form and simple contact twin. Untwined orthoclase is more common than twinned orthoclase. Simple contact twin of orthoclase crystal in microcline (Figure 4d). Some orthoclase is altered to sericite and zircon inclusion in orthoclase (Figure 4b). Sericitization is well developed in orthoclase (Figure 4c). Microcline exhibits cross hatched twinning (Figure 4d). Microcline intergrowth with sodic plagioclase from microcline perthite (Figure 5a). Perthitic texture is fromed by the intergrowth of alkali feldspar and albite. Perthitic

textures indicate that the feldspars are formed at high temperature and cooled slowly, result in unmixing as the solvus curve.

Biotite occurs as subhedral flakes form and it shows yellowish brown to dark brown. Biotite is observed as inertial the orthoclase feldspar and edge of orthoclase crystal (Figure 5b). In some biotite is altered to chlorite along the cleavage plane, (Figure 5c) and it sometime associated with muscovite, (Figure 5d).

Figure (3) a**.** Photograph showing nature of exposure and close up view of biotite granite at Zaw Pho Kyin Prospect, b. Photograph showing nature of exposure and megascopic view of greisen at Zaw Pho Kyin Prospect

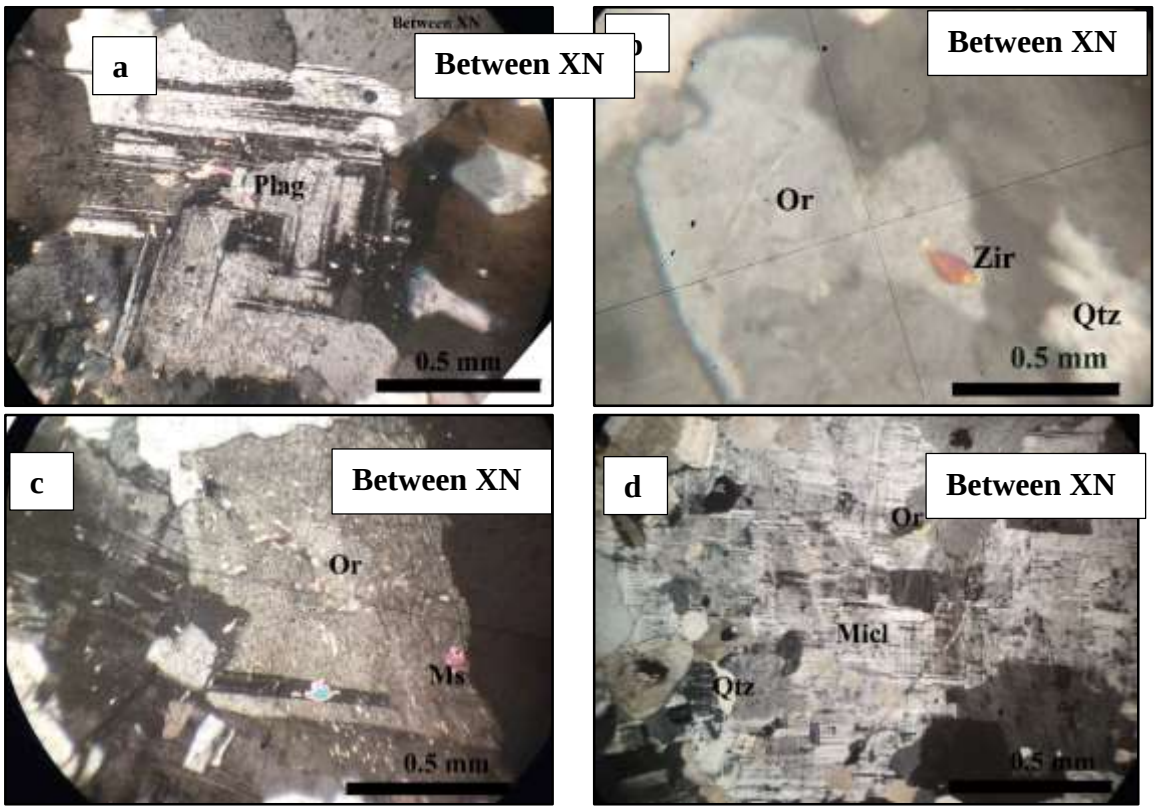

Figure (4) a. Photomicrograph showing normal zoning plagioclase in biotite granite (Between X.N, 10X), b. Photomicrograph showing zircon inclusion in orthoclase crystal in biotite granite (Between X.N, 10X), c. Photomicrograph showing secritization in orthoclase crystal (Between X.N, 10X), d. Photomicrograph showing simple contact twin of orthoclase in microcline at biotite granite (Between X.N, 10X)

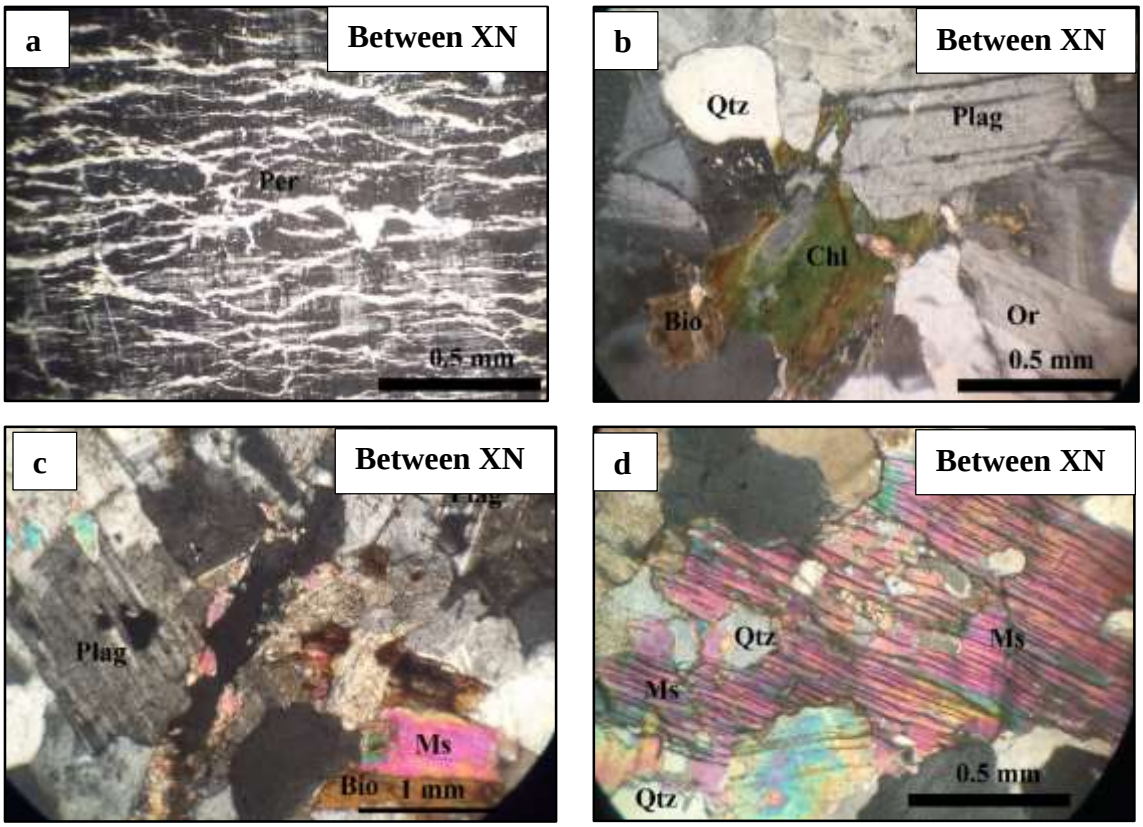

Figure (5) a. Photomicrograph showing string perthite in biotite granite (Between X.N, 10X) b. Photomicrograph showing chloritization along the biotite cleavage planes in biotite granite (Between X.N, 10X & 4X), c. Photomicrograph showing the biotite (Bio) associated with muscovite in biotite granite (Between X.N, 10X), d. Photomicrograph showing quartz grain observe as muscovite (Ms) flakes in greisen (Between X.N, 10X)

Geochemical characteristics of granitic rocks

Major elements oxide

The granitic rocks of the study area are biotite granites and greisen. The concentration of major element oxides of biotite granite rocks from the study area include SiO_2, Al_2O_3, FeO, MgO, CaO, K_2O, Na_2O, TiO_2 and P_2O_5 are presented in table (1). The SiO_2 contents range between 75.641 and 78.058 wt.% corresponding to a felsic composition. The contents of Al_2O_3(11.534-13.17wt.%), K_2O (3.942-4.631wt.%) and Na_2O (0.386-2.836wt.%) respectively. Total alkali contents (Na_2O + K_2O) ranges from 4.328 to 7.467 wt%. MnO and P_2O_5 concentrations are less than 0.5 wt%.

The concentration of major element oxides of greisen rock from the study area include SiO_2, Al_2O_3, FeO, MgO, CaO, K_2O, Na_2O, TiO_2 and P_2O_5 are presented in table (1). The SiO_2 contents range between 75.441 and 83.423 wt.% corresponding to felsic composition. The contents of Al_2O_3 (8.647- 12.538 wt.%), K_2O (2.924-4.279wt.%) and Na_2O (0.357-0.398wt.%) respectively. Total alkali contents (K_2O+Na_2O) ranges from 3.281 to 4.677 wt%. MnO and P_2O_5 concentrations are less than 0.5 wt%.

Table 1. Concentration of major (in wt.%) and trace elements (in ppm) by XRF for the Granite and Greisen sample in study area

Sample No	**zpl-1/16**	**zpl-5/19**	**zpl-6/19**	**zpk-3/20**	**zpk-5/20**	**zpk-8/20**	**zpk-11/20**	**zpl&zpk-5/21**	**zpl-6/21**
Major elements (wt%)									
SiO_2	76.33	77.908	78.058	83.423	75.641	75.649	76.108	75.672	75.441
TiO_2	0.019	0.018	0.013	0.029	0.034	0.03	0.068	0.029	0.019
Al_2O_3	12.897	12.201	12.181	8.647	11.534	13.17	12.701	13.136	12.538
FeO	1.138	0.977	0.821	2.229	3.969	1.299	1.412	1.287	3.373
MnO	0.153	0.074	0.044	0.167	0.271	0.101	0.081	0.106	0.37
MgO	0.297	0.288	0.285	0.311	0.289	0.304	0.346	0.303	0.295
CaO	0.542	0.29	0.302	0.049	0.045	0.655	0.622	0.76	0.817
Na_2O	2.597	2.812	2.836	0.357	0.386	2.755	2.414	2.802	0.398
K_2O	5.309	4.631	4.49	2.924	3.947	4.749	5.306	4.56	4.279
P_2O_5	0	0.009	0.015	0.005	0	0	0.005	0	0
Total	99.862	99.878	99.885	99.871	98.856	99.872	99.857	99.875	99.66
Trace elements (ppm)									
S	**0.0046**	**0.0075**	**0.0064**	**0.0081**	**0.9578**	**0.0074**	**0.0067**	**0.0057**	**0.0811**
Cl	5	1	2	2	1	4	4	1	1
V	0	0	0	1	1	9	0	6	0
Cr	19	23	21	28	26	22	17	22	24
Co	10	34	9	0	15	26	20	8	15
Ni	42	38	37	38	35	39	35	38	43
Cu	6	3	6	8	303	3	1	3	324
Zn	8	27	19	59	0	20	41	18	516
Pb	89	88	75	0	472	44	65	36	83
Hg	0	0	0	0	0	0	0	0	0
As	17	15	13	11	0	11	16	14	0
Sb	15	17	14	23	25	19	20	24	28
Sn	29	23	24	143	244	51	43	53	295
Bi	0	0	0	0	0.013	0	0	0	0.007
Mo	12	12	12	14	34	16	18	16	10
W	53	45	33	25	281	37	15	34	70
Rb	687	694	676	848	1239	741	775	703	1330
Sr	8	6	5	0	0	0	25	0	0
Ba	172	162	126	106	133	106	233	100	158
Y	229	173	174	202	227	199	187	205	324
Zr	75	78	75	95	87	92	109	94	73
Th	27	21	22	38	88	38	29	41	57
U	18	19	13	9	0	18	19	10	18

Harker diagrams are used for some major elements relative to SiO_2. Most of the major elements are negatively correlated with SiO_2 whereas the exception of the P_2O_5 contents which are slightly positively correlated (Figure. 6). Classification of the rock with SiO_2 & (K_2O + Na_2O) geochemical rock classification diagram of Middlemost (1994) for plutonic rocks shows that they plot in the granite field (Figure.7).

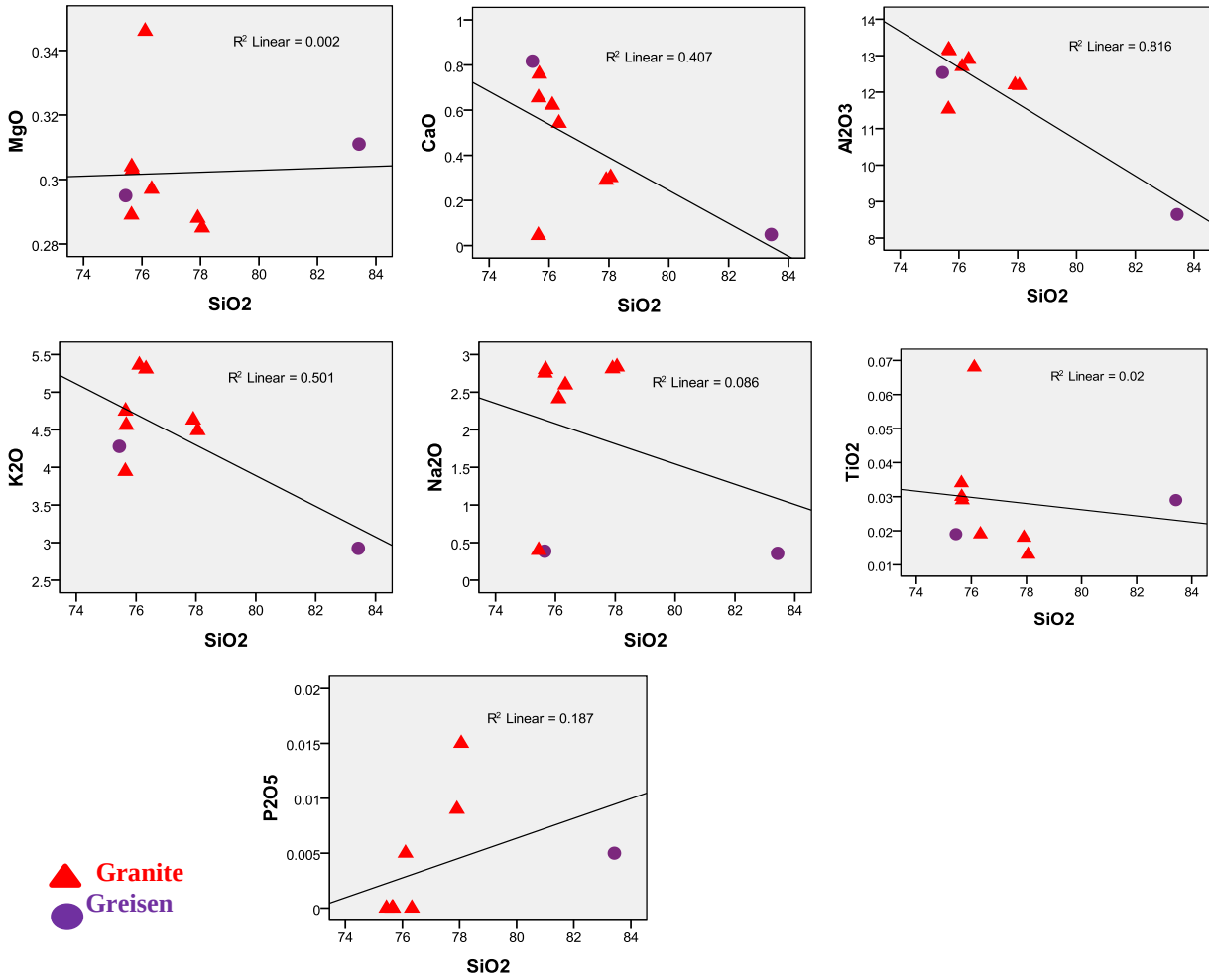

Figure 6. Harker's variation diagrams showing the correlation between major oxide (wt%) and SiO_2 (wt%) of the granitic rocks from the study area

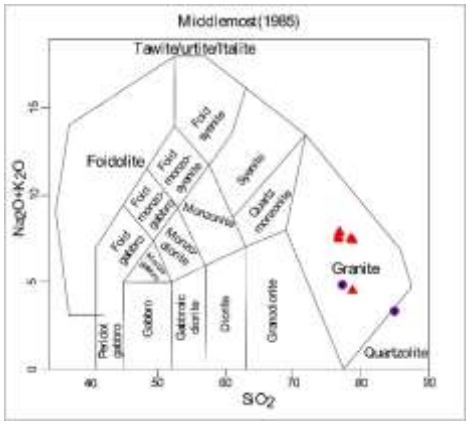

Figure 7. TAS diagram of Middlemost (1985) showing the granitic rock of the study area.

All granitic samples from the study area fall in the high-potassium calc-alkaline series in the K_2O and SiO_2 diagram after Peccarillo and Taylor (1976) Figure 8. The alumina saturation index (ASI) defined by molecular ratio Al_2O_3 / (Na_2O + K_2O + CaO) is greater than one in all the granitic samples ranging from 1.18 to 1.22 implying that the granitic rocks are peraluminous and S-type (Shand, 1943) Figure. 9.

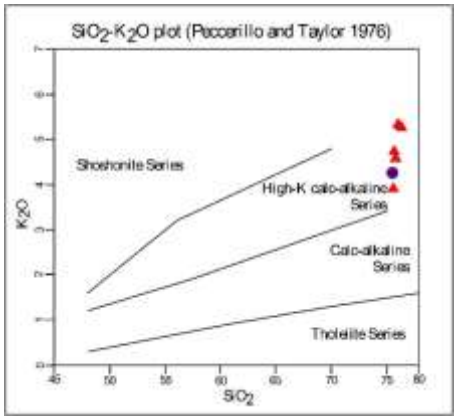

Figure 8. K_2O Vs SiO_2 plot for the granitic rocks of the study area are fitted as in the high-K cala- alkaline Series (after Peccerillo & Taylor, 1976)

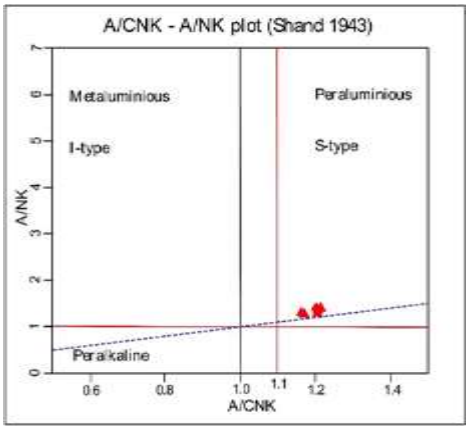

Figure 9. Alumina saturation index diagram of Shand (1943)

Trace elements

Trace elements concentration of the granitic rocks from the study area are listed in table (1) which shows the concentration of large ion lithophile elements (LILE) such as Rb (676-1330 ppm), Sr (0-25 ppm) and Ba (100-233 ppm). The ternary diagram Rb-Ba-Sr is applied for classification of genetic types of plutonic rocks (EL Bouseily and EL Sokkary, 1975) Figure.10. Trace elements discrimination (Y+Nb) and Rb diagrams (Pearce et al., 1984) indicates that the granitic rocks of study area fall in the WPG setting (Figure. 11).

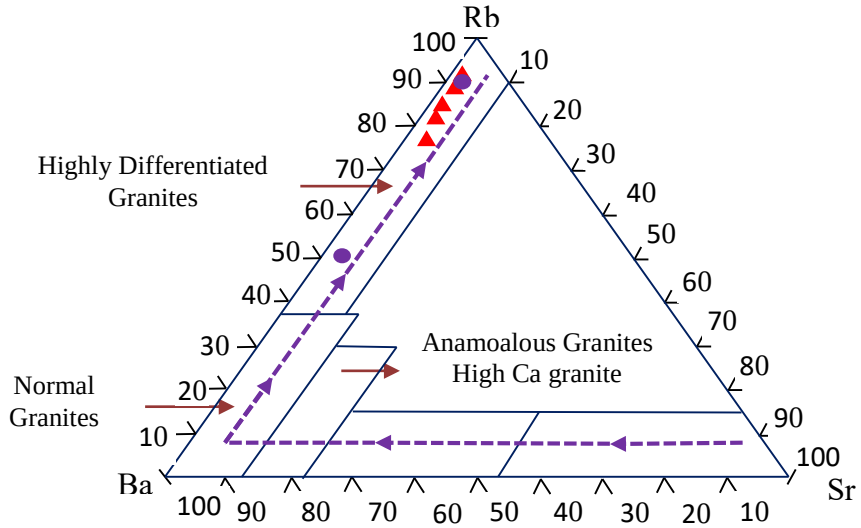

Figure 10. The different fields of the ternary relation Rb-Ba-Sr on some investigated granitic rocks. Arrow indicates differentiation trend. (after EL Bouseily and EL Sokkary, 1975)

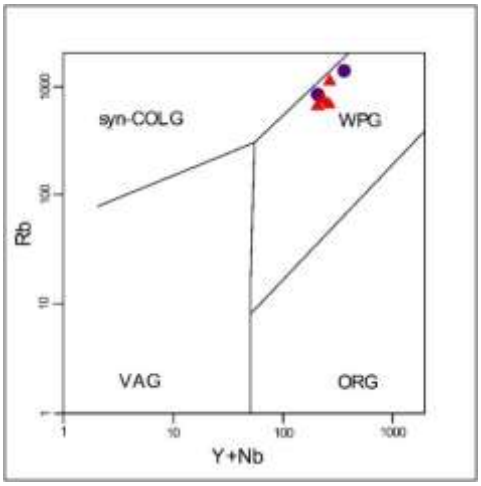

Figure 11. Y+Nb Vs Rb diagram showing the tectonic setting of granitic rocks (after Pearce et al, 1984)

Discussions

In the study area, P_2O_5 of biotite granite is slightly increased with SiO_2 contents. It reveals a highly fractionated S-type granitic magma. The Rb-Ba-Sr relationship also exhibits that highly differentiated granite. The granitic rocks of the study is characterized as highly fractionated, peraluminous, and exhibiting a tectonic signature of WPG setting, which confirms that they were formed on the continent relation to the subduction of an oceanic plate beneath the continent. Yang *et al.* (2018) stated that the strongly peraluminous S-type granite formed from the partial melting of peraluminous clastic sediments are common in the continental collisional zones, even during the Neoarchean time.

According to the previous literatures stated that the content of P_2O_5 in highly fractionated S-type granites is high compared to I-type granites (Chappel and White, 1992; Champion and Bultitude, 2013). In the present result recorded that the granitic rocks of the study area is characterized by the A/CNK [molar Al_2O_3/ ($CaO+Na_2O+K_2O$)] value ranging from 1.18 to 1.22. The SiO_2 is negatively correlated with Na_2O, MgO, TiO_2, Al_2O_3, K_2O and CaO except for P_2O_5.

Conclusion

The granites within the WGP of the Southeast Asia are mostly S-type and I-type granites which are related to collision following the westward subduction of the West Myanmar Terrane beneath Sibumasu during the Cretaceous to Tertiary. Tin-tungsten mineralization in the Central Granitoid Belt of Myanmar occurs dominantly as near-vertical and parallel, greisen bordered, quartz vein-type deposits at the cusps of small granitoid plutons or along the granitoid metasedimentary rocks contact or exclusively in the adjacent metasedimentary country rocks. The granitic rocks of the Zaw Pho Kyin tin-tungsten deposit are mainly composed of quartz, feldspar (plagioclase, orthoclase, and microcline), and mica (muscovite and biotite). They are strongly peraluminous and highly fractionated S-type granites formed in a WPG setting. Thus, S-type granites of Zaw Pho Kyin Mine area were produced through partial melting of the metasedimentary rocks and parental magma may have been derived from a crustal source.

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THE STUDY ON NATURAL RESOURCES RELATED TO THE MICRO, SMALL, MEDIUM AND HEAVY ENTERPRISES AROUND KYAUKSE AREA, KYAUKSE DISTRICT, MANDALAY REGION

Hnin Min Soe[1]

Abstract

The study area is between Latitude 21° 31' N to 21°47' N and Longitude 96°07' E to 96°17' E, Kyaukse District, Mandalay Region. In fact, this area is located 45 km SSE of Mandalay, occupying parts of UTM map sheets N0.2196-02 & 06. Firstly, field works have been undertaken for systematic sampling of rocks and minerals for the geological study of the research area. Second tasks dealt with the physical and chemical properties of rocks and minerals and their uses. Some geologists have attempted to describe the geology and mineral resources of the present research area and its environs. However, natural resources related to the Micro, Small and Medium Enterprises (MSME) and Heavy Enterprise (HE) have not yet been done. Therefore, the focus point of research works attempts to explore the MSME and HE with the natural resources of industrial raw materials (quartzite, marble, limestone, and dolomitic limestones units), decorative stones as well as road materials and construction materials (Biotite granites, Microgranite, Leucogranite, Hornblende diorites, Hornblendite, Calc phyllite, Schist, Gneiss, Calc-silicate, Quartzite, Marble, and Limestone) of the study area. Besides, ore minerals Copper, Cu (0.01 % to 8.01 %), Iron, Fe (2.52 % to 24 %) Lead, Pb (0.01 % to 5.01 %), Barite, Ba (0.03 % to 10.66 % and Manganese, Mn (0.13 % to 6.11%) around the Kyaukse area are to be studied. Final task has been done for the Environmental Impacts affected by the MSMHE factories, especially production of limestones for cement plant will take the quarry mine life last about 50 years for the Thandawmywet Taung and also 20 years for the Nwalagauk Taung in the future. According to the geological and environmental perspectives, the benefits of research works are to be applied for the production of natural resources of the study area, to fulfill the academic needs of Kyaukse University, and to use the findings in the teaching programs for the Department of Geology.

Keywords: *Natural Resources, Micro, Small, Medium and Heavy Enterprises.*

Introduction

Location, Areal extend and Accessibility

The study area is situated between Latitude 21° 31' N to 21°45' N and Longitude 96°07' E to 96°15' E and it also lies in Kyaukse District in the central part of Myanmar. This area is approximately 10.5 km long in north-south direction and 7 km wide in east-west direction, covering about 73.5 square kilometers. The study area falls in UTM map sheets N0.2196-02 & 06. The research area is well-known for its quarries producing the road metals, industrial minerals, construction materials and decorative stones.

Method of Study

This research has been carried out by using field methods and laboratory investigations. Collecting of fresh representative samples of rocks in the study was selected and prepared to analyze the characters of the rocks. Photographing of necessary minerals, rock types and structures had been done in the field. The laboratory work comprises the representative rock samples primarily for the identification of mineral species and elements content of the rocks. Mineralogy

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of ores and rocks were carried out by using Wavelength Dispersive X –Ray Fluorescence (WDXRF) Spectrometer.

Purpose of Study

Purposes of the investigations are as follow:

To prepare a modified geological map of the research area,

To mention the geology and geochemical of the study area,

To describe the natural resources related to the Micro, Small, Medium, Heavy Enterprises of the study area, and

To study the environmental impacts of the research area

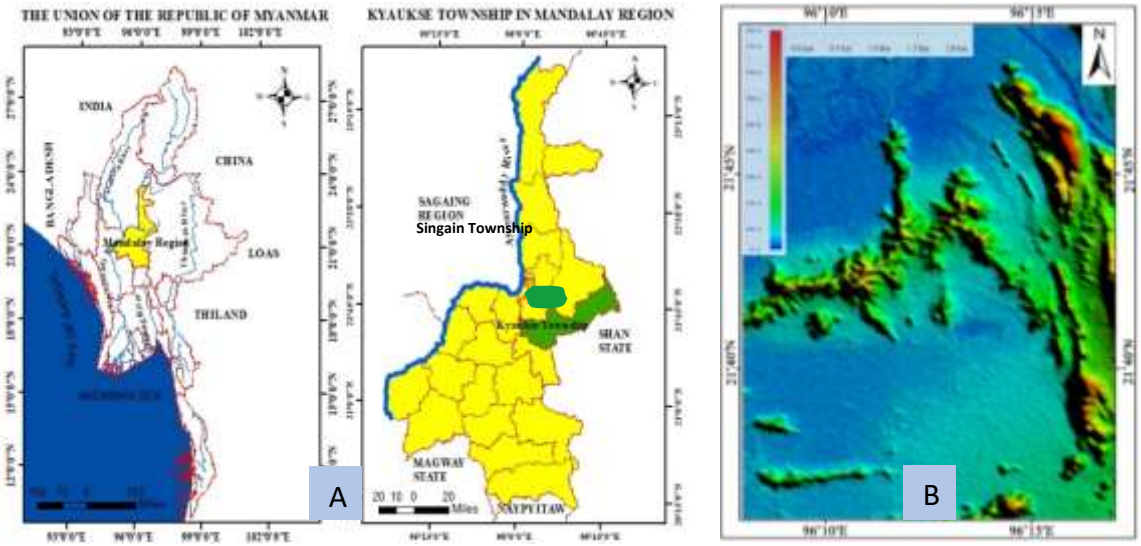


Figure 1.1 (A) Location map and **(B)** Satellite map showing the relief of the study area.

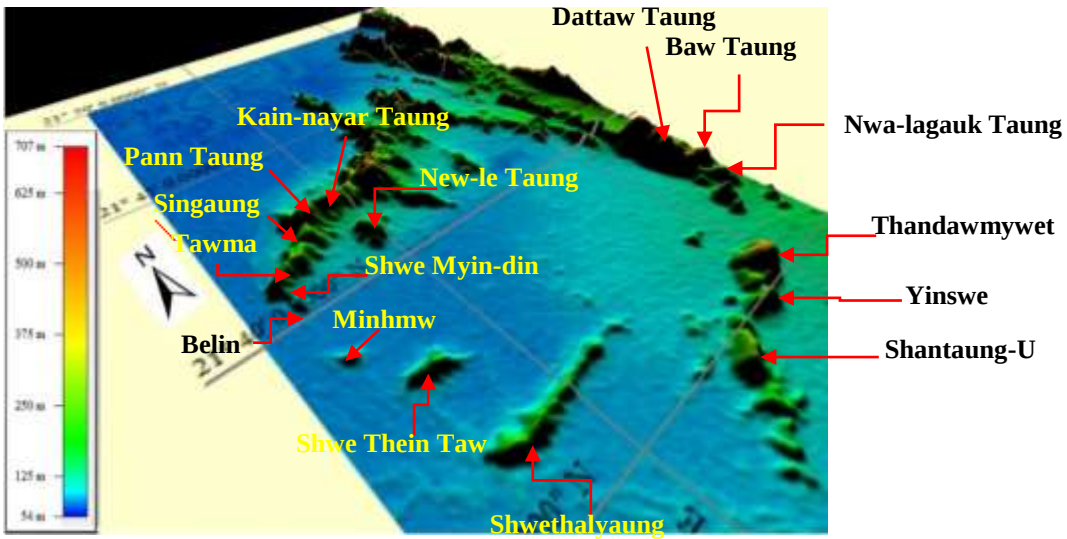


Figure 1.3 DEM (Digital Elevation Model) image showing the physiography of the study area and its environs.

General geology of the study area

The area consists of Upper Paleozoic and Jurassic-Cretaceous sedimentary rocks and their metamorphic equivalents. These rocks are intruded by biotite granites, hornblende diorites, and a few hornblendites. The youngest granite in the Kinda area yielded K/Ar ages of 82 ± 2 m.y and 58 ± 1 m.y (Brook and Snelling, 1976 in Myint Thein, 2004). The biotite-augen gneisses of the Shwethalyaung Hill are regarded as orthogneiss transformed probably from the Mesozoic granite (Myint Thein, 1984). The age of metamorphism affected in this area was probably at the end of Cretaceous (Myint Thein, Bo San and Myint Thein, 1984).

Stratigraphically, in the study area, the sedimentary units can be divided into two groups: they are Upper Plateau Limestone and Pan Laung Formation. The Pan Laung Formation (Middle Jurassic to Early Cretaceous) overlies unconformably the Permian Triassic Upper Plateau Limestone. The name Pan Laung Formation was introduced by Garson *et. al* (1976). Myint Thein and Win Myint (1988) redefined the term. According to Myint Thein (2004) at the eastern margin of this project area, the formation begins on the Plateau Limestone with a unit of red clastics with basal limestone. Boulder and cobble conglomerate unit which passes upwards into red to purplish red sandstones, siltstones, calcareous mud rocks with Mytilus and argillaceous limestone bearing discycladacean algae. This unit is followed by orthoquartzite and quartzite with interlayers of red purples slates which pass upwards into a sequence of cross-bedded sandy allochemical limestones containing Pseudo cyclammina, Haurania and minute gastropods which form nuclei of ooids and oncoids. The upper part of the formation is dominated by pelitic, psammitic and calcareous rocks.

As the grade of metamorphism increases westwards, the rocks of the Plateau Limestone and the Pan Laung Formation becomes low and medium grade marbles (found in west of Dattaw valley, Thandawmyet, Taungni and Nwalegauk Hills, east of Thin-dwe cannel, Kyauk-hte Hill and eastern tip of the Shwethalyaung Hill, and micaschists, phyllites, marble, and calc-silicate rocks and quartzites respectively. Myint Thein, Bo San and Myint Thein (1984) deciphered the structure of this area, the regional synclinal structure, intruded by a stock of granite near Belin is obvious and it is cut across by a north- south fault passing through the Sunye village. Small folds trending north-south occurred near the Sunye Fault between the Kyauk-hte Hill and Htonbo village. The Plateau Limestone makes an anticlinal flexure at Dattaw Hill area. Geological map of the study area is shown in (figure 2.26)

Rock units succession of the study area

Table 2.1 Succession Rock unit of the study area (After Myint Thein, 1984)

Quaternary

Alluvium

Jurassic-Cretaceous

Panlaung Formation

Upper Pelitic-Calcareous Unit: Phyllites, limephyllites, schists, calc-silicates and some quartzites.

Upper Psammitic- Pelitic Unit: Slates, metawacke, quartzites, phyllites and some calc-silicates.

Psammitic- Calcareous Unit: Clastic limestones, calcareous siltstones and sandstones, marbles, limephyllites and phyllites, calc-silicates and schists.

Lower Psammitic- Pelitic Unit: Red and purple clay-slates, siltstones, sandstones, limestone-conglomerates, silty limestones, mica schists, quartzites and some marbles.

Lower Pelitic-Calcareous Unit: Dark schists, phyllites, limephyllites and banded marbles.

Permian-Trassic

Plateau Limestone: Limestones, dolomitic limestones and marbles (low to medium grade), overlying the rocks of the lime clastics (S3qtz).

Quartzite (Upper Silurian)

Granite (Early Tertiary)

Augen Gneiss (Possibly Up. Cretaceous-Paleocene)

Natural Resources of the Study Area

The present research area is well-known for its quarries producing industrial raw materials, decorative stones, construction materials and road metals (Mines of Burma, 1976 in Maung Thein, 1984). The present research works are focusing on (1) Decorative Stones, Road Metals and Construction Materials (2) Industrial Raw Materials (3) Ore minerals: Copper, Iron, Lead-Barite and Manganese ores with Micro, Small and Medium Enterprise (MSME) and Heavy Enterprise (HE) of the study area.

Micro, Small and Medium Enterprises (MSME)

Decorative stones, Road metals and Construction materials

In the study area, exposed metasedimentary units are calc-silicate rock, phyllite, schist, gneiss, quartzite and marble and their age are (199ma - 65ma). Sedimentary unit of limestone (299 ma -199 ma) and intrusive igneous units are granitic rocks, hornblende diorite, hornblendite and dykes and veins units and also their age are (65ma-55.8ma). All of these units are used as Decorative stones, Road Metals and Construction Materials.

In the study area, Belin quarries are well-known from 1915 to 1984, these quarries were worked by private contractors. From 1948 to 1978, Quarry Corporations of the Construction Ministry worked those quarries. From 1979 up to present the Quarry Section of Prison Department has taken over the works (Figure.2.1). The existing quarries are situated in the calc-silicate rocks at south-west of Kyaukkyi Taung in the Belin area. The rocks quarried are mainly calc-silicate rock

with subordinate phyllite, schist, quartzite, marble and granite. They are used as road metals, construction materials and decorative stones. The annual output is estimated as 600,000 cubic feet.

Rock Slab Factory of Belin is famous in the last decade and it stands as a Heavy Enterprise at that time. Because of the easy accessibility, availability of suitable rock types in large amount make quarrying in this area a profitable enterprise. But in recent time, Rock Slab Factory of Belin has been closed down.

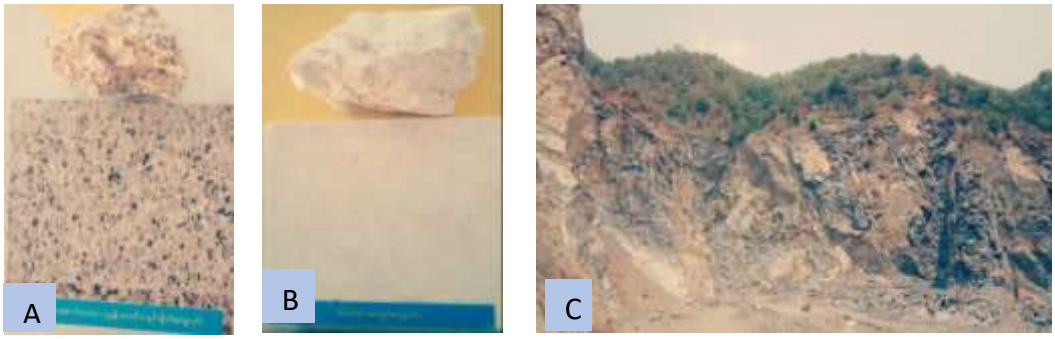
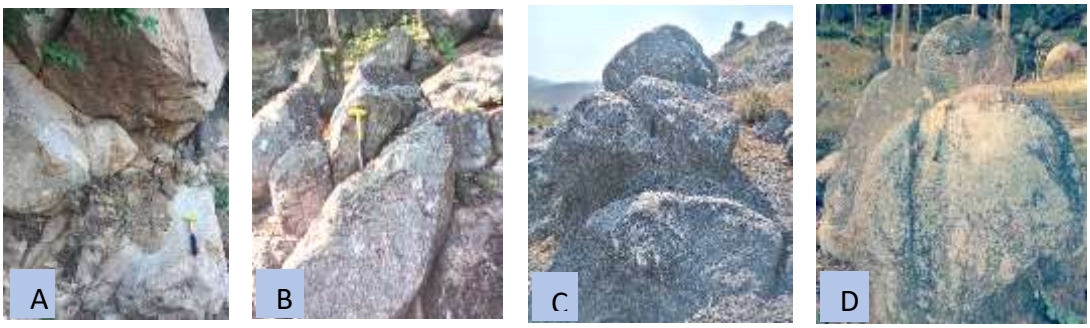


Figure 2.1 (A & B). Granite and Marble slabs are used as decorative stones from Rock Slab Factory of Belin. (C) Calc-silicate rocks, phyllite, and schist exposed at Quarry Section of Prison at Belin area used as road metals.

Biotite granites exposed at Singaung, Pann and Kyaukyi Taungs are used as decorative stones. These marbles are metamorphosed part of the Plateau Limestone Unit. At the eastern end of the Shwethalyaung Hill, exposed white marble, has been quarried and gneiss from this hill also quarried for use as road metals and decorative stones. Compact greenish-gray calc-silicates with micaceous quartzite quarries situated in the study area are Shwemyindin Taung, Kyaukyi Taung, Belin Taung, Nwa-le Taung and near Ngazu and Patta villages. Patta village rocks are known as "Patta Kyauk (Patta Rock)" (Figure 2.2). They are used as road metals as well as construction materials and decorative stones. Local people are still work at Small Enterprise there.

Figure 2.2 (A, B, C & D) Calc-silicates with micaceous quartzite (Patta Kyauk) occur at quarry of Shwemyindin Taung are used as decorative stones.

Granite, Hornblende Diorite and Hornblendite exposed near Tawma village are also being quarried for decorative stones, construction materials and road metals (Figure.2.3 A, B, C, D).



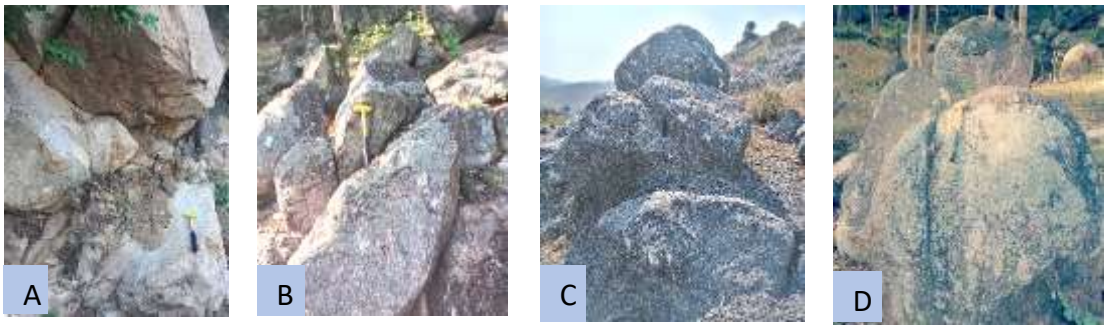


Figure 2.3 (A & B) Leucogranite, Microgranite and Biotite granite exposed at Tawma Taung are used as decorative stones, (C & D) Boulder natures of Hornblendite, Hornblende Diorite units cropped out near Mogaung village are used as decorative stones.

Schists and phyllites units of the research area are also being quarried for decorative stones. Local name of these rocks is called as "Shwelar-Ngwelar decorative stones" in the study area (Figure 2.3 A, B, C, D). Cutting machine for rock slab is shown in (Figure 2.4 A & B). These decorative stones are still popular among the enterprises of Micro and Small in the research area. Sale for current prices is as shown in Table 2.1.

Figure 2.3 (A, B, C, D) Packages of Shwelar-Ngwelar decorative stones.

Figure 2.4 (A, B) Hand cutting machine for decorative stones (Shwelar-Ngwelar Slab)

Table. 2.1 Current prices for Shwelar-Ngwelar decorative stones

Sr. No	Rock Slide Numbers	Package	Size in Box	Price (Kyats)
1	160	1	6″× 1.25″	12000
2	450	1	8″× 4″	98000
3	2880	1	4″× 1.25″	280000
4	1800	1	4″×2″	280000
5	1200	1	4″×2″	95000

In the study area, sand from the Pan Laung River, Zawgyi River, Myitnge River and Ayeyarwady River are used in construction of roads and buildings. Sand of Ayeyarwady River is the best quality. Current price for a pile of sand is 45000 kyats for sand of Ayeyarwady River (Figure 2.5).

Figure 2.5 Sand of Ayeyarwady River is used as construction for roads and buildings.

Rock powder of brecciated limestones and dolomitic limestones and limestones of this area are used in manufacturing of handmade bricks /sun-dried bricks. The size of the brick is (11″×5″×4″) and current price for brick is 200 kyats/ per piece (Figure 2.6).

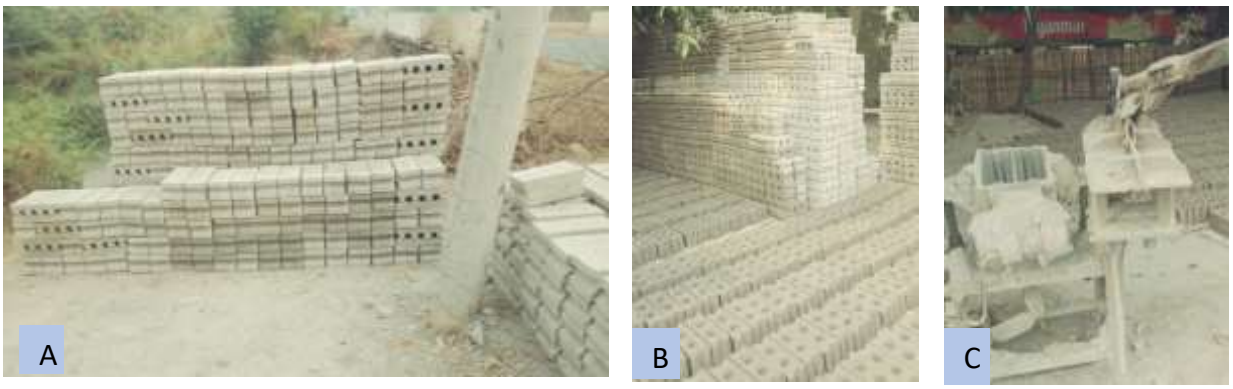


Figure 2.6 (A, B & C) Hand-made bricks /Sun-dried bricks (Byone-brick) are used as construction of buildings.

Various sizes of chippings of brecciated limestone, dolomitic limestones and limestones are used as foundation stones as well as road stone. Current price for One-pile is depending on the rock chippings size: (Big-sized 6″×9″), (Medium-sized 2″×4″), (Small-sized 1″×2″) and (Ngar-Moo Size 1/2″) (Figure 2.7).

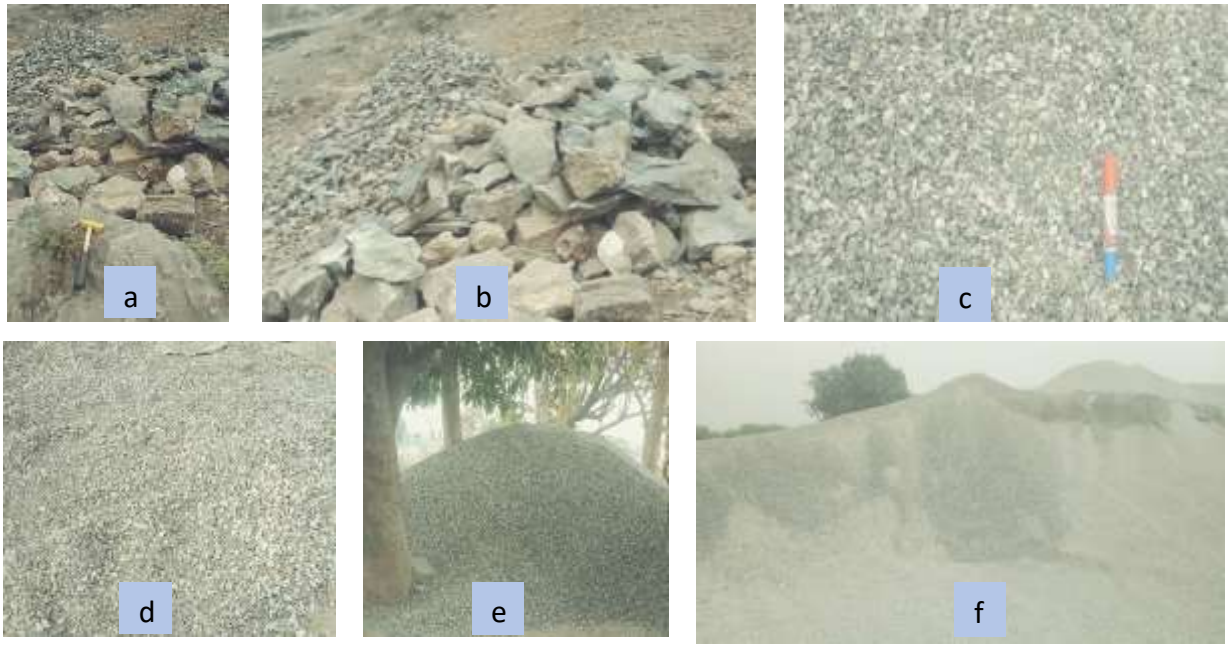


Figure 2.7. (a, b, c, d & e) Various sizes of chippings of brecciated limestone, dolomitic limestones and limestones of the study area are used in construction purposes.

In making concrete materials such as various diameter sizes of concrete barrels, concrete cover slabs, concrete tunnel pipes, and concrete fence poles (Figure 2.8). For making concrete materials using three kinds of materials ratio is 1 package of cement: 2 packages of sands: 4 packages of rock powders (3/4″ & 1/2″ Size). The best powder of rock is limestone unit. They all are registered in the list of Micro and Small Enterprises and some local people depend for their life by doing such jobs in the study area.

Sand and Rock Powders.

Figure 2.8. (A, B, C, D & E) Various forms of concrete materials can be made by using Cement, Sand and Rock Powders.

Heavy Enterprise (HE)

In the Kyaukse area, Heavy Enterprise (HE) are situated especially in SE of Kyaukse city such as cement factories of Alpha, Double Rhino and Sin Minn and one glass Factory. All factories need non-renewable resources like limestones, dolomitic limestones, dolomite, marbles and quartzites of the study area. Permian to Triassic age Plateau Limestones are well exposed at Thandawmywet, Nwalagauk and Taungni Taungs. It is a high-quality limestone mining area in Myanmar.

Geological information of industrial raw materials

Industrial Raw Materials in the study area are limestones, dolomitic limestones, marbles and quartzites. Age of the limestone and dolomitic limestones is (299 ma -199 ma), marble and quartzites is (199 ma-65 ma).

Depending on the application, the quartz must have varying grain sizes. Due to its weather-resistant and robust properties, quartzite is very well adapted as natural stone for floor coatings, gravel and other uses in the construction industry. The stone is also used as a raw material in the glass industry. Quartzite from Yinswe Taung is extracted for glass factory in the research area.

Low grade marbles of Thandawmyet and east of Shwethalyaung Hills are being used in cement factories. These rocks are metamorphosed Plateau Limestone. The marbles exposed near Sunye have been quarried for lime burning. The Plateau Limestones exposed at Dattaw Ridge and Thandawmyet Hill is also suitable for used in the cement industry. The limestones of the Pan Laung Formation and some limestones of the Plateau Limestone can be used in cement factory and in baking limestones for lime. Limestone has numerous uses: as a chemical feedstock for the production of lime used for cement, as aggregate for the base of roads, as fluxing materials in iron and steel industry, as white pigment or filler in products such as toothpaste or paints, as a soil conditioner, and as a popular decorative addition to rock gardens. Limestone is also used in sugar industry in the preparation of mill of lime which is added to cane juice to remove impurities. Lime is also an essential raw material in chemical industries and fertilizer manufacture. Analyzed results of limestones are shown in (Table 2.2) and analyzed samples locations is in (Figure 2.22).

Cement is a fine powder made up of calcium silicates, aluminates, and alumino ferrites, which are all hydraulic, cement components. There are 30 unripe components in all, divided into four categories (calcium, silica, alumina, iron).

Alpha Cement Plant

Alpha Cement Plant is situated in the Latitude 21° 36' 39″ N and Longitude 96°10'13″ and it also lies in Kyaukse area, NW of Thandawmywet Taung. Myanmar Conch Cement Co., Ltd. was incorporated by Anhui Conch Cement Co., Ltd. (China). The company is located at the industrial zone, Kyaukse. It is about 2 km to Kyaukse city and about 45 km to Mandalay city (Figure 2.9). Lime Quarry near the plant is shown in (Fig.2.10). Limestone and Dolomitic limestone exposed there (Figure 2.11 A & B).

Alpha Cement Limited is also famous for its low Alkali Content, Low Heat of Hydration, Excellent Consistency, Good Resistance to Sulphate and Chloride, High Early Strength, Low Expansion, No Cracks and Good Workability. Alpha Cement can be used in all general constructions especially in Major Projects where cement is to meet stringent requirements; it can

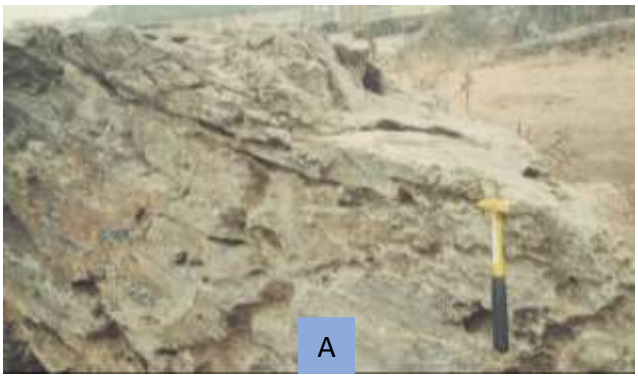
be used in concrete motors and grouts, concrete blocks and bricks, High rise buildings, dams, bridges etc.

Demand for cement in the construction of development of the Kyaukse District, Mandalay Region and Country Myanmar. Conch Cement Company was produced Alpha cement 5000 ton per day. Distribute two types of cement such as 50 kg bag and 30 Tons silo truck to users (Figure 2.12 A & B).

Figure 2.9. Cement plant of Alpha, Kyaukse

Figure 3.10 Lime Quarry for Alpha cement plant.

(GPS. 21° 36' 39″ N and 96°10'13″E)



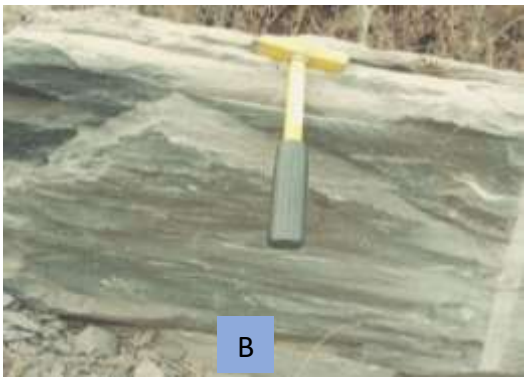


Figure 2.11. (A & B) Dolomitic Limestone and Limestone for Alpha cement at NW of Thandawmywet Taung (GPS. 21° 36' 39″ N and 96°10'13″E).

Figure 2.12. (A) Distribute type of Alpha cement 30 tons silo truck, and (B) 50 kg bag.

Double Rhinos Cement Plant

Double Rhinos Cement Plant is situated in the Latitude 21° 34' 38″N and Longitude 96°14' 57.83″ and it also lies in Kyaukse District, SE of Thandawmywet Taung (Figure 2.12 A). Lime Quarry near the plant is shown in (Figure 2.12 B). Limestone and dolomitic Limestone exposed there (Figure 2.13 A & B).

In Myanmar, this site is the best quality limestone mine-lot area, covers an area of 400 acres, it is currently the only one largest capacity 10,000 Ton per day of cement clinker production project in Myanmar country. Distribute two types (A) Casting-bricked and (B) 50 kg Bag (Figure 2.14 A & B&C).

The classes of this cement are 32.5 and 42.5. 32.5 class cement is suitable for ordinary concrete works, i.e., general housing construction, concrete pavement, concrete tiles, drain pipes, water tanks, masonry and plastering works, etc. 42.5 class cement is suitable for high permeability and high temperature resistant engineering, highways roads, high-rise buildings, all kind of concrete piles and poles, it is also recommended for construction projects, general industrial and civil construction.

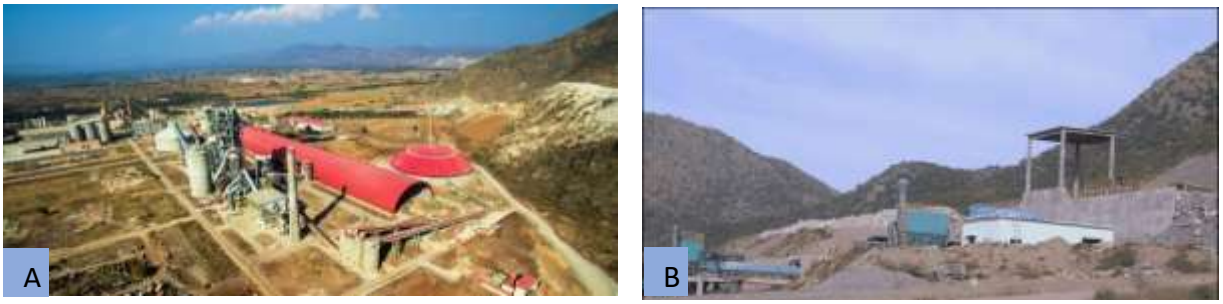


Figure 2.12. (A) Cement plant of Double Rhino in the Kyaukse Industrial Zone.

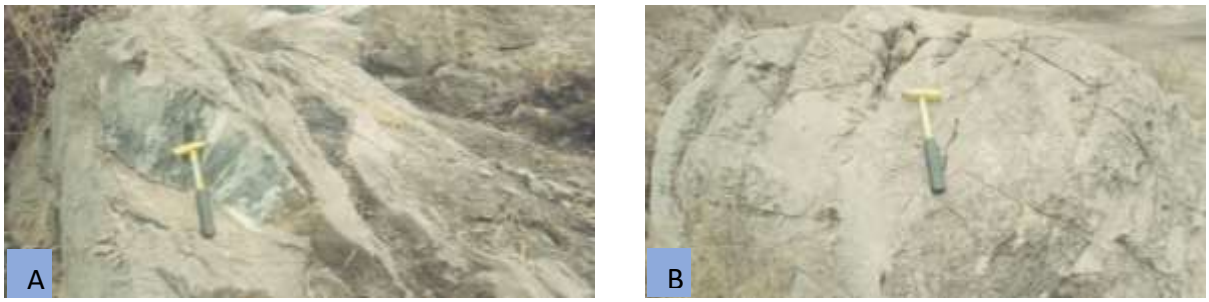


Figure 2.13. Exposures of Limestone and Dolomitic limestone for Double Rhino cement Plant at SE of Thandawmywet Taung (GPS. 21° 34' 38″ N and 96°14' 57.83″E).

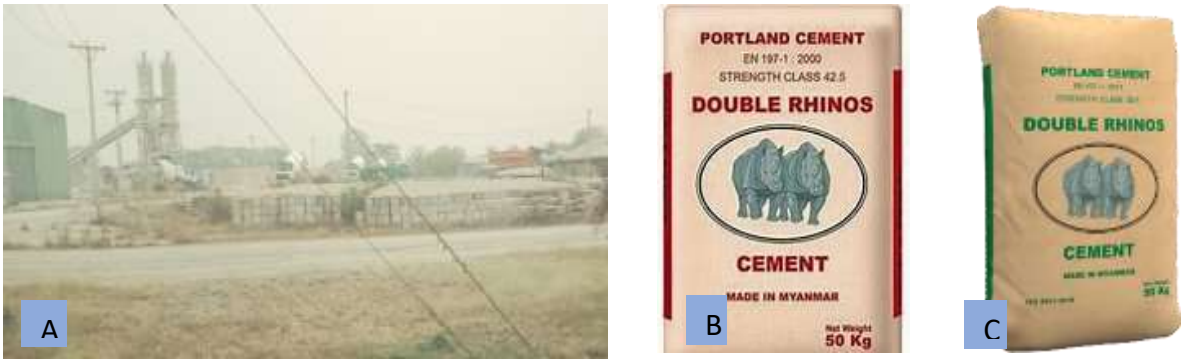


Figure 2.14. Distribute type (A) Casting-brick and (B & C) 50 kg Bag of Double Rhino cement.

Sin Minn Cement Plant

Sin Minn Cement Plant is situated in the Latitude 21° 36' 08″ N and Longitude 96°15' 13.83″E and it also lies in Kyaukse District, west of Nwalagauk Taung (Figure 2.15) Outcrops of

Limestones and Dolomitic limestones are shown in (Figure 2.16 A&B). Sin Minn cement is symbolized by the strength of an Elephant. It offers more than just a solution to the general construction industry but also a brand of imperceptible standard, resonates quality, power, maturity, resilience, durability and reliability. Distribute type of Sin Minn cement is shown in (Figure 2.16 C).

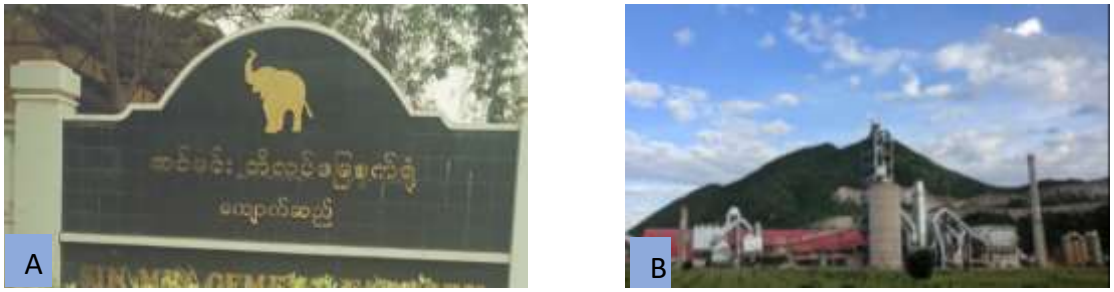


Figure 2.15. (A&B) Cement plant of Sin Minn in the Kyaukse Industrial Zone (21° 36' 08″ N and 96°15' 13.83″E).

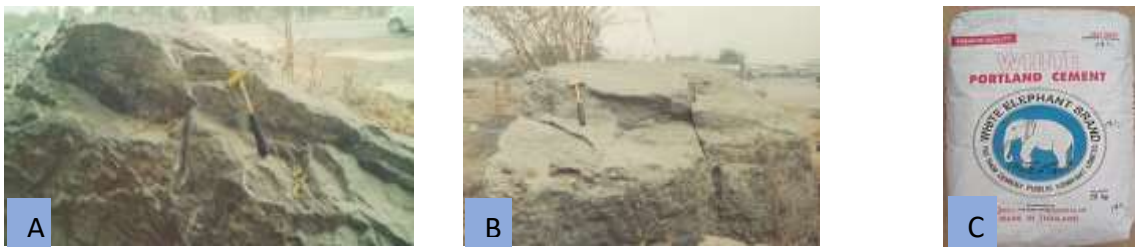


Figure 2.16. (A & B) Outcrops of Limestone and Dolomitic limestone for Sin Minn cement Plant at west of of Nwalagauk Taung (21° 36' 08″ N and 96°15' 13.83″E). (C) Distribute type of Sin Minn cement.

Glass Factory

Glass factory is situated in the Latitude 21° 35' 31.28″ N and Longitude 96°12' 47.56″E and it also lies in Kyaukse District, south of Yinswe Taung (Figure 2.17). Phyllite, schist and quartzite units are well exposed at the Yinswe Taung. Quartzite from Yinswe Taung is extracted for glass factory (Figure 2.18). The important Industrial Raw Materials for manufacture of glass are quartzites, dolomites, limestones, feldspars, Ssodium sulphates (salt cake), carbons and soda ashes. Mirror stands in the Glass Factory is shown in (Figure 3.20).

Cullet occurs as a byproduct from the Glass Factory and it can be reused in the manufacture of glass (Fig.2.19).

Figure 2.17. (A, B) Glass Factory, near Yinswe Taung, Kyaukse Industrial Zone. (C) Quartzite unit from Yinswe Taung (21° 35' 31.28″ N & 96°12' 47.56″E).

Figure 2.18. (A) Bullet-protect mirror, (B & C) Mirror Stands in the Glass Factory.

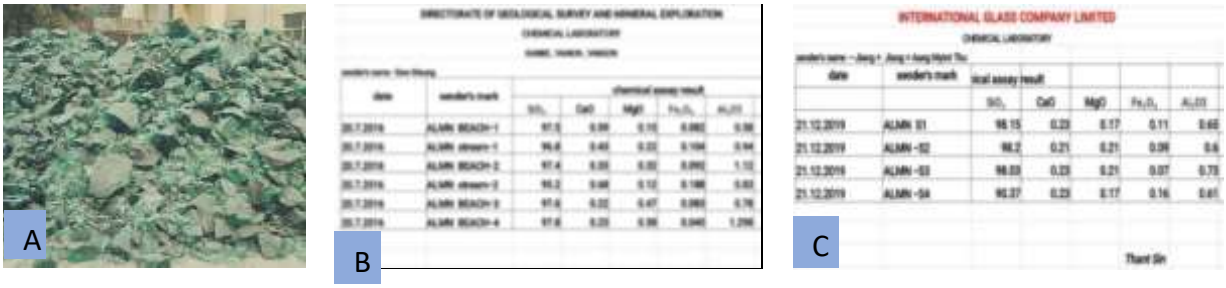


Figure 2.19. (A) Cullet is the byproduct of manufacture of glass in the Glass Factory. (B&C) Geochemical analysis of raw materials for Glass Factory.

Ore minerals in the study area

Copper ore

In the study area, copper ore is found as veins and in the Ye-Yaman tract, south-east of Kyaukse city, copper are is associated with barites. These veins are small and impersistent. Copper mineralization is also found in the Lower Paleozoic rocks as fissure-filled deposits, (Maung Thein, 1984). Copper mineralization occurs in this district is confined to the Late Precambrin and Lower Paleozoic rocks. Common copper ores occur as chalcopyrite, azurite and malachite (Fig.2.20). The concentration of copper (Cu) content ranges from 0.01 % to 8.01 % in (Table 2.3). Copper is ductile and a great conductor, its main use is in electric generators, household/car electrical wiring, and the wires in appliances, computers, lights, motors, telephone cables, radios and TVs. The old adit is found in the vicinity of Kyauk-Aii village.

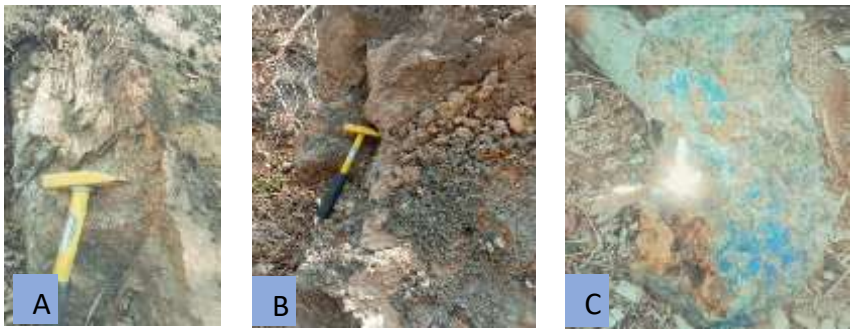


Figure 2.20. Fissure-filled types copper mineralization in Old adit, vicinity of Kyauk-Aii village (N 21° 37'19.82" and E 96° 16' 30.13")

Iron ore

Iron commonly found at the south of Kyauk-Aii village and the ore minerals are magnetite, hematite and limonite. These metallic ore found in phacoidal limestone unit of Nyaungbaw Formation of Silurian age. The ore is a coarse-granular mixture of magnetite and manganese ore.

It occurs as small, lens-shaped massed (Figure 2.21 A & B). The mode of occurrence suggests that the ore lenses had been originally sedimentary iron nodules or lenses in limestone. The concentration of iron (Fe) content ranges from 2.52 % to 24 % (Table 2.3). It is the most useful industrial raw mineral and the primary use of iron ore (98%) is to make steel**,** for home and other construction process.

Figure 2.21. (A&B) Massive type Iron ores occurred in phacoidal limestone unit of Nyaungbaw Formation (N 21° 37'19.82" and E 96°16' 30.13")

Lead-Barite ore

Barites mineralization found in the west of Kyauk-Aii village and at Tha-mone-ye-htwet, barite associated with lead in the host rocks of thinly bedded limestones and siltstones (Fig.2.22 A, B & C). of Ordovician age, it is about 12 km from north-east Kyaukse city.

The barite deposits also observed as fissure-filled epigenetic veins, although the source probably was endogenous (Maung Thein, 1984). The concentration of barite (Ba) content ranges from 0.03 % to 10.66 %. Lead (Pb) is ranges from 0.01 % to 5.01 % (Table 2.3). It is an important industrial raw mineral and it is used as manufacture of white paint, in the production of wall paper and in the drilling mud.

Figure 2.22. (A, B & C) Barite associated with lead in the thinly bedded limestones and siltstones at west of Kyauk-Aii village and at Tha-mone-ye-htwet, (N 21° 37'19.82" and E 96°16' 30.13").

Manganese ore

At the southern part of Shantaung-U Taung, the manganese ore bands occurred in the dark blue marbles layers and the ore bands found as widely distributed (Fig.2.23 A&B). These manganese ore bands are interlayered with thin-bedded blue marble and calc-silicate rocks. The bands are generally two inches to six inches in thickness. There are at least six ore bands having a cumulated thickness of about four feet. The thickest is traceable along the strike of the enclosing marble which is approximately east-west direction at that locality. According to Maung Thein and Soe Win, 1969, these ores can be said that they have been originally manganese of sedimentary origin later metamorphosed together with the host rock. The grade of ore is pyrolusite (MnO_2). The concentration of manganese (Mn) content ranges from 0.13 % to 6.11 % (Table 2.3). Manganese is a grey-white, pinkish-gray, chemically active element**,** hard and very brittle metal. Manganese ore is primarily used in steel and iron production.

Figure 2.23. (A, B & C) manganese ore bands occurred in the dark blue marbles layers at the southern part of Shantaung-U Taung (N 21° 37'19.82" and E 96°16' 30.13").

Estimate annual production of limestones from the Thandawmywet and Nwa-la-gauk Taungs in the study area

Major oxide composition of cement is SiO2 (20.87 %), CaO (60.63 %), MgO (1.51 %), Fe_2O_3 (3.53 %), Al_2O_3 (5.29 %), Free Lime (0.86 %), SO_3 (2.25 %), IR (2.01 %). Production rate of limestones from NW of the Thandawmywet Taung (**Work Site I**) is 2000 metric tons /per day and for annual production rate of limestones (One year /300-days) probable reserves is approximately 600,000 metric tons. Limestones quarry mine life is last about 50 years for NW of Thandawmywet Taung and that is about 30,000,000 metric tons limestones will be used in future (Fig. 2.22). Production rate of limestones from SE of the Thandawmywet Taung (**Work Site II**) is 1800 metric tons /per day and for annual production rate of limestones (One year /300-days) probable reserves is approximately 540,000 metric tons. Limestones quarry mine life is last about 30 years for SE of Thandawmywet Taung and that is about 16,200,000 metric tons will be mined in future (Figure 2.22). Production rate of limestones from the Nwalagauk Taung (**Work Site III**) is 800 metric tons /per day and for annual production rate of limestones (One year /300-days) potential reserves is approximately 240,000 metric tons. Limestones quarry mine life is last about 20 years for Nwalagauk Taung and that is about 12,000,000 metric tons will be consumed in future (Figure 2.22).

Table (2.2) Major oxides (%) composition of limestone units from the Thandawmywet and Nwa-la-gauk Taungs in the study are

Sample No/ Symbol	Th-1 ▮	Th-2 ▮	Th-3 ▮	Th-4 ▮	Th-5 ▮	Th-6 ▮	Th-7 ▮	NL-1 ▮	NL-2 ▮	NL-3 ▮
CaO	44.78	44.68	48.4	50.63	48.4	50.63	46.58	40.4	39.68	40.25
SiO_2	8.96	10.2	4.6	3.69	4.6	3.69	9.2	4.5	6.96	9.2
Al_2O_3	0.42	0.13	3.9	0.34	3.6	0.34	0.13	3.6	0.42	0.13
Fe_2O_3	0.53	0.15	0.12	0.2	0.1	0.2	0.17	0.1	0.33	0.16
MgO	1.94	1.91	1.98	2.00	1.45	1.41	1.58	0.67	1.46	0.51
K_2O	0.3	0.3	0.36	0.15	1.44	13.56	10.46	11.67	9.67	9.97
Na_2O	0.03	1.03	0.03	0.3	0.3	0.03	0.03	0.03	0.3	0.3
Cl	1.09	0.12	0.31	0.03	0.03	0.06	0.12	0.31	0.03	0.03
SO_3	0.03	0.02	0.02	0.05	0.23	0.03	0.02	0.02	0.05	0.043
LOI	41.48	41.37	40.00	42.35	40.30	31.38	32.39	39.00	41.3	39.54
Total	99.56	99.91	99.72	99.74	100.4	100.3	99.6	100.3	100.2	100.1

Figure 2.24. Map showing the analyzed sample locations of limestones and Limestone Quarry Work Sites of Thandawmywet and Nwa-la-gauk Taungs in the study area.

Table 2.3 XRF analyzed results of major and minor elements constituents in rock units of study area

Elements	L9	L14	L15	L16	L17	L20	L21	L28	L29	L30	L32	L38	L43	L48	L50	L53	L56	L57	L64	L66
Mg	1.01	0.16	2.93	0.69	0.82	0.72	0.21	0.29	1.70	0.40	0.87	0.46	0.77	0.30	0.14	0.10	9.64	1.81	-	3.94
Al	9.59	8.11	10.10	8.55	10.30	8.40	8.22	8.49	9.72	7.36	9.20	9.46	9.29	8.35	9.16	9.38	5.12	9.16	8.43	10.60
Si	55.60	66.80	44.30	59.20	57.30	58.90	64.50	64.70	56.80	66.20	59.60	59.40	60.40	63.10	62.00	56.30	38.60	53.90	63.60	38.70
P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S	0.23	0.15	0.11	0.20	0.11	0.13	0.16	0.15	0.11	0.14	0.13	0.13	0.24	0.25	0.12	0.15	0.10	0.13	0.12	0.30
Cl	0.66	0.33	0.71	0.78	0.30	0.69	0.37	0.36	0.31	0.89	0.63	0.79	0.32	0.40	0.30	0.71	0.53	0.75	0.27	0.25
K	13.80	11.30	6.40	9.30	10.50	9.71	12.30	12.70	9.58	10.40	9.50	11.80	9.37	13.20	12.20	8.34	2.55	6.22	17.10	1.49
Ca	5.38	3.70	8.25	6.15	6.27	6.87	3.53	4.02	4.91	4.65	5.74	5.40	5.63	3.98	4.44	7.74	18.30	7.78	1.06	16.30
Ti	0.70	0.44	2.01	0.91	0.85	0.83	0.44	0.36	1.17	0.62	0.87	0.75	0.88	0.53	0.46	1.10	1.13	1.27	0.07	1.85
V	0.01	0.01	0.04	-	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.03	0.02	0.02	-	0.01	0.01	-	0.00	0.09
Cr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	-	-	-
Mn	1.23	6.11	0.38	3.21	1.19	5.21	0.13	0.13	5.21	0.14	3.24	0.17	1.24	0.15	0.13	2.22	1.41	0.28	0.19	2.38
Fe	5.46	2.88	17.30	7.19	5.46	5.51	4.14	3.45	10.70	4.32	7.79	6.07	7.04	3.99	5.18	9.01	21.00	12.70	2.52	24.00
Co	0.02	0.01	0.06	0.03	0.01	0.03	0.01	0.00	0.01	0.02	0.03	0.02	0.01	0.00	0.01	0.03	0.09	0.04	0.00	0.02
Ni	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.14	0.03	0.01	0.01
Cu	5.01	1.01	8.01	0.01	3.01	4.01	2.01	0.01	8.01	0.01	6.01	3.01	0.01	6.01	2.01	0.01	1.05	0.01	5.01	1.01
Zn	0.02	0.01	0.05	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.02	0.04	0.03	0.01	0.04
Ga	1.01	0.01	2.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ge	0.00	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00	-	-	0.00	-
As	-	0.00	0.00	-	-	-	-	-	-	-	-	-	0.00	0.00	-	-	0.00	-	0.00	-
Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Br	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rb	0.11	0.13	0.06	0.09	0.11	0.08	0.11	0.11	0.09	0.08	0.08	0.10	0.10	0.08	0.11	0.05	0.02	0.05	0.16	0.01
Sr	0.17	0.08	0.25	0.01	0.17	0.17	0.09	0.10	0.08	0.09	0.12	0.14	0.16	0.14	0.09	0.23	0.22	0.19	0.02	0.26
Y	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01
Nb	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	-	0.01	0.00	0.00	0.00	0.05	-
Mo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ru	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh	-	-	0.00	-	-	-	-	-	0.00	-	-	-	-	-	-	-	-	-	-	-
Pd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Environmental impact of cement factories in the south-east of Kyaukse City

In the study area, Heavy Industries Enterprise of Cement Plants and Glass Factory are located about the 2 km SE of Kyaukse City. Cement is the primary material for building and civil engineering constructions. Therefore, the cement manufacturing sector plays a vital role in the nation’s economic development.

The main environmental issues associated with cement productions are the consumption of raw materials and energy use as well as emissions to air. Negative impacts of cement production plants are primarily loss of vegetation in quarry areas, SE of Kyaukse.

The increasing harvesting of raw materials for mounting cement manufacturing causes reduction in quantity of the non-renewable resources of limestones and dolomitic limestones exposed at Thandawmywet, Taungni and Nwa-la-gauk Taungs, (Figure 2.24) and (Figure 2.25 A & B). The cement factory activities linked to harvesting of the non-renewable resources from limestones exposures natural surroundings, damages the green landscape in the SE of Kyaukse City which is the habitat of flora and fauna exposing to the risk of ecological imbalance. The continuous extraction of these precious resources of limestone outcrops it to the risk of depletion in future. Besides the processing phases of raw materials in the factory releases dusts, noises, greenhouse gases especially carbon dioxides that contaminates the environment and aggravates the climate change (Figure 2.26 A & B).

In the cement industry areas, SE of Kyaukse City there are some major environmental impacts such as solid waste, wastewater, flue gas, and noise. The cement industry requires a large amount of energy to use in the whole process and to generate this. Even though some renewable resources use in some industries still a large amount of fossil fuel use for this process. So due to this matter, major impacts on the environment are CO_2, CO, NO_2, SO_2, and Volatile Organic Compounds (VOCs).

The emissions of those gases into the atmosphere can pose environmental problems but also affect public health. Climatic changes, global warming, ozone depletion, acid rain and biodiversity loss can cause the reduction of crop productivity, etc. It integrates the effects of various variables, such as soil properties, topographic attributes, tillage, and plant population. Water is used at some stages in the cement production process. Wastewater discharge to the environment causes to contaminate water sources such as rivers of Pan Laung, Zawgyi, Myitnge and Ayeyarwady and groundwater sources in the research area.

Noise pollution occurs during the whole process of the cement production process. From preparing raw materials, from the clinker burning and production process, from material storage, the heavy machines large fans used in the process. There are some impacts on rock-quarries near populated areas in Kyaukse. It can be directly affected high-story buildings and especially concrete buildings. The repeated blasting of mine quarries can cause the vibrating and crushing of building walls and then all structures can collapse.

Dust emission pathways can be minimized by the frequent spraying of water, oil, or other materials on soil stabilization. Air pollution caused by particular matters (PM) is one of the major problems of environmental pollution.

In view of the points mentioned above, it is suggested that the continuous environmental impact assessment studies are to be carried out in the cement industry areas, SE of Kyaukse, to be able to obtain environmental clearance. In addition, the production plants should be established in accordance with the Federal Environmental Protection Agency's rules and guidelines for predicting the ground level concentration of pollutants.

Figure 2.25. (A&B) Depletion of non-renewable resources of limestone and dolomite exposures near cement plant, Kyaukse area.

Figure 2.26. (A) Dust emission can be seen in limestone quarry of cement plant in the Kyaukse area. (B) Flue gas and smoke emitted from the cement plant in the Kyaukse area.

Conclusion

The study area is situated in Sintgaing and Kyaukse Townships, Kyaukse District, Mandalay Region. It lies between Latitude 21° 31' N to 21°47' N and Longitude 96°07' E to 96°17' E in UTM Map Sheet No.2196-02 and 06. In the project area, Upper Paleozoic Permian-Triassic (299ma-199ma) age rock units and Jurassic-Cretaceous (199ma - 65ma) metasedimentary rocks and their metamorphic equivalents units are well exposed. These rocks are intruded by biotite granites, microgranite, leucogranite, hornblende diorites, and a few hornblendites units of Early Tertiary in age (65ma-55.8ma).

All these above units are cropped out in the northern part of Singaung, Pann Taung and Tawma Taung, western and south-western part of Nwa-le Taung. Hornblende diorite is exposed in the northern part of Pann Taung and hornblendite and pegmatite dykes occur in the western part of Pann Taung (Near Mogaung). Limestones are exposed at eastern part of the study area (Dattaw, Thandawmyat, Taungni and Nwa-la-gauk Taungs.

This research work is aimed to explore the needs of MSME and HE with the natural resources of industrial raw minerals such as quartzite, marble, limestone, and dolomitic limestones units and also for the decorative stones as well as road materials and construction materials such as biotite granites, microgranite, leucogranite, hornblende diorites, hornblendite, calc-phyllite, schist, gneiss, calc-silicate, quartzite, marble and limestone of the study area are attempted. Besides, the non-renewable ores such as lead-barite, copper, iron and manganese ores are observed and their content percentage and uses are described.

Production of limestones for cement plants will take the quarry mine life last about 50 years for the Thandawmywet Taung and 20 years for the Nwa-la-gauk Taung in the future. The important

things to be dealt with in this area is the prohibition of the over harvesting of non-renewable rocks such as limestones and dolomitic limestones for mounting cement manufacturing in the future and the control of the tasks for repeated blasting of quarries near populated areas. Moreover, the main environmental issues associated with cement productions need to be detected annually and to mitigate these impacts with the aid of advanced technological methods.

The results of this research works are to be applied for the proper production of natural resources. The research will fulfill the academic needs of Kyaukse University, and use the teaching programs for the Department of Geology. This research is the first to study the Micro, Small and Medium Enterprises (MSME) and Heavy Enterprise (HE) with the observation of the natural resources found in the Kyaukse area, especially from both views of geological and environmental perspectives.

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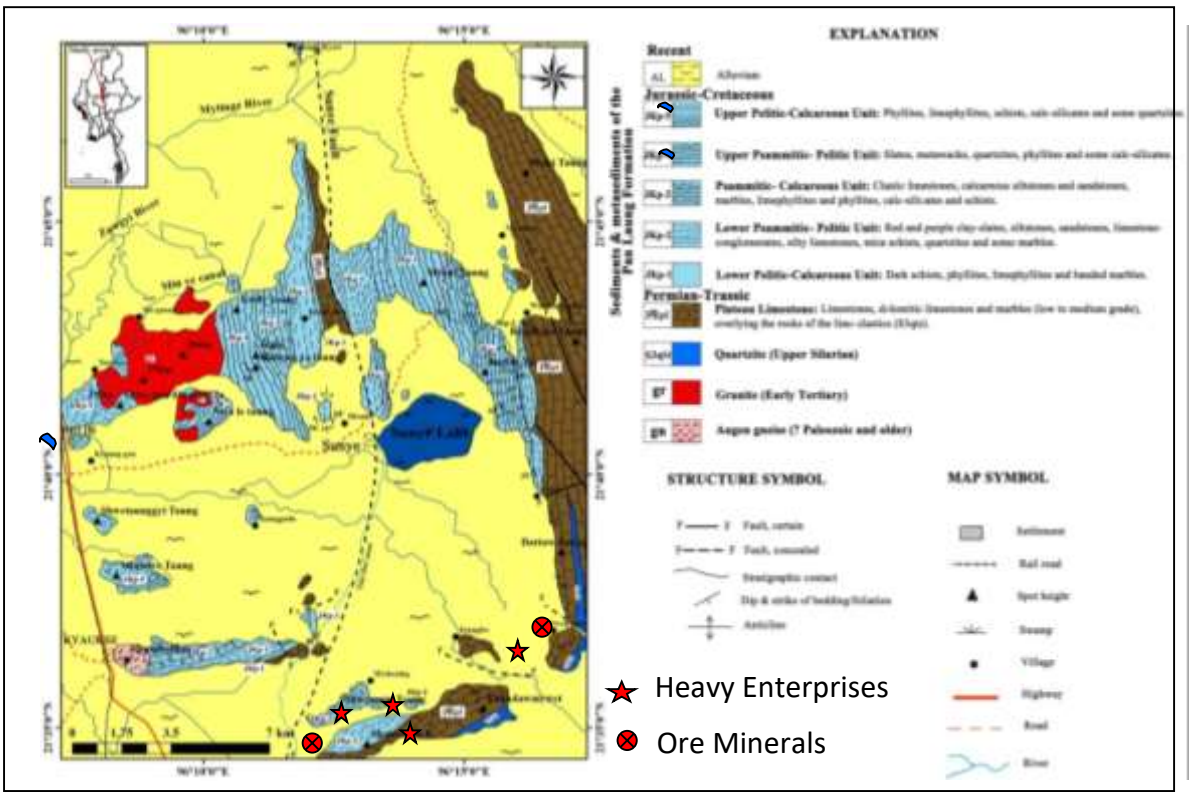

Figure 2.26. Geological map of the study area (Modified after Myint Thein,1984).

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ASSESSMENT OF THE GROUNDWATER QUALITY IN NYAUNG U TOWNSHIP, MANDALAY REGION

Min Han Nyein[1], Nay Nwe Myo[2], Hnin Ei Hlaing[3], Ye Zaw[4]

Abstract

The research area is underlain by the Recent Pliocene age. It is mainly composed of yellow to red, fine to coarse sand, gravel, yellow to red of lateritic soil and yellowish clay. The main aquifer is Irrawaddy aquifer and Alluvial aquifer. The specific yield of the groundwater is 1000 to 2500 gallons per hour for 4 inches diameter well and the depth of the water-bearing horizon ranges from 40 ft to 350 ft in the aquifer. The concentration of hydrogen ions (pH) is between 6.5 and 7.7. Mostly, the total dissolved solids are 1900 ppm in the research area. Total salinity is low and electrical conductivity (E.C) is 160-2820 μ mho/cm. The concentration of Chloride ions is widely distributed in most of the water of the studied region and the amount present in groundwater is relatively higher than in other anions. The results of chemical data analyzed by the SSP% method, the SAR method and the MAR method show that some tube wells can be assessed as not suitable for use as irrigation water. Kurlov's Method and PIPER method can be classified the water types, drinking water, domestic use and Irrigation water. If high amount of Iron concentration, it can be reduced of amount with aeration methods and sand filtering methods. According to the KURLOV's Method and Piper Method, Water Types can be classified into 6 water types. According to the above methods and the WHO Drinking Water Standard, the data can assess whether the groundwater of the research area is suitable for drinking water, domestic use, and irrigation water, except that some tube wells are not suitable.

Keyword: SSP%, SAR and MAR

Location, Size and Accessibility

The study area is situated in the middle part of the Dry Zone of Central Myanmar. The study area is bounded by Taungtha Township in the Northeast, Kyaukpadaung Township in the Southeast and East, Chauk Township in the West and Pakkoku Township in the West and Northwest. The study area is lying between North Latitude 20° 51' 38" to 25° 18' 33" and East Longitude 94° 39' 32" to 95° 13' 50" respectively. The whole area is roughly about 572.75 square miles. The study area is easily accessible. The study area can be traveled by car, train, ship and motorcycle. The location map is shown in Fig. (1). Nyaung U Township has flat plains and hills. The main ridge is Thurain Taung. It is located in 9.6 km away from township. It is 16 km long and about 350 m high.

Purpose of the study

The purposes of this research are described as follows;

- To detail the study of the major rock types.
- To draw the geological map and hydrogeological map.
- To classify the quality of groundwater, groundwater movement and types of groundwater in the study area.
- To analyze the chemical characteristics of the groundwater of the study area.
- To interpret groundwater for drinking, domestic, agricultural and industrial use.

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Method of Study

Before the fieldwork, I collected township data, UTM map data, population data, Literature and Laboratory techniques for the study area. Method of study has two methods. There are Field Methods and Laboratory Methods.

Field Methods

During the fieldwork, the location of the well by G.P.S (Global Positioning System), the measurement of water level, well depth, well logging and Collection of rocks and water samples, to set the information from the local people who need and well water was taken into the recorded.

Laboratory Methods

In the laboratory of Utilization of Water Resources Department, measurement of Cations (Ca^{2+}, Mg^{2+}, Na^{+}, $K^{+,}$ and Fe^{2+}) and anions (HCO_3^-, SO_4^- and Cl^-) total dissolved solids (TDS), total hardness (TH), p^H and electrical conductivity (EC), smell, salinity, color, were made.

Previous Investigation

Most previous works in this study area emphasized on geology, hydrogeology and others. They are listed as follows:

- Khin San (1993). Geology of Gwegyo-Taungzin Area, Chauk and Nyaung-U Townships, Magway and Mandalay Division M.Sc Thesis, MU.
- Khin Thet Oo (2005). Geology and Paleontology of Yenangyaung Oil Field, Magway Division M.Sc Thesis, MGU.
- Win Swe (1981). A major strike-slip fault in Burma. Contribution to Burmese Geology, vol.1, pp-63-72.
- Duny. L.W (2017). Hydrogeology of the dry zone- Central Myanmar.

Drainage Pattern

Nyaung U of the main river is Ayeyarwady. Nyaung U has many streams. All streams are freshwater type. The drainage pattern of the study area is dendritic and parallel pattern. According to the pattern, the bedrock of the study area is shale and sandstone. The drainage map of the study area is shown in Figure (2).

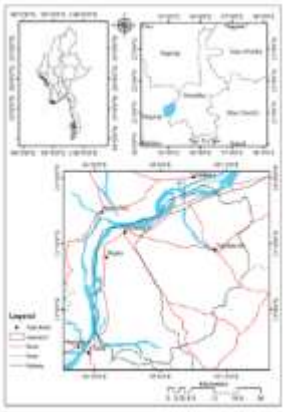

Figure 1. Location Map of the Study area

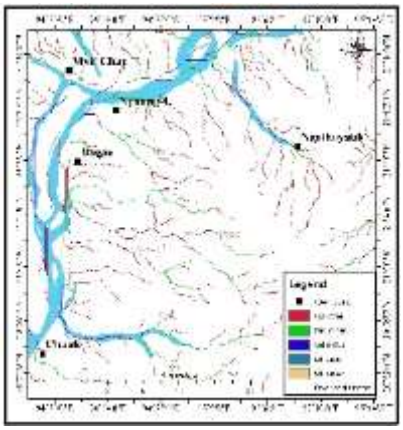

Figure 2. Drainage Map of the Study Area

Climatological Features

The study area is located in the dry zone of Myanmar. This area has two seasons. They are a dry season (mid-November-mid-May) and a wet season (remainder of the year). Annual rainfall sets during the May to October monsoon period. The study area receives rainfall from the southwest monsoon which moves from the Bay of Bengal and Andaman Sea. The average annual rainfall is about 638 mm (25 inches). The monthly mean minimum Temperature (°C) of the study area are 13.9°C, 13.3°C and 13°C in January 2017,2018 and 2019. The monthly mean maximum Temperature (°C) of the study area is 40.7°C, 44.5°C in May 2017,2019 and 42°C, in April 2018. The humidity falls from 71% to 78% during the months of March, April and May. The average annual humidity was 82% in 2017, 2018 and 2019. The monthly total rainfall, monthly mean maximum Temperature (°C), monthly mean minimum temperature and monthly mean humidity (%) graph during the period of 2017, 2018 and 2019 are shown in Figures (3,4,5 and 6) respectively.

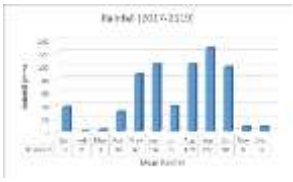

Figure 3 Average Monthly Rainfall,

Figure 4 Average Mean Maximum Temperature,

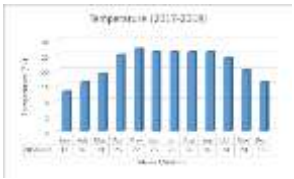

Figure 5. Average Mean MinimumTemperature,

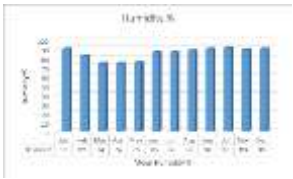

Figure 6. Average Meam Humidity

Natural Vegetation and Agriculture

Natural vegetation that can be found in the study area are Tamar, Tanaung, Tamarind, Kokko, Mango and Plum etc. Agriculture crops are bean, pulse, rice, groundnut, onion, maize, cotton, pea, plum, sugarcane, sunflower and watermelon. The grazing of cattle, cheap and goats occur in most villages.

Population and Land Use

Nyaung U township is composed of 17 urban wards, 75 village tracts and 220 villages. Total household of the Nyaung U is 53,896. The total population of the Nyaung U is 255,875. The total land used of the Nyaung U is 366,563 acres.

Regional Geologic Setting

General Geology

The study area is situated in the Central Cenozoic Belt of Myanmar. The area has a lowland topography. The regional trend of the strata is NWW-SSE parallel to the major tectonic structure. The formations in this area are recognized by the sandstones, clays, shales and conglomerates. According to the geological Map (2014), the present area and its environs are divided into the three stratigraphic units: (1) Alluvium (Holocene), (2) Irrawaddy Formation (Late Miocene-Pliocene) and (3) Upper Pegu Group (Mid-Miocene) as shown in Fig. (7) and geological succession and hydrogeological significance of aquifer of the study area are shown in Table No. (1). The aquifer types and well location map are shown in Fig. (8). The present area falls within the Minbu basin which is a segment of the western margin of Central Myanmar and Comprises an almost completely Cenozoic succession, dominantly of sandstones and days or shales. The study area comprises mainly Paleogene-Neogene sediments, deposited in shallow marine to fluvial and deltaic environments. The strata can be differentiated into four lithologic Units.

1. Upper Pegu Group (Oligocene - Miocene)
2. Irrawaddy formation (Late Miocene - Pliocene)
3. Older Alluvium (Pleistocene)
4. Younger Alluvium (Recent)
5. Igneous Rocks (Pre -Tertiary)

Upper Pegu Group (Oligocene-Miocene)

The Oligocene and Miocene layers of molasse facies of the Minbu Basin in Central Myanmar are referred to as the Pegu Group which is exposed in a north-south trending linear belt fringing the Eocene belt nearly along the whole stretch of the Minbu Basin. Each subgroup is subdivided into at least three formal lithostratigraphic units, and it is divided into the Lower Pegu Group in the Oligocene, and the Upper Pegu Group, in the Miocene. The Oligocene strata of the Lower Pegu Group are exposed only at the Gwegyo Hills west of Kyaukpadaung in the extreme western part of Mandalay Region.

Irrawaddy formation (Late Miocene- Pliocene)

Poorly consolidated. Thick-bedded or massive, large-scale cross-bedded. Medium to coarse-grained, locally pebbly, non-marine gritty sandstone and interbedded minor shale or clay of Late Miocene-Pliocene age of Central Myanmar is referred to as the Irrawaddy Formation. They are also exposed in small patches near Singu and northeast of Tagaung, both in the northern part of the Mandalay Region.

Quaternary Deposits

The Quaternary deposits are exposed in the Magway Region and Mandalay Region. River terrace deposits and plateau gravels are the older Quaternary. The Ayeyarwady River is exposed to younger alluvium, especially in the Chauk-Yenangyaung region.

Igneous Rocks

The volcanic rocks of Mt. Popa area include andesites, basalts, rhyolites and ignimbrites which range in age from Late Miocene to Late Quaternary.

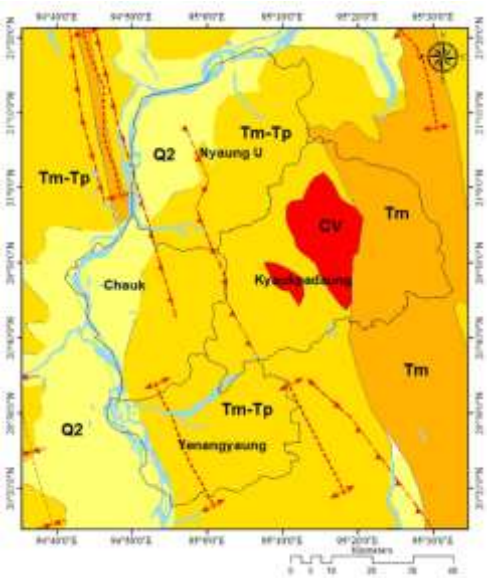

Figure 7 Regional Geological Map of the study Area. (MGS-2014)

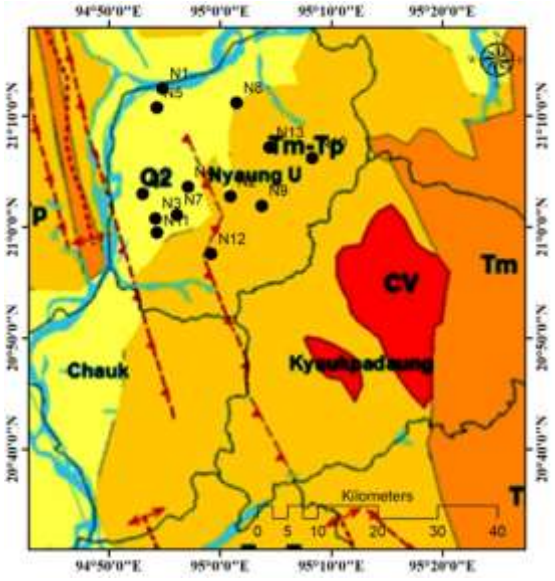

Figure 8. Aquifer types and well location map

Table 1. Geological Succession and Hydrogeological Significance of Aquifer

Unit		Stratigraphic Units	Lithology	Hydrogeological Significance
Quaternary	Recent	Younger Alluvial	Alluvial clay, silt, sand and gravel	Bearing Aquifer
	Pleistocene	Older Alluvium	Clay, silt, sand, gravel and plateau gravel	Aquifer with high yield
Tertiary	Late Miocene to Early Pliocene	Irrawaddy Formation	Loosely cement sandstone with minor clay and shale	Aquifer with moderately to fairly high yield
	Oligocene-Miocene	Pegu Group	Rapid alternation of shale, siltstone and sandstone	Limited aquifer with low yield
	Pre-Tertiary	Igneous Rocks	Andesite, Olivine Basalt and Basalt	Fractures and joints

Hydrogeologic Characteristics of the Research Area

Collection of Data

One-inch topographic map was used in the collection of the water samples of the area under investigation. The collected samples were analyzed at Health Department. Tube-wells data were collected from the Water Resources and Utilization Department (W.R.U.D) and the Ministry of Agriculture and Irrigation Department. There are two main types of lithology and aquifer, i.e. there are Alluvial Aquifer and the Irrawaddy Formation. According to lithologic logs the water-bearing horizons consist of yellow and blue-colored sand, sand with clay and gravel. According to well-log data, the aquifer type is confined type. The depth of the aquifer ranges from 40 ft to 340ft from the ground surface. The groundwater chemical analysis of the research area is shown in Table No. (2).

Table 2. Chemical analysis of the research area

Tube Well No.	TDS mg/l	EC micro mho/cm	pH	Na mg/l	k^+ mg/l	$Ca2^+$ mg/l	$Mg2^+$ mg/l	$Fe2^+$ mg/l	Cl^- mg/l	$SO4^=$ mg/l	$HCO3^-$ mg/l	Aquifer Types
N1	100	160	6.66	6	2.94	21.64	6.72	4.5	37	20.16	24	Alluvial
N2	770	1190	6.76	63	7.5	216.43	13	3.5	239	141	84	Irrawaddy
N3	1900	3000	7.76	0	0	117	110	0	499	330	84	Alluvial
N4	1300	2000	7.67	0	0	19	27	0	300	81	220	Alluvial
N5	660	1030	7.7	108	0	33.67	16	0	58	65	66	Alluvial
N6	1800	2820	7.2	34	0	53	139	0.01	461	391	80	Alluvial
N7	1590	2480	7.8	125	0.53	72	23	0.3	360	53	40	Alluvial
N8	830	1300	7.6	0	0	44	19	0.02	200	34	98	Irrawaddy
N9	1000	1550	7.52	75	0.62	192	26	1	230	161	40	Irrawaddy
N10	430	660	7.8	112	0.45	72	40	0	80	34	104	Irrawaddy
N11	780	1200	7.6	19	9.53	106	16	0.3	138	357	28	Alluvial
N12	660	1007	6.8	110	1.67	78	34	0.1	123	138	100	Irrawaddy
N13	900	1016	7.4	40	2.67	104	44	0.65	155	80	82	Irrawaddy

Aquifers

The Irrawaddy aquifer found the eastern part of the research area. The alluvial aquifer mainly composed of the western part of the research area. Irrawaddy rocks mainly composed of siltstone, clay, shale and sandstone. The water bearing horizon of Irrawaddy Formation is encountered at the depth ranging between 40 feet and 350 feet. In the research area, well no. N8 with the depth yields 2500 gallons per hour from the depth 570 ft.

Chemical Composition of Groundwater

The collected samples are analyzed at the Water Resources and Utilization Department (W.R.U.D) and Ministry of Agriculture and Irrigation Department, the cations and anions and TDS, EC, pH, total alkalinity and total hardness.

Classification by KURLOV'S (1928) Method

Kurlov's formula is written by using ionic concentrations that are expressed in milliequivalent percent (meq/l). The highest amount of ion is expressed first and the lesser ion in second and so on. The anions are written above the line and cations are written below the line. The degree of mineralization (m) is placed in front of the format while pH, temperature, Fe^{++} etc., is placed behind. Based on Kurlov's Method, the chemical classification of groundwater types in the research area is shown in Table No. (3).

Classification of Piper Diagram, (Piper, 1944) and Hill, 1940)

This method was proposed by Piper (1944) and Hill (1940). This method of the tri-linear diagram is widely used to depict chemical data and show the relative concentrations of the major cations (Ca^{+2}, Mg^{++} and K^{+}) and anions (CO_3^{-},HCO_3^{-},Cl^{-} and $SO_4^{=}$). Cations are plotted on the left triangle and anions on the right triangle. Piper diagrams are shown in Fig. (9). Kurlov's method and Piper method compare the results of the research area in groundwater types shown in Table No. (3).

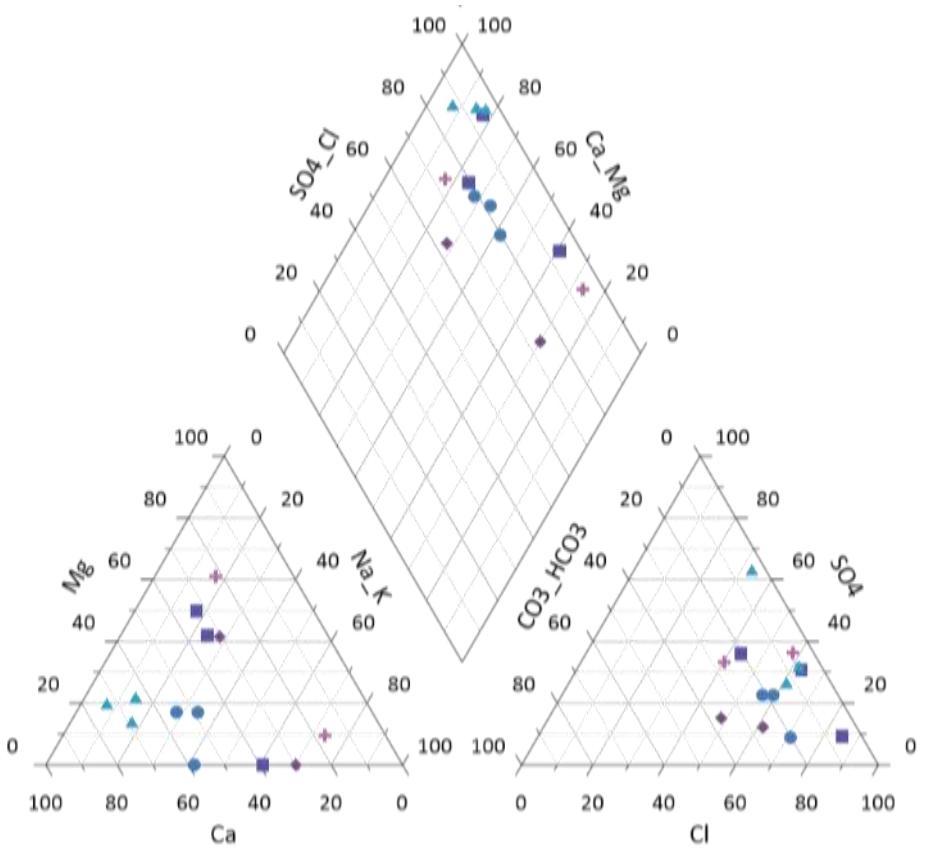

Figure 9. Classification of the Piper diagram, after Piper (1944) and by Hill (1940)

Table 3. Compares ion with Kurlov's (1928) Method and Piper Method

Tube Well No.	Kurlov's Method	Piper Method
T_1	Cl Ca Mg	Ca Mg Cl
T_2	Cl Ca	Ca Mg Cl
T_3	Cl Ca Mg	Ca Cl
T_4	Cl Ca Mg	Mg Cl
T_5	Cl SO_4 Na	Ca Mg HCO_3 Cl SO_4
T_6	Cl	Ca Mg Na Cl
T_7	Cl Na Ca Mg	Ca Mg Na SO_4
T_8	Cl Ca Mg	Ca Mg Cl
T_9	Cl Ca	Ca Cl
T_{10}	Cl HCO_3 Ca Mg	Ca HCO_3 Cl
T_{11}	SO_4 Na Ca	Ca HCO_3 Cl
T_{12}	Cl SO_4 Na Ca	Ca Mg Cl SO_4
T_{13}	Cl Ca Mg	Mg Cl

Chemical Analysis of the Groundwater

Chemical Analysis is important to specify the actual characteristics of groundwater. Determination of pH, total dissolved solids, T.D.S, electric conductivity E.C, dissolved cations of Na^+, K^+, Ca^{++}, Mg^{++} and Fe^{++} and dissolved anions of are made in the laboratory.

In groundwater resources evaluation, the quality of groundwater is as important as its quantity. The chemical and physical constituents of groundwater determine its usefulness for municipal, commercial, industrial, agricultural and domestic water supplies.

Major Cations

Cations, that are commonly contained in tube wells including iron Fe^{++} cations have been determined. Common cations are Sodium Na^+, Potassium K^+, calcium Ca^{++} and Magnesium Mg^{++} are represented shown in Fig. (10,11,12 and 13).

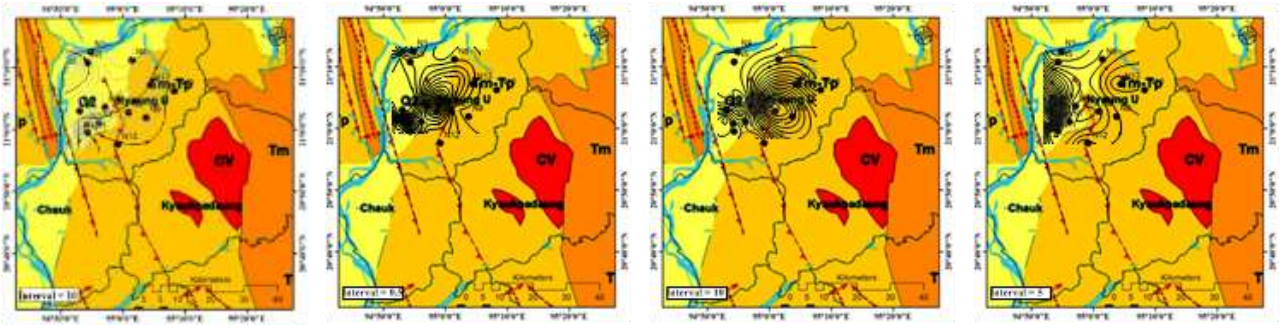

Figure 10,11,12 and 13 Distribution map of the Major Cations in research area.

Major Anions

Anions play a vital role in the quality determination of groundwater. Only major anions of Bicarbonate (HCO_3^-), Sulphate ($SO_4^{=}$) and Chloride (Cl^-) ions should be taken into account are represent shown in Fig. (14,15 and 16).

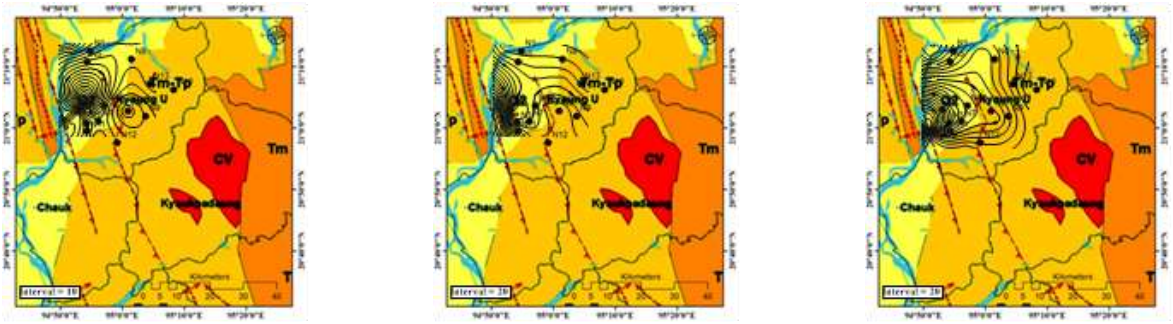

Figure 14,15 and 16. Distribution map of the Major Anions in the research area.

Domestic Purposes for Water Quality

The pH values of water samples are ranging from 6.6 to 7.8. The electrical conductivity (E.C) of groundwater samples is ranging from 160 to 2820 µmhos/cm. TDS concentration is ranging from 100 ppm to 1800 ppm. Sodium concentration is more than 125 ppm which makes the water salt taste and health problems. Calcium concentration is ranging from 19 ppm to 216.43 ppm and does not exceed the W.H.O standard of 200 mg/L. The value of iron in the research area is ranging from 0.0 ppm to 4.5 ppm. Bicarbonate concentration in the research area is ranging from 24 ppm to 220 ppm. The concentration of sulfate in the research area is ranging from 34 ppm to 357 ppm. The water quality shows the domestic uses and drinking water of water quality in Table No. (4).

Table 4. WHO standard guideline for the drinking water in research area

Characteristics	Guideline value		The range obtained from groundwater	Remark
	Desirable	Max Permissible		
Calcium	75 mg/l	200 mg/l	19-216 mg/l	Good
Magnesium	30 mg/l	150 mg/l	13-139 mg/l	Good
Sodium	0- mg/l	200 mg/l	6-125 mg/l	Good
Potassium	0- mg/l	200 mg/l	0.53 –9.53 mg/l	Good
Sulphate	0- mg/l	400 mg/l	34 – 357 mg/l	Good
Chloride	200 mg/l	600 mg/l	37-499 mg/l	Good
Iron	0.5 mg/l	1.5 mg/l	0 – 4.5 mg/l	Poor
TDS	0- mg/l	1000 mg/l	100-1800 mg/l	Poor
pH	6.5	8.5	6.6 – 7.8	Potable
EC	0-micro mho/cm	1500 micro mhos/ cm	160–2820 micro mhos/cm	Doubtful

Agriculture purposes for Groundwater Quality

Agriculture is the basis of the Myanmar economy. The quality of water for irrigation is classified by Sodium Adsorption Ratio (SAR), Magnesium Adsorption Ratio (MAR) and Soluble Sodium Percentage (SSP or Na %). The respective values of all water quality parameters are summarized in each table.

Sodium Adsorption Ratio (SAR), (Richardson, 1954)

Sodium Adsorption Ratio (SAR) is most commonly used to assess the suitability of irrigation water and classification based on the SAR values is expressed in Table (5). The SAR measures sodicity in terms of the relative concentration of sodium ions to the sum of calcium and magnesium ions in a water sample. Sodium concentration in water affects the deterioration of the soil properties reducing permeability. SAR is calculated using the following formula:

$$S.A.R.\frac{Na+}{\sqrt{Ca+2+Mg2+/2}}$$

Table 5. Water classification based on the SAR Values

Class	SAR	Hazard and limitation
S1	<10	No Harmful effect of sodium
S2	10-18	An appreciable sodium hazard in fine-textured soils of high critical flocculation concentration but could be used on sandy soils with good permeability
S3	18-26	Harmful effects could be anticipated in most soils and amendments such as gypsum would be necessary to exchange sodium ions
S4	>26	Generally unsatisfactory for irrigation

Where the ionic concentrations are expressed in meq /L. The result of the Sodium Adsorption Ration (SAR) is shown in the Fig. (17) and Table No. (8).

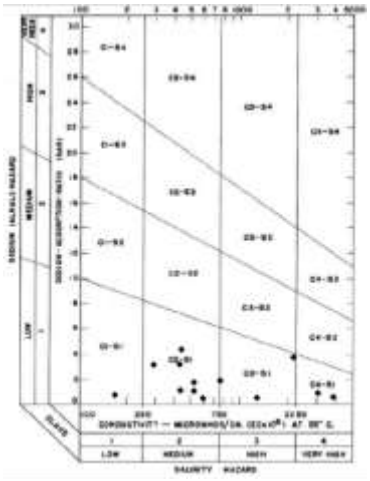

Figure 13. Sodium Adsorption Ration (SAR)

Magnesium Adsorption Ratio (MAR), (Raghunath, 1987)

The magnesium content of water is considered one of the most important qualitative criteria in determining the quality of water for irrigation. Generally, calcium and magnesium maintain a state of equilibrium in most water. More magnesium in water will adversely affect crop yield as the soil becomes more saline, Raghunath, H. M., (1987). The values of the magnesium adsorption ratio of each aquifer are tabulated in Table No. (8).

$$\mathrm{M.A.R.} = \frac{\mathrm{Mg2+}}{\mathrm{Ca2+ + Mg2+}} \times 100$$

The value of the magnesium adsorption ratio of the research area ranges from 21.52 to 76.77 %. The acceptable limit of the magnesium adsorption ratio is 50 %.

Soluble Sodium Percentage (%), (Wilcox,1955)

Sodium concentration plays an important role in the evaluation of groundwater quality for irrigation because sodium causes an increase in the hardness of the soil as well as a reduction in its permeability. The sodium percentage (Na %) is calculated using the formula given below:

$$\mathrm{SSP} = \frac{(\mathrm{Na+ + K+})}{\mathrm{Ca\,2+ + Mg2+ + Na+ + K+}} \times 100$$

The methods of classification of groundwater based on Na% (Wilcox,1955) is shown in Table No. (7). The result of the SSP% are shown in Table No. (8).

Table 7. Classification of Groundwater based on Na % (Wilcox, 1955)

(Na %) Percentage of Sodium	Classification
< 20	Excellent
20 - 40	Good
40 -60	Permissible
60 - 80	Doubtful
> 80	Unsuitable

Table 8.0 Summary Table for various methods of irrigation water quality in the Research Area

Tube Well No.	EC	SAR	MAR	SSP%
1	160	0.3	34	17
	Good	Good	Suitable	Good
2	1190	1.1	9	20
	Permissible	Good	Suitable	Good

Tube Well No.	EC	SAR	MAR	SSP%
3	3000	0	61	0
	Doubtful	nil	Suitable	Permissible
4	2000	0	20	0
	Doubtful	nil	Suitable	Doubtful
5	1030	3.8	44	61
	Permissible	Good	Suitable	Doubtful
6	2820	0.6	81	10
	Doubtful	Good	Suitable	Excellent
7	2480	3.3	35	50
	Doubtful	Good	Suitable	Doubtful
8	1300	0	42	0
	Permissible	Nil	Suitable	Excellent
9	1550	1.3	18	22
	Permissible	Good	Suitable	Good
10	660	2.6	48	41
	Good	Good	Suitable	Doubtful
11	1200	0.5	20	14
	Permissible	Good	Suitable	Good
12	1007	2.6	42	42
	Permissible	Good	Suitable	Permissible
13	1016	0.8	41	17
	Permissible	Good	Suitable	Good

Results and Outcomes

The results of chemical data analyzed by the SSP% method, the SAR method and the MAR method show that some tube wells can be assessed as not suitable for use as irrigation water. The crop should be irrigated by the tolerable plants. Kurlov's Method and PIPER method can be classified the water types, drinking water, domestic use and Irrigation water. If high amount of Iron concentration, it can be reduced of amount with aeration methods and sand filtering methods. According to the KURLOV's Method and Piper Method, Water Types can be analyzed into 6 water types. According to the above methods and the WHO Drinking Water Standard, the data can assess whether the

groundwater of the research area is suitable for drinking water, domestic use, and irrigation water, except that some tube wells are not suitable.

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REVIEW ON THE PRE-PALEOZOIC TO EARLY MESOZOIC STRATIGRAPHY OF YE-NGAN TOWNSHIP, SOUTHERN SHAN STATE, MYANMAR

Aung Myo Zaw[1] & Aye Ko Aung

Abstract

The Pre-Paleozoic to Early Mesozoic rocks are well exposed in northwestern part of the southern Shan State. It is located about 13 km, NE of Ye-U, Ye-ngan Township, southern Shan State which lies in northwestern part of the Pindaya Range, located in the Eastern High Land (Shan-Tanintharyi Block). Ye-ngan Township is a geologically very well-known to a great number of geologists. The present paper focused on the newly defined Pre Paleozoic to Early Mesozoic stratigraphy with two types of approach; data collection in the field and literary work in the library. The Ye-ngan Township comprises seven type sections and four reference sections of the Pre-Paleozoic to Lower Mesozoic strata such as, Chaung Magyi Group (Late Neoproterozoic), Molohein Group (late Jiangshanian to Furongian), Lokepyin Formation (Trmadocian to Floian), Wunbye Formation (Darriwilian), Nan-on Formation (Katian/ Caradocian), Tanshauk Member (Hirnantian), Linwe Formation (Silurian-Lochkovian), Zebingyi Formation (Pragian-Emsian), Maymyo Formation (Eifelian-Frasnian), Thitsipin Formation (Early to Upper Permian), and Nwabangyi Dolomite Formation (late Upper Permian-Induan to Olenikian). Most of the Paleozoic rock units are fossiliferous in Ye-ngan Township the Upper Cambrian is represented by the Molohein Group, bearing saukiid trilobites. The Lokepyin Formation which contains small brachiopods. The Wunbye Formation includes the nautiloids, brachiopods, sponge and receptaculitid algae. The Nan-on Formation is rich in fossils, among with cystoids, orthid brachiopods, bryozoans, sponges and trilobite have been recorded. The Tanshauk Member yields brachiopods and trilobites. The Linwe Formation (in the limestone) includes nautiloids, crinoids, cystoids, ostracods, conodonts; trilobites and graptolites occurred in the siliciclastic horizon. Some Devonian outcrops of the Zebingyi Formation and? Maymyo Formation are narrowly exposed in the Myogyi area where there are [2]minor amounts of tentaculitids and ammonoids. There are so far no Carboniferous strata are discovered in this area. The Thisipin Formation contains rugose and tabulate corals, foraminifers mainly fusulinids, brachiopods, bryozoans, crinoids, gastropods and leaf fossils. The Nwabangyi Dolomite Formation has some fossils, ammonoids, conodonts, bivalves, and shark teeth. In completion of this works, detailed stratigraphic investigations are still needed in a matter of urgency.

Keywords: Pre-Paleozoic to Early Mesozoic stratigraphy, southern Shan State, Myanmar

Introduction

The first field trip to around Ye-ngan Township, especially Ye-U, Linwe, Thitsipin, Ingyi, Hsinsapya, Padongaing, Yechanbyin, Nwabangyi and Myogyi areas (Figure 1) was made by the authors. The field work was done with two purposes: Firstly, to let the students learn practically the stratigraphic characters of the type sections of the Pre Paleozoic to Early Mesozoic units in the western part of the southern Shan State formally established by Dr Myint Lwin Thein in 1973. The Paleozoic units designated in this area are, (1) Chaung Magyi Group (Pre Cambrian to ?Cambrian), (2) Molohein Group (late Middle to Late Cambrian), (3) Lokepyin Formation (Lower Ordovician), (4) Wunbye Formation (Middle Ordovician), (5) Nan-on Formation (Upper Ordovician), (6) Tanshauk Member (late Upper Ordovician), (7) Linwe Formation (Lower Silurian to early Devonian), (8) Zebingyi

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Formation (Early Devonian) (9) possible Devonian unit (?Maymyo Formation), not mentioned in the previous reports, (10) Thitsipin Formation (Early to Upper Permian), and (11) Nwabangyi Dolomite Formation (late Upper Permian to Lower Triassic).

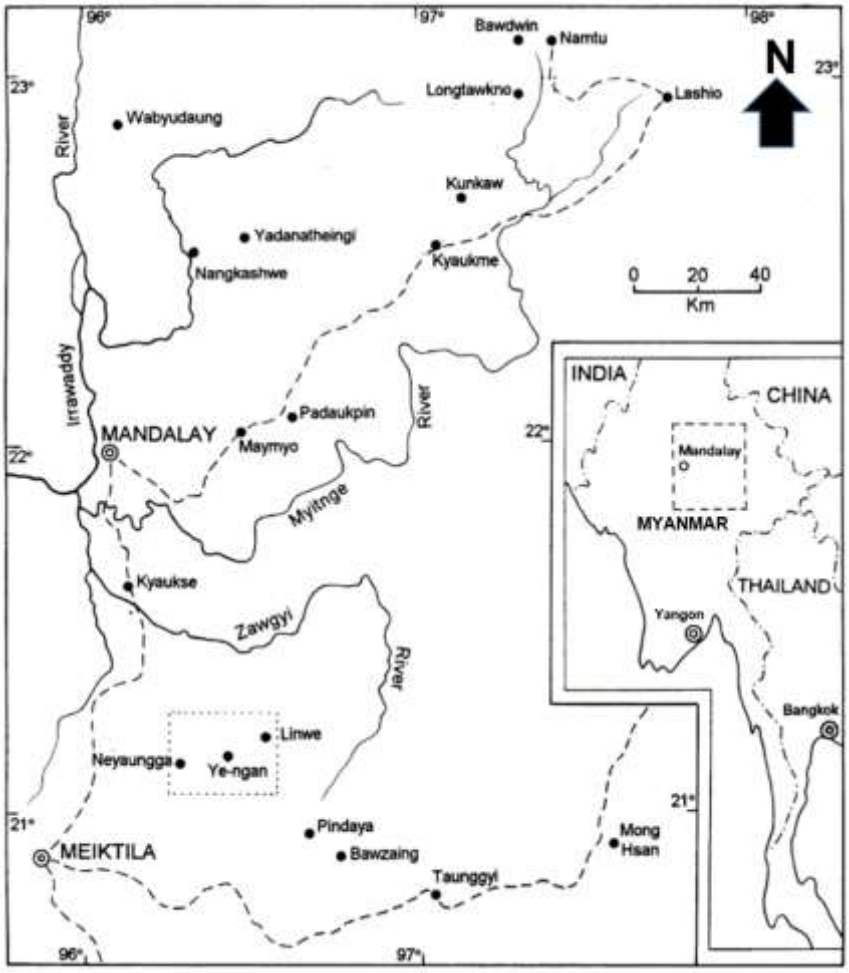

Figure 1. Locality Map of the study area, with dotted blocks showing the study area

Secondly, to achieve the biostratigraphic knowledge on basis of the Paleozoic to Early Mesozoic fossils as most stratigraphic units in this area are fairly fossiliferous which yield a variety of micro and macro fossils (foraminifers, fusulinids, conodonts, tentaculities, ostracods, corals, brachiopods, trilobites, bryozoans, cephalopods, graptolites and crinoids, receptaculitid algae, and leaf fossils). These faunas greatly assisted the geological mapping of the area which led to the understanding of the geological history of the western part of the southern Shan State.

A number of the research papers and M.Sc, Ph.D Theses completed in the present area are, such as; Myint Lwin Thein (1973) re-classified the Lower Paleozoic units of the western part of the southern Shan State which includes the Ye-ngan, Ye-u, and Linwe areas. Garson *et al.* (1976) wrote a comprehensive report on the geology of the Neyaungga-Ye-ngan area. The geology of the Myogyi area was investigated by Ko Ko Myint (1989) in the frame of a M.Sc study and for the preparation of a detailed geological map of the area. Cocks and Zhen Ren Bin (1998) described and reviewed the brachiopod fauna of the Nan-on Formation. In the following years the undergraduate geology students

from the universities of Yangon and Taunggyi have made field mapping in the present area. Aye Ko Aung (2002) wrote a depermental unpublished paper, guide to the stratigraphy of the Ye-U, Linwe, Thisipin, and Nwabangyi areas, Ye-ngan Township, southern Shan State. Maung Maung and Aye Ko Aung (2005) described the first record of the late Silurian conodonts of the Linwe Formation, Ye-ngan Township, southern Shan State. Aung Myo Zaw (2014) mentioned *Ipciphyllum subtimoricum dilatum*, sub sp. nov from the Permian Thitsipin Formation, Pegin-Kyauktaw area, Ywa-ngan Township, Shan State (south). Aung Myo Zaw (2015) first described five coral species belonging to four genera *Yatsengia hangchowensis, Iranophyllum* sp. cf. *caracinophylloides*, *Ipciphyllum subelegans, Pavastehphyllum* sp., *Pavastehphyllum* (*Thomasiphyllum*) sp., from the Thitsipin Limestone of the Pegin-Linwe area, Ye-ngan Township, southern Shan State. Wernette *et.al* (2021) firstly systematically described the Cambrian Trilobite from Linwe-Padongaing areas. Aung Myo Zaw and Aye Ko Aung (2024) described a rugose coral new subspecies *Ipciphyllum subtimoricum dilatum,* of Middle Permian from Ye-U area, southern Shan State. Kyi Soe (1983), Ko Ko Myint (1989), Me Me Thein (2000), Tun Naing Zaw (2005), Maung Maung (2005), Aung Myo Zaw & Chit Thet Mon (2007), Kyi Kyi Maw (2010), Yin Min Htwe (2011) studied their M.Sc and Ph.D Theses in the present research area.

Regional Geological Setting

The area lies to the east of the great Shan Scarp and it occupies a large part of the plateau to the north and south-east of Ye-ngan consists of wide shallow valleys and broad low grassy hills formed by the Nwabangyi Dolomite Formation. The elevation is between 4000 and 4250 feet, dropping to about 3500 feet in some valleys. To the north-west and west Ye-ngan, the Natteik Limestone Formation forms more rugged and rocky country. The western margin of the area is bounded by the prominent Panlaung escarpment. The Panlaung valley is composed largely of Jurassic (Panlaung Formation, Loi-an Group), and questionable Cretaceous sediments (Kalaw Red Bed Formation). The area is bounded to the north by the dissected area covered with Chaung Magyi sedimentary rocks which forms an anticlinal structure (Yechanbyin anticline). The major folding of the area is dissected in the middle of the area, by Ingyi-Ingaung Fault. The block of the Lower Paleozoic sediments in the east of the area (Molohein Group, Lokepyin Formation, Wunbye Formation, Nan-on Formation, Linwe Formation, and a possible Devonian unit) is faulted (Karani Fault) against the "Plateau Limestone Group". The Cambrian rocks in the south-east corner of the area (Molohein Group) thrusted (?) against the other Lower Paleozoic units in the Linwe and Kyaukhnget areas (Figure 2).

Stratigraphy

The sequence of the rock units well exposed in this area is shown in (Figure 2). The sequence comprises five groups, seven formations, and two members of Precambrian to Early Triassic in age. All of the Lower Paleozoic (Cambrian to Silurian) units in the area were established by Myint Lwin Thein (1973).

Chaung magyi group

The group name is taken from Chaung Magyi river (locally named as Nampek river) flowing from north to south in the area to the east and north-east of Sedawgyi, in Madaya Township, Mandalay Region. The rock sequences in the type area was firstly mapped by Aye Ko Aung (1981). The Chaung Magyi rocks consists of a sequence of phyllite, slate, quartzite, and greywacke which is well exposed in the east and north-east of Sedawgyi. The different lithologies are recognized in the area north of Ye-U where the group consists of a series of slightly metamorphosed, folded sediments include brown, fine-grained, cross-laminated sandstones and mudstones, green argillites, and turbiditic sediments, forming a thickest unit in the group. The sedimentary structures strongly suggest that the sandstones are turbidites.

The Chaung Magyi Group is overlain unconformably by the Cambrian unit (in the type area) and? comformably (Yechanbyin area) the relationship with the older unit, in the present area, however, is indeterminable due to thick soil covering. Although no fossils have been recorded, the age of the group should be considered better be of Late Precambrian or Early Cambrian as it is unconformably overlain by the Late Cambrian saukiid trilobite- bearing sand unit of the Molohein Group of southern Shan State. According to Dew et.al (2019), based on the youngest detrital zircons present in sandstone samples from the western Yeywa Dome area, at least the upper part of the Chaung Magyi Group is of late Neoproterozoic age. The age of the Chaung Magyi Group is still controversial. Further detailed work is needed to better constrain stratigraphic, metamorphic and igneous ages associated with the Chaung Magyi Group.

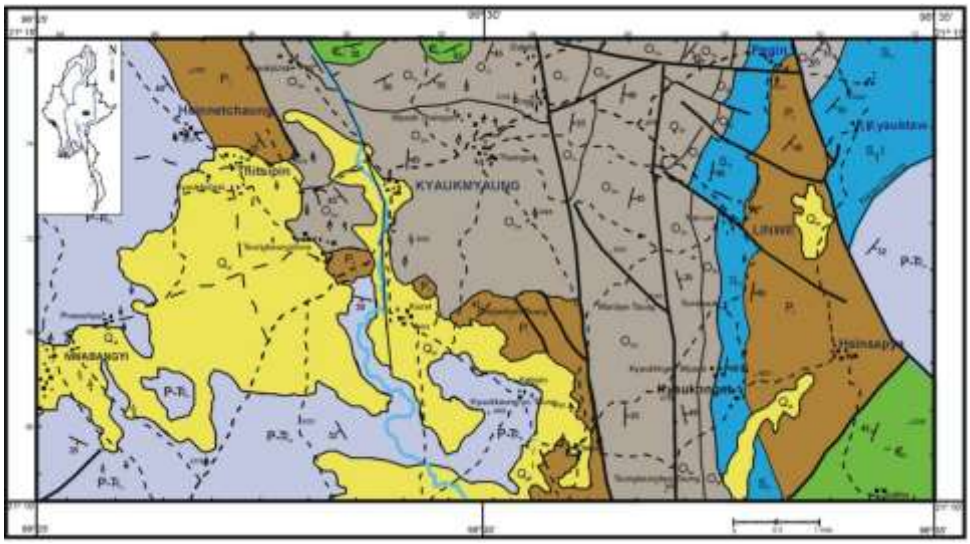

Figure 2. Geological map of the Nwabangyi-Kyauktaw area, Ye-ngan Township, southern Shan State (from, Garson *et.al* (1976); Kyi Soe (1983)

Molohein Group

The Group consists of a thick succession of orthoquartzites, siltstones, and sandstones. It is best exposed in the south-east corner of the area. The orthoquartzite unit of the Group (informally known as the Pandung Formation) in the vicinity of the Satthe village gradually passes upwardly into

whitish, pinkish, purplish, and yellowish brown micaceous sandstones and lenticular siltstones (Myetye Formation). In this tract, the fossiliferous horizon, which bears a great number of saukiid trilobites of the Padongaing area (Figure 3). The first Cambrian fossils to be formally described from Myanmar: *Eosaukia buravasi* (Myint Lwin Thein, 1973), which was previously reported but not figured; *Asioptychaspis asiatica*, previously known from North China; and *Asioptychaspis lata*, a new species endemic to Myanmar (Wernette *et.al* 2021). Considerable additional diversity may exist within the Myet-Ye Formation, which will permit further test of the above biostratigraphic and paleogeographic conclusions.The present faunas indicate the age of Lower to Middle Cambrian (Late Furongian).

Pindaya Group

Myint Lwin Thein (1973) introduced this Group as a formal stratigraphic unit, comprising essentially of thick-bedded, burrowed, pelletal or silty limestones with irregular silt specks or laminae, and the grey or yellow siltstones. The Group comprises three Formations (Lokepyin, Wunbye, and Nan-on) and one Member (Tanshauk). All of these rocks are well exposed in the Ye-Ngan Township. The lower limit of the group is the lower boundary of the Lokepyin Formation which in contact with the micaceous, pinkish or purplish sandstones of the Molohein Group and the upper limit is marked at the upper boundary of the Tanshauk Member of the Nan-on Formation. The type locality of the group is established at the north-western part of the Pindaya range, in Ye-ngan Township. The age of the Pindaya Group is regarded to be (Lower Ordovician to late Upper Ordovician).

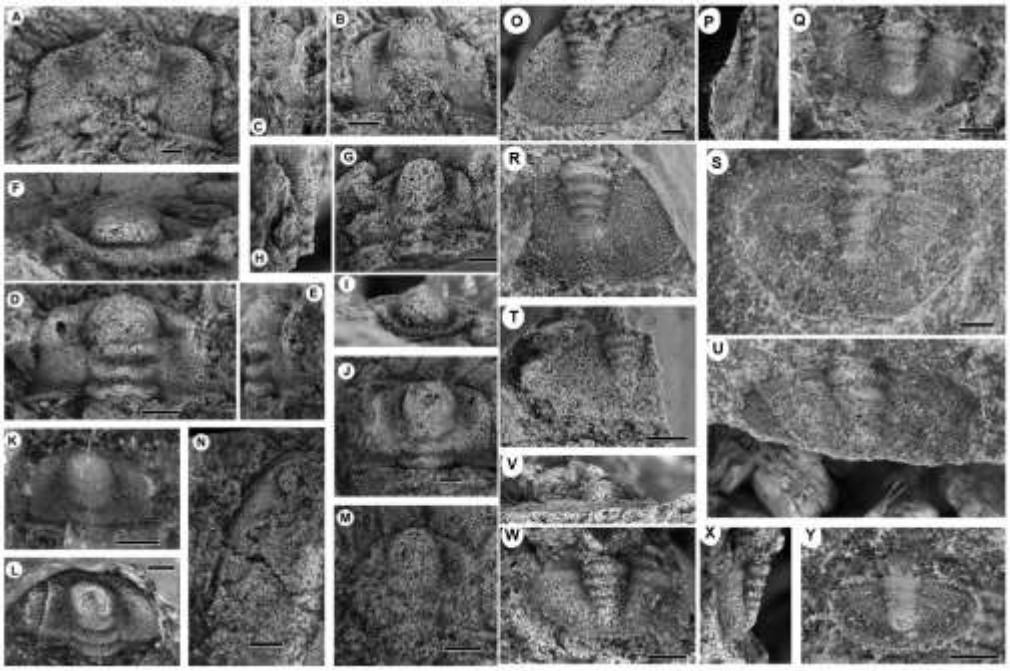

Figure 3. Late Cambrian trilobites of the Myet ye Formation, Molohein Group, Padongaing area, Ye-ngan Township, southern Shan State. A-M *Asioptychaspis lata* n. sp. all Cephalon. O-Y *Asioptychaspis lata* n. sp. all pygidium. All scale bars 2 mm.

Lokepyin Formation

Myint Lwin Thein (1973) first named this unit after Lokepyin village, 6km NE of Myaing, Ye-ngan Township, southern Shan State. This unit occurs at Theingon and Ingyi villages, located at about one mile east of Ye-U. It is also exposed at the road cutting between Kabyin and Kyaukhnget villages. It comprises a thick succession of medium- to thick-bedded, grey to buff, soft to indurated, micaceous siltstones. Subordinate rock types are yellowish, buff to greenish marl, and hard bands of micaceous and brownish sandstones interbedded with the yellowish siltstones that occur at the lower horizon. The carbonate content increases towards top of the unit and the upper boundary of the Lokepyin Formation is determined at the first appearance of limestone bed of typical Wunbye character. In this area the lower boundary is obscured due to thick soil cover. In the type area (Lokepyin village, about three miles north-east of Myaing, in Ye-ngan Township). The contact between this unit and the underlying micaceous siltstones of the Molohein Group is transitional. The unit yields abundant orthid brachiopods. Although no age determination of the fauna has been reported by the original author it should better be regarded, on basis of the stratigraphic position, as the age of Early Ordovician. The present authors trying to discover the conodonts fauna from the lime-mudstone of Lokepyin Formation at the Dalapin Village but no conodonts have been detected yet.

Wunbye Formation

The type section of this formation is established in this area between the point located at the eastern margin of the Ingyi village (GR 045 748) and the point (GR 078 728) of one-inch topographic map 93 C/12, situated at about one half mile north of western margin of Nan-on village. This formation has the largest distribution and thickness among the units exposed in this area. It occurs in the east and south-east of Ye-U, Ingyi, Theingon, and Wunbye Hill. In the type locality, the Wunbye Formation consists of a succession of thick-bedded limestones, siltstones, and dolomites. The limestones are finely crystalline, grey to bluish grey, sometimes oolitic and with pink, buff or yellow coloured silty materials in the forms of burrows, specks, pellets or irregular and regular laminations; burrow structure is most typical of these limestones. The minor siltstones are thin, medium- to thick-bedded, yellow to light grey and soft to indurated, sometimes thin bands of hard and light greenish siliceous claystone occur within the siltstones. The dolomite subunits are usually thick-bedded, often massive, but generally with laminations. The lithologies of the subunits of limestones, siltstones, and dolomites of the Wunbye Formation may vary from place to place. The lower and upper boundaries of the formation is marked by the first and last appearances of distinctive beds with the burrow structures or silty specks.

Faunally the formation is characterized by abundant orthid brachiopods, nautiloid cephalopods (*Actinoceras*, *Michelinoceras*, and *Wuthinoceras*), receptaculitid algae and stromatoporoids. The Wunbye fauna from Phyaukseikpin area, east of Kyaukse includes new species, *Michelinoceras burmese*, *M. kyaukse*, and *Actinoceras moeseini* identified by Myint Lwin Thein (1968), and receptaculitid algae, *Fisherites burmensis* by Rietchel and Niteckei (1984) indicates lower Middle Ordovician age of the Wunbye Formation. Niko & Sone (2014, 2015), reidentified the above nautiloid cephalopods from the Wunbye Formation as *Ordosoceras theini* sp. nov, *Armenoceras myanmarense* sp.nov, *Paratunkuskoceras* sp. *Wutinoceras moeseini* indicate the age of late Early or early Middle Ordovician (Floian or Dapingian) (Niko & Sone, 2014) (Figure 4). In 2015, they described

Gondwanan Nautiloid Cephalopods, *Sibumasuoceras langkawiense* (Kobayashi), *Ormoceras langkawiense, Tasmanoceras* sp. (Figure 5) which indicate late Middle Ordovician (Darriwillian).

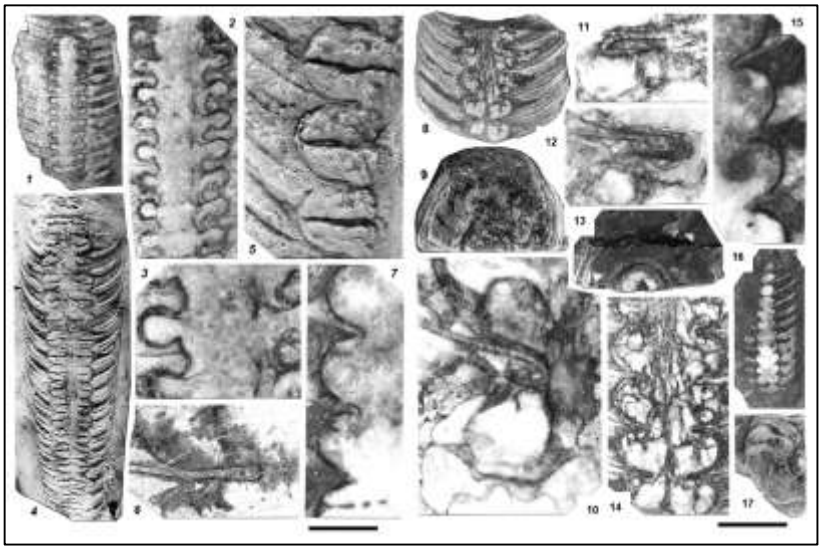

Figure 4. 1-3**,** *Ordosoceras theini* sp. nov., 4-6, *Armenoceras myanmarense* sp. nov.: 4, 5, holotype; 6, paratype, longitudinal thin section, showing details of septal neck. **7,** *Paratunkuskoceras* sp., Scale bar is 20 mm in Figure ; 8-12, 14**,** *Wutinoceras moeseini* (Thein, 1968); 13, *Armenoceras myanmarense* sp. nov., 15, 16, *Paratunkuskoceras* sp.; 17, *Ordosoceras theini* sp. nov., holotype, Scale bar is 30 mm

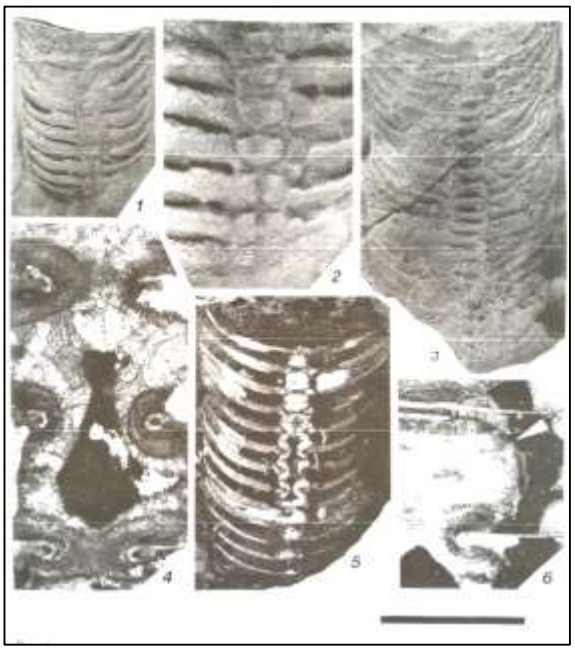

Figure 5. 1-6. *Sibumasuroceras lankawiensis* (Kobayashi, 1959). Middle Ordovician, Wunbye Formation, southern Shan State.

Nan-on Formation

The unit is named after Nan-on village, about one-mile south-west of Linwe village in Ye-ngan township. The type section of the unit is chosen at the area located between the grid coordinates of 078728 and 080728 (93 C/12) where both the upper and lower boundaries are being exposed. It has limited distribution in this area, extending southwardly to Tanshauk and Kyaukhnget areas. The Nan-on Formation at the type locality consists of yellow to buff or light orange, thin- to medium-bedded siltstones, mudstones, and claystones, generally subindurated to soft. Occasional occurrences of micaceous siltstone of light buff to whitish colour with pink or purple specks differentiate this unit from the yellow siltstones of the Wunbye Formation. In some areas, thin bands of laminated and argillaceous limestones occur interbedded with the siltstones. The lower boundary of the formation is marked at the contact of the siltstones with the top-most limestone bands of the Wunbye Formation which have burrow structures filled with silty materials, or silty laminations. The upper boundary, at the point of first appearance of purple band of the overlying Tanshauk Member. The Nan-on Formation is highly fossiliferous yielding abundant orthid brachiopods, cystoids, bryozoans, sponges, and trilobites. The brachiopod fauna of the Nan-on Formation was described and reviewed by Cocks and Zhen Ren Bin (1998). The fauna includes 31 genera, of which *Dirafinesquina* is a new genus, and *D. globosa* and *Leptellina (Leptellina)* minor are new species. The assemblage of the fauna indicates the age of Caradocian (Late Ordovician) (Figure 6).

Tanshauk Member

This unit is a member of the Nan-on Formation and was named after Tanshauk village, situated at about one-mile south of Nan-on village. The type section of the Tanshauk Member is located along the cart road situated at about one quarter of a mile north-west of Linwe village. This is also exposed at west of Nan-on and Pegin, at Tanshauk, and Kyaukhnget south villages. The member consists of purple or pink, soft, laminated, thin- to medium- bedded siltstones, calcareous shales and mudstones, the unit is characterized by its purple-coloured siltstones and shales. The lower boundary of the Tanshauk Member is determined at the horizon of the first occurrence of purple or pink shales or siltstones overlying the yellow or grey siltstones of the Nan-on Formation. The upper boundary is marked at the horizon of the last occurrence of purple siltstones or shales below the horizon of the first occurrence of phacoidal limestones or grey shales of the Linwe Formation. This member is correlated with the Hwe Maung Purple Shale Member of northern Shan State. This member is well exposed in the Pa-thin area, between Mandalay and Pyin Oo Lwin, Mandalay Region (Rong *et.al* 2020). Chen & Rong (2019) Discovered the brachiopods assemblage, *Xenocrania haimei* (Figure 7) that indicate the age of late Ordovician (Hirnantian). It is reasonable to take the age of Tanshauk Member of southern Shan State same as the Hwe Maung Purple Shale Member of northern Shan State.

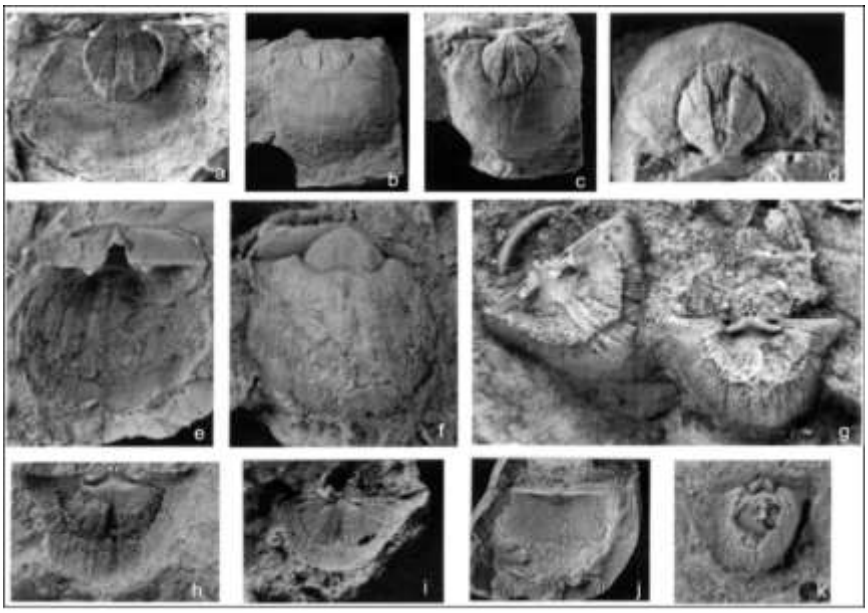

Figure 6. a-d, *Dirafinesquina globose* gen.et sp. nov. Linwe area. x2; e-k, *Leptellina* (*Leptellina*) *minor* sp. nov., Linwe area, x5. g-h, Holotype, x5 (Cocks and Zhen Ren Bin, 1998).

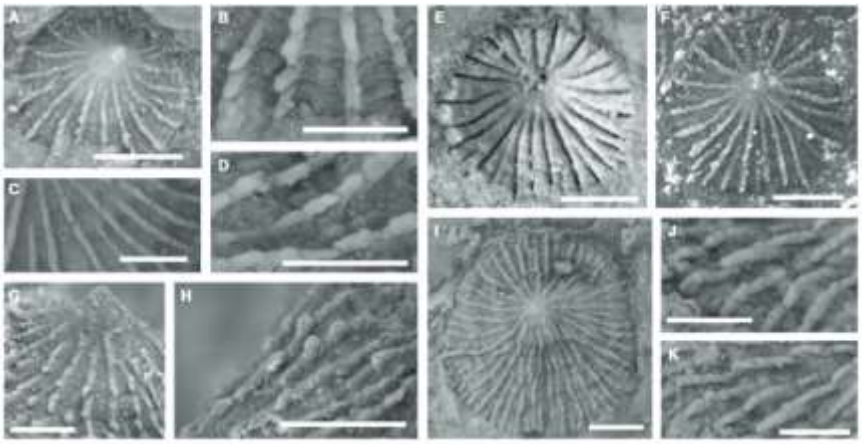

Figure 7. *Xenocrania haimei* (Reed, 1915). All specimens illustrated come from Hwe Mawng Purple Shale Member (Hirnantian), Mandalay Division, Myanmar.

Mibayataung Group

Myint Lwin Thein (1973) proposed this new stratigraphic unit which comprises essentially of phacoidally textured, pink, purple or bluish grey, argillaceous limestones (Linwe Formation), calcareous mudstones, buff-coloured mudstones, pink or purplish shales and slates (Wabya Formation), and orthoquartzites (Taungmingyi Member). The name of the group is taken from the Mibayataung Hill (GR 351 961 on topographic map 93/D11), situated at about 9 miles south-southwest of Heho. In exception of the Taungmingyi orthoquartzites the rock sequence of the group is well exposed at Linwe and Pegin areas. The lower limit of the group is marked at the last appearance of the purple or grey shale or siltstone of the Tanshauk Member which is the top of the Pindaya Group. The

upper boundary however, is placed at the base of possible Devonian unit or where it is absent, of the Thitsipin Limestone Formation of the "Plateau Limestone Group".

Linwe Formation

This unit is named after Linwe village in Ye-ngan Township, southern Shan State. The type section of the unit is located at and neighborhood of Linwe village (GR 085 725 on topographic map 93/C12), the total thickness of the unit at the type section is 1725 ft. (Myint Lwin Thein, 1973). In Linwe area the unit is widely distributed at Linwe and towards north, in the vicinity of Pegin village. Lithologically it is characterized by a thick succession of purple, pink, and grey-coloured limestones showing phacoidal structure on the weathered surface. Argillaceous limestones and calcareous mudstones constitute as minor account. Faunally straight and simple-cone nautiloid cephalopods (*Michelinoceras*) are locally fairly abundant in some outcrops of the calcareous mudstones or argillaceous limestones, crinoid stems and cystoid plates are quite often present but are of fragmentary nature. The Linwe limestone is easily distinguishable from the Ordovician Wunbye Limestone by having phacoidal character and purple or pink coloration. The latter distinctly has burrowed structure and lateral variation of the sedimentary facies is not common as in the Linwe Formation.

Myint Lwin Thein (1973) regarded the age of the Linwe Formation as Lower Silurian by judging from the graptolites and stratigraphic horizon. These graptolites were latter identified by Chit Sein (1998). The fauna indicates Early Silurian (Llandoverian) so that it is advisable to include all of the graptolite-bearing whitish silty shales (may be a member rank) into the Linwe Formation instead of taking as a separate unit that is the Wabya Formation. The youngest age for the Linwe Formation is now known to be accepted by the evidence of conodonts (*remscheidensis* Zone) (late Ludlovian to late Lochkovian), which is moving up into the Early Devonian by the presence of *Ozarkodina remscheidensis*, *Ozarkodina* sp., *Panderodus unicostatus*, *Dapsilodus obliquicostatus* (Figure 8) (Maung Maung & Aye Ko Aung 2005, 2009).

Wabya Graptolite Shale of the Linwe Formation

The mainly graptolite-bearing whitish, micaceous, silty, slaty shales well exposed at Wabya hill in Pindaya township are recognized as a new stratigraphic unit by Myint Lwin Thein (1973). The unit has limited distribution in the western part of the southern Shan state, Kyauktap, Mibayataung, Mwetaw Taung and Pegin areas.

The stratigraphic position of the unit is to be questioned as in places, it is sandawished between two phacoidal limestones of the Linwe Formation. In addition to that the shale unit may disappear laterally to be replaced by the phacoidal limestones showing lateral facies changes locally as well as regionally. The different faunal facies due to the above changes might as well pose problems in the stratigraphic correlation (e.g. *Michelinoceras* and crinoid species are common in phacoidal limestones whilst graptolites in shales). The age of the unit indicated by the graptolite faunas (orthograptids, climacograptids, glyptograptids, monograptids, and cyrtograptids) is of Early Silurian (Llandoverian to Wenlockian) (Figure 9).

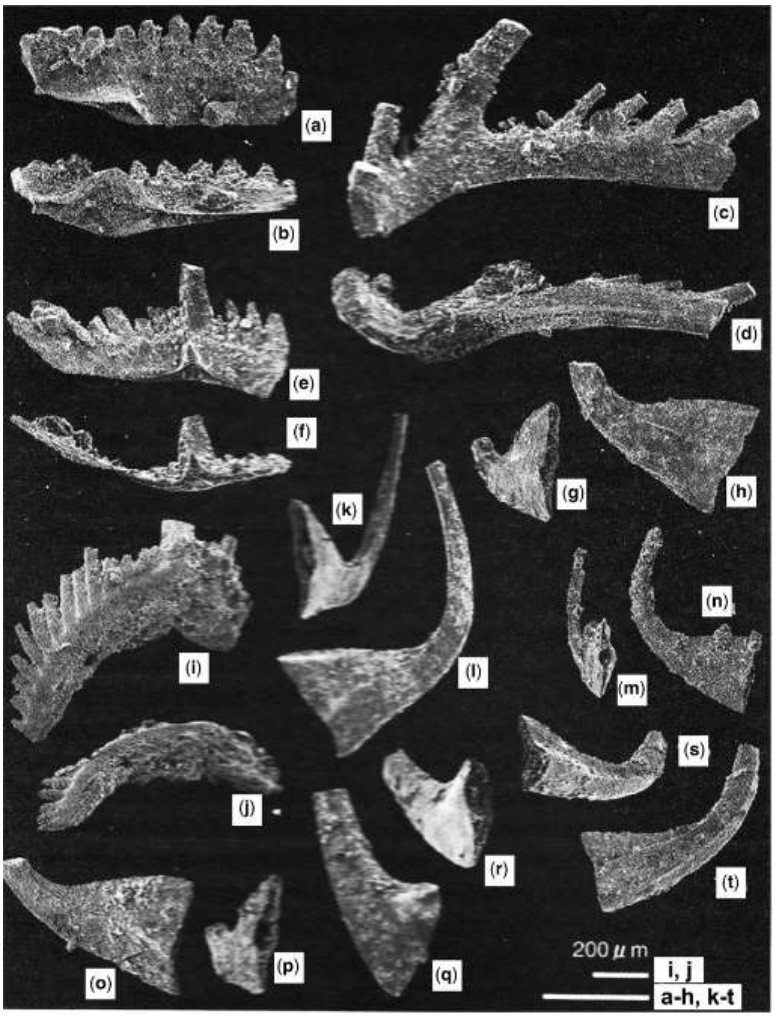

Figure 8. Early Devonian (Lohkovian) conodonts from the upper most part of the Linwe Formation, east of Pegin village, Ye-ngan Township, southern Shan State. (From Maung Maung, 2005; Maung Maung & Aye Ko Aung, 2005). 1) (a, b) *Ozarkodina remscheidensis* Ziegler; (c–f, i, j) *Ozarkodina* sp.; (g, h, k–r) *Dapsilodus obliquicostatus* Branson & Mehl; and (s, t) *Dapsilodus unicostatus* Branson & Mehl.

Devonian units of the Myogyi area

The Devonian units including Zebingyi Formation and ?Maymyo Formation are narrowly exposed in the Myogyi area, western part of the southern Shan State. The sequence consists of alternating purplish-red, unindurated calcareous siltstone and medium-bedded, light grey, argillaceous limestone with some ammonoids, gastropods, nautiloids, and ostracods. This Devonian sequence is tentatively considered as part of the Maymyo Formation since it overlies conformably the proper Zebingyi Formation of Pragian-Emsian age. This sequence requires future mapping. The Zebingyi Formation is recognized by the presence of a number of tentaculitids and the ?Maymyo Formation is of the presence of Late Devonian (Frasnian) ammonoids. The ammonoids include *Beloceras shidianese* Yang, *Tornoceras* cf. *contractum* Glenister (Figure 10). (Aye Ko Aung *et.al* 2010)

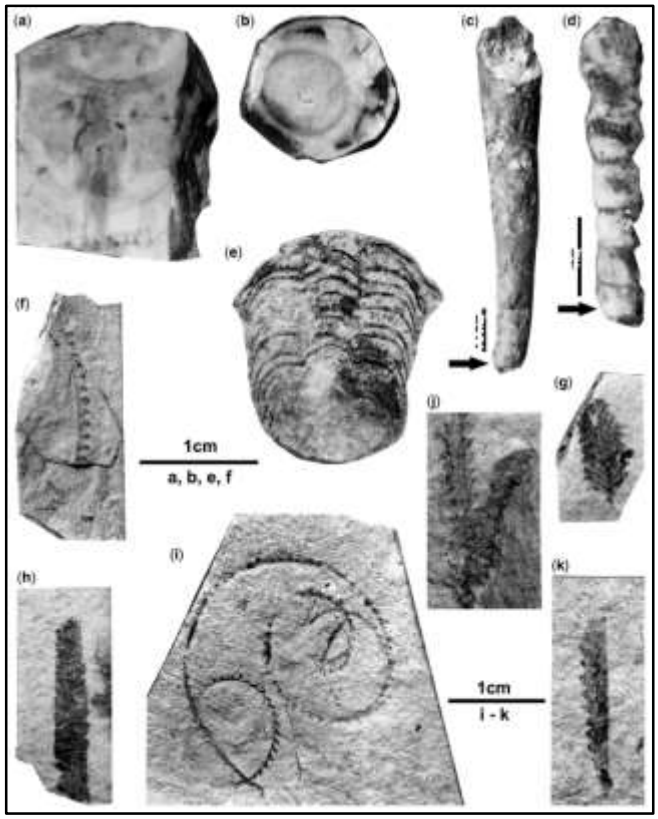

Figure 9. Silurian fossils of the Linwe Formation. (a-d) *Michelinoceras* sp.: (a) longitudinal section; (b) transverse section; and (c, d) surface specimens; (e) indeterminate dalmanitid; (f) *Monograptus priodon* (Bronn); (g) *Phyllograptus* sp.; (h)*Normalograptus normalis* (Lapworth); (i)*Cyrtograptus* sp.; (j) *Neoglyptograptograptus* sp.; and (k) *Pristiograptus variablis* (Perner).

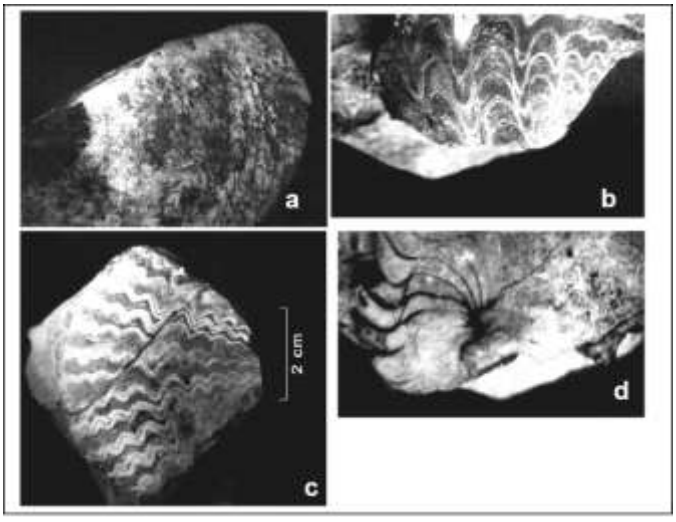

Figure 10. Late Devonian ammonoids of the Maymyo Formation, Myogyi area, Ye-ngan Township, (a-c) *Beloceras shidianense* Yang; (d) *Tornoceras* cf. *contractum* Glenister.

Thitsipin Limestone Formation

The unit was firstly named by Garson *et al*. (1976) after Thitsipin village, north of Ye-ngan. The formation is well exposed at the type area and the areas to the east and south-east of Pegin and Linwe villages. The largest outcrops of the unit are in the east of the Linwe area where more than half of the outcrop has been intensely dolomitized, resulting brecciation. The outcrop of which is easily distinguishable from the undolomitized or non-brecciated calcitic part of the formation. The undolomitized portion of the unit consists entirely of limestones. Five coral species belonging to four genera *Yatsengia hangchowensis*, *Iranophyllum* sp.cf. *caracinophylloides*, *Ipciphyllum subelegans*, *Pavastehphyllum* sp., *Pavastehphyllum* (*Thomasiphyllum*) sp., are discovered from the Thitsipin Limestone Formation (Aung Myo Zaw 2014) (Figure 11). At the base of the Pegin Pagoda Hill, a nearly ten-meter-thick sequence of well-bedded, dark grey, crinoidal limestone and micritic limestone is well exposed. In this interval, a typical Middle Permian foraminifer, *Multidiscus padangensis* is recovered (Aung Myo Zaw 2024). Middle Permian Limestone exposures of Pegin area and Htam sang area are closely similar not only lithology but also fauna occurrences (Aung Myo Zaw, 2023). Garson *et al.* (1976) divided the Thitsipin Limestone into three main facies: a massive limestone facies with abundant big brachiopods; a massive cherty limestone facies; and a well-bedded calcarenite facies.

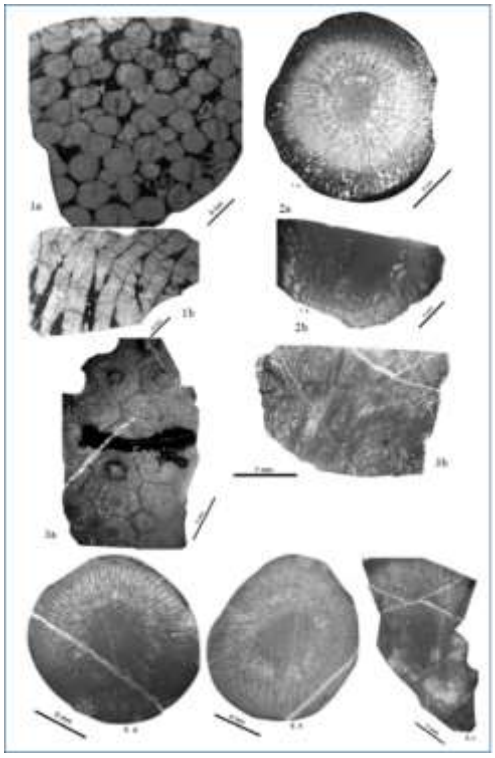

Figure 11. Permian corals of the Thitsipin Formation, Pegin-Linwe area, Ye-ngan Township.1a-*Yatsengia hangchowensis* ; transverse section,1b) *Yatsengia hangchowensis*; longitudinal section, 2a) *Iranophyllum* sp.cf *carcinophylloides*; transverse section, 2b) *Iranophyllum* sp.cf *carcinophylloides*; 3a) *Ipciphyllum subelegans*; transverse section, 3b) *Ipciphyllum subelegans*; longitudinal section, 4a, b) *Pavastehphyllum* sp.; transverse section, 4c) *Pavastehphyllum* sp.;longitudinal section.

Nwabangyi Dolomite Formation

Garson *et al*. (1976) first used the name after the village of that name situated in Ye-ngan township. It wide spreadly outcrops in north and north-west of Nwabangyi village and outside the study area the unit is extensively distributed in Pindaya, Heho, Shwenyaung, and Taunggyi townships. Garson *et al*. (1976) recognized four main facies in the formation: thin- bedded foraminifera limestone; laminated and turbiditic limestone; sedimentary breccia; and light and dark grey, fine-grained limestone.

In Myogyi area the Nwabangyi Dolomite Formation is chiefly composed of dolomitite carbonate rocks with the common features of surficial shattering and brecciation. In the present area, however the rock sequence consists of grey intraformational oligomitic conglomerate in combination with sedimentary breccia, bedded, grey lithic packstone, thin to medium bedded bioclastic wackestone with ammonoids, brachiopods, conodonts and shark fossils and thinly bedded, grey, tyrbiditic and dolomitic packstone-grainstone. The ammonoid-bearing unit crops out extensively in the Myogyi area which is situated approximately 20 km southeast of Kyaukse town, Mandalay Region. It is located at the triple junction of Kyaukse-Myitha and Ye-ngan townships.

The ammonoids species are identified as, *Ussurflemingites* cf. *abrekentisis* Shigeta & Zakharov; *Ussurflemingites* cf. *primoriensis* Shigeta & Zakharov; *Arctoceras* cf. *subhydaspis* (Kiparisova) (Figure 12), and a nautiloid species *Trematoceras* cf. *subcampanile* (Kiparisova); conodonts, *Neospathodus pakistanensis* Sweet, Neo. Dineri Sweet (Figure 13); and shark teeth, *Acrodus* cf. *cuneocostatus* Cuny, Rieppel & Sander, *Polyacrodus* sp.indet., *Hybodus* sp. and Placoid scale of Euselachii order, family., gen. et sp. indet, from the sequence. These faunas indicate Lower Triassic (Induan-Olenekian).

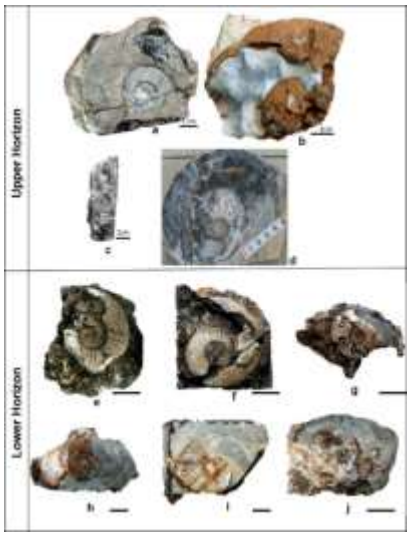

Figure 12. a) *Ussuriflemingites* cf. *primoriensis* Shigeta & Zakhrov, 2009; b, d) *Arctoceras* cf. *subhydaspis* Kiparisova, 1961; c) *Trematoceras* cf. *subcampanile* Kiparisova, 1954; Upper Horizon of the Nwabangyi Dolomite Formation, Myogyi area; e-j) *Paleokazakhstanites* cf. *ussuriensis* Zakharov, 1968; Lower Horizon of the Nwabangyi Dolomite Formation; scale bar=1cm for all figures.

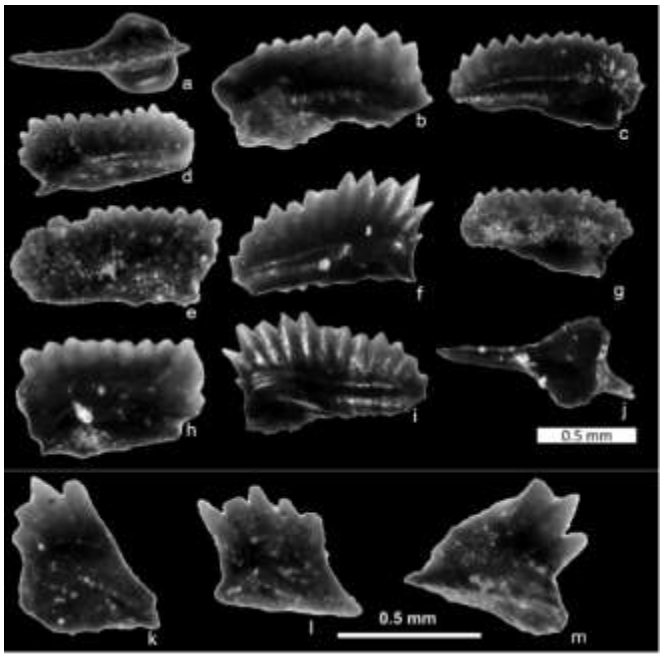

Figure 13. a-j *Neospathodus pakistanensis* SWEET; k-m *Neospathodus dieneri* SWEET, Nwabangyi Dolomite Formation, Myogyi area, Ye-ngan Township, southern Shan State.

Conclusions

Based on the experience in the study of Pre-Paleozoic to Early Mesozoic stratigraphy in southern Shan State, it is known to be believed that most of the rock units require comprehensive revision. The age of the Chaung Magyi is reviewed as Precambrian-Cambrian (Aye Ko Aung & Cocks, 2017) and Late Neoproteozoic-? Cambrian (Dew *et.al*., 2019). The existence of Late Cambrian trilobites has been acknowledged since 1970's, but no formal descriptions of such fossils have been published. Wernette *et.al* (2021) provide such descriptions of some trilobites from Molohein Group's "Myet-Ye Formation" from the Linwe area, Ye-ngan Township of the southern Shan State. Three species from two genera are reported: *Asioptychaspis lata* n.sp.Wernette, *A.asiatica* Zang & Jell, and *Eosaukia buravasi* Kobayashi. These fossils indicate Furongian (Lower to Middle Cambrian) stage 10. The Early to Middle Ordovician nautiloids are identified by Niko & Sone (2014, 2015) provide new species: *Ordoceras theini* n.sp. Niko & Sone, (Floian or Dapingian, late Early-early Middle Ordovician), *Armenoceras myanmarensis* n.sp. Niko & Sone (Darriwillian, early Middle Ordovician), *Wutinoceras moeseini* (Thein) Niko & Sone (late Early Ordovician), two species of Gondwanan cephalopods: *Sibumasuoceras langkawiensis* (Kobayashi) (Dariwillian). The Nan-On Formation consists abundant brachiopods (*Dirafinesquina globose* gen.et sp. nov. & *Leptellina* (*Leptellina*) *minor* sp. nov., suggests Late Ordovician (Katian/Caradocian). Latest Ordovician (Hirnantian) Tanshauk Member, revealing typical *Hirnantia* Fauna. The Silurian Linwe Formation is revised with the evidence of conodont fauna, *Ozarkodina remcheidensis*, an important zone fauna which indicates the age of Early Devonian (Lochkovian). In the Devonian system, Late Devonian (Frasnian) unit,

previously not known in the Shan State is recognized in the Myogyi area, Ye-ngan Township, southern Shan State. It is evidenced by the recovery of the Late Devonian (Frasnian) ammonites (*Beloceras* sp.). There are so far no Carboniferous strata found in the present study area. The Permian rocks are the most widely distributed in the Shan Plateau. They are rather fossiliferous, comprising corals, foraminifers, brachiopods, bryozoans, and crinoids, which are very useful tool for stratigraphic correlation locally as well as regionally with the neighboring countries such as, Thailand, Malaysia, and Yunnan. The Early Triassic (Induan-Olenikian) boundary bed is recognized in the Myogyi area by the occurrence of ammonoids (*Ussuriflemingites* cf. *primoriensis*) and conodonts (*Neospathodus pakistanensis*), which are useful tools for global correlation.

Acknowledgements

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COMPARISON OF AQUIFER CHARACTERISTICS IN THE PART OF CENTRAL DRY ZONE, MYANMAR

Hnin Ei Hlaing[1], Nay Nwe Myo[2], Min Han Nyein[3]

Abstract

The study area, Mahlaing Township is located in the central dry zone of Myanmar. Historically this area has been hydrogeologically renowned for its high salinity, low groundwater yielding aquifers and low success rate in locating potable water suitable for irrigation and human consumption. Although this is the case in some of the fractured marine shale and fine sandstone aquifer of the Obogon and Kyaukkok Formations of Lower to Middle Miocene age. The study area is classified into two types of aquifer. There are Irrawaddian aquifer and Peguan aquifer. The recovery test shows that the transmissivity of the Irrawaddian aquifer is (89.47 m^2/day) in 4" diameter tube well and (2.8 m^2/day) in 6" diameter tube well of Peguan aquifer.

Keywords: aquifer, the transmissivity of the aquifer (KD) m^2/day, the constant well discharge (Q) m^3/day, drawdown difference (Δs) m, the residual drawdown (Δ s') m

Introduction

Water is essential commodity to mankind and the largest available sources of water lie in the underground. Extended irrigated lands, industrialization and increasing population will demand both underground and surface water. Adequate supplies of clean, safe fresh water are fundamental for human survival and well-being.

Location and Size

Mahlaing area is located near the northern extent of the Bego Yoma Anticlinorium. The majority of the ranges trend NNW-SSE in the direction of the strike of the rocks. The study area lies between North Latitude 20° 55′ to 21° 00′ and East Longitude 95° 25′ to 95°50′. It is bounded on the east by Wundwin Township, on the west by Taungtha and Kyaukpadaung Township, on the south by Meiktila Township and on the north by Ngwethoegyi Township. It is bounded by Taungtha range at the western part of the area. Generally, its length is about 20 miles from south to north and the width is about 24 miles from east to west. Roundly the whole area is approximately about 428.70 square miles. The location map of the study area is shown in Figure1.

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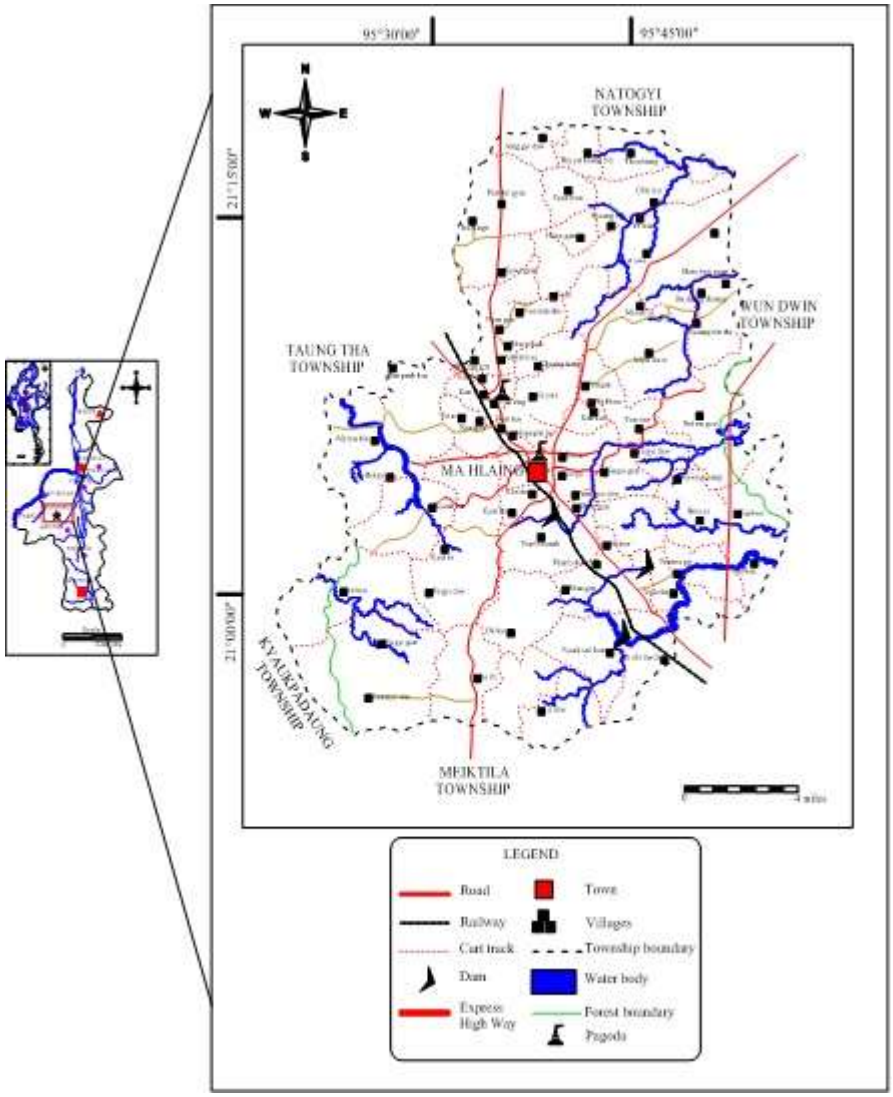


Figure 1. Location Map of Mahlaing Township

Materials and Methods

Types of Aquifer

On the basis of the data of the tube wells drilled by the Irrigation Department, in conjuction with the author's own investigation and hydrogeologic testing and the lithologic composition and stratigraphic position, the following aquifers are classified into two types.

- Groundwater in Irrawaddian Aquifer
- Groundwater in Peguan Aquifer

Groundwater in Irrawaddian Aquifer

The exposures of the Irrawaddian aquifer is found at the east and west of the study area. Depth of the Irrawaddian aquifer lying in eastern and western parts of the area is variable in place to place. Irrawaddian aquifers are found at the depth range from 180 ft to 260 ft below the surface of the study area. In the Irrawaddian aquifer sediments are composed of unconsolidated, distinguishable blue to bluish grey colored sand and gravel of various sizes with stiff blue silty clay and yellow to yellowish brown clay as a confining bed. However, blue clay is not always present

as confining bed at some places. The alternation of sand, gravel and thin clay layers are also found in some well logs.

Usually medium to coarse sand are found at the top of the aquifers. Gravel mixed with sand is found at the lower portion. Most of the aquifer materials are well rounded to sub-angular. Sand and gravel of the aquifer materials are quartz, chert, fragments of sandstone and quartizite.

Recharge to the Irrawaddy Formation aquifers occurs by direct rainfall on the permeable sandy soil, surface runoff along chaungs during the wet season or throughflow of saline water from the Pegu Group. The salinity of the groundwater in aquifers of the Irrawaddy Formation is quite variable and is largely dependent on its relative location to that of the Pegu Group. The discharge rate of the study area is 1314 gph for 4 inches diameter tube well. According to well log data, the water bearing horizon is at 180-260 ft depth.

Groundwater in Peguan Aquifer

The groundwater flow system within aquifers of both the Irrawaddy Formation and Pegu Group is complex. Due to their low hydraulic characteristics, the relatively impermeable marine shale of the Obogon Formation forms distinct hydrogeological boundaries throughout the area. This is especially so along the major anticlines. Flow is mainly from the anticlines to the Irrawaddy Formation located on the anticlinal flanks and thin the synclines. The exception appears to be the highly faulted region north of the Indaw Anticline, where groundwater flow is east through rocks of the Kyaukkok and Obogon Formations towards Thinbon Chaung. The rater of the rock and hydraulic gradient.

The Pegu Group rocks are highly fractured, especially along the anticlinal fold axis and fault systems. Recharge to the fractured aquifer systems of the Pegu Group occurs as rainfall and intermittent surface flow on rock outcrops. Discharge occurs as salt springs

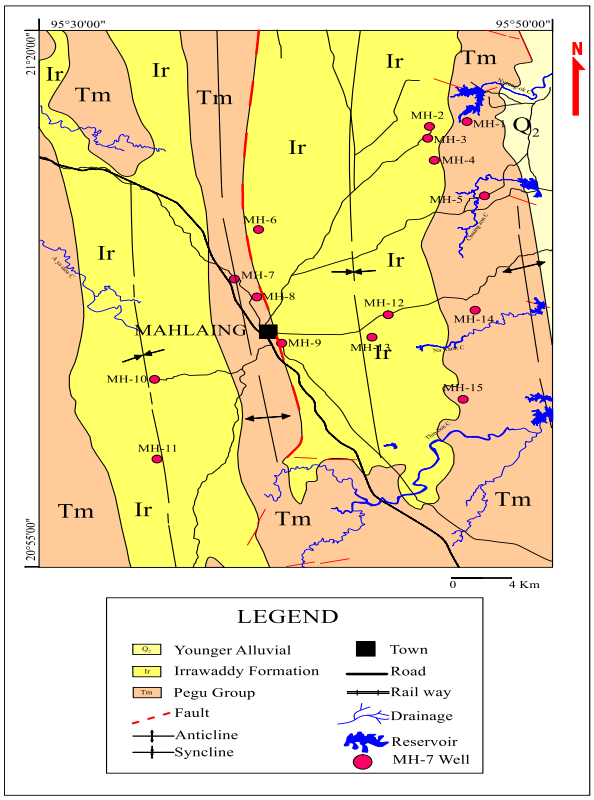
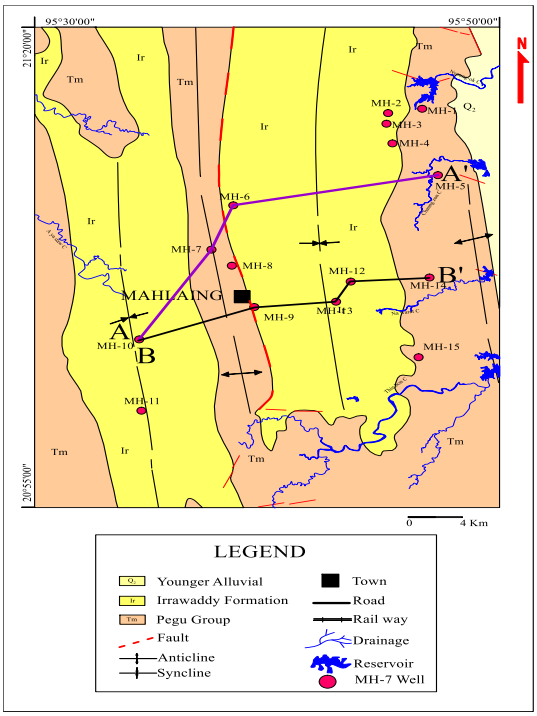


Figure 2. Map Showing Existing Tube-Well on the Geological Map of the study area

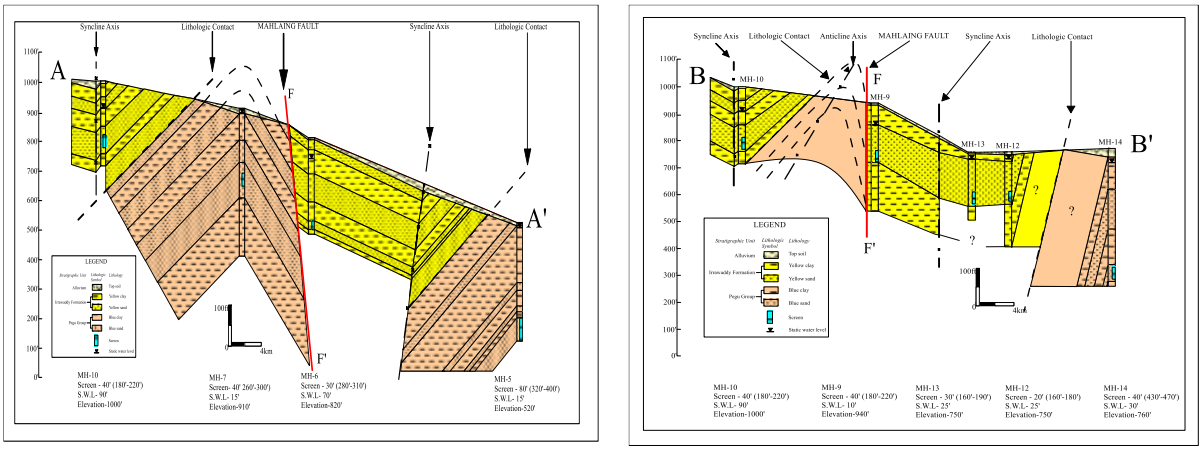
along fault lines, through flow into the more permeable aquifers of the Irrawaddy Formation and artificially from tube wells and dug wells. The elevation of aquifers in the Pegu Group is largely controlled by geological structure. It is located at the center of the study area. The lithology is noted as sandstone interbedded with blue shaly sand and clayey sand. Generally, the colors of the rocks are white, yellowish brown, greenish blue and blue. Thick bedded sandstone is usually interbedded with clay and shale. The discharge rate of the study area is 1998 gph for 6 inches diameter tube well. According to well log data, the water bearing horizon is at 455-485 ft depth. Static water level is at 50 ft depth.

Fence Diagram

Fence diagram of the study area shows that sand and clays are dipping towards the north and north-east (Figure 3).



Map showing line of cross-section in the study area



Hydrogeological Cross-section along (A-A') section and (B-B') section

Figure 3 Fence diagram of the study area

Results

Pumping Out Test in Mahlaing Area

Pumping out test is the measurement of discharge rate and drawdown that occur in a pumped well and also measuring the drawdown of the piezometers surrounding the pumped well in certain time during pumping of the well. This measurement indicates how much volume of water that store in the aquifer can be release and cone of depression at a certain time during pumping. Theis's measurements can show hydraulic characteristics of the aquifer of storativity and transmissivity after proper calculation.

Discharge rate of the well was measured by container method. In study area, pumping out test for 1 hours and 25 minutes and recovery test for 3 hours duration were performed at 4 inches diameter tube well in Irrawaddian aquifer at Yon Daw Village, Ma Hlaing Township, Fig. (4) and Fig (5).

Pumping Out test in Irrawaddian Aquifer

The Jacob's method (Copper and Jacob's), 1946 is based on the Theis's equation.

$$KD = (2.3\ Q) / (4\pi\Delta s) \text{ (Jacob's Straight Line Method)}$$

$Q = 217.92\ m^3/day$

$\Delta s = 0.3m$

$KD = 2.3Q/4\pi\Delta s = (2.3\times217.92)/ (4\times3.14\times0.3) = 501.22/3.77 = 118\ m^2/day$

The constant well discharge is 217.92 m^3/day and the drawdown difference is (0.45) m and the transmissivity of the aquifer is 118 m^2/day.

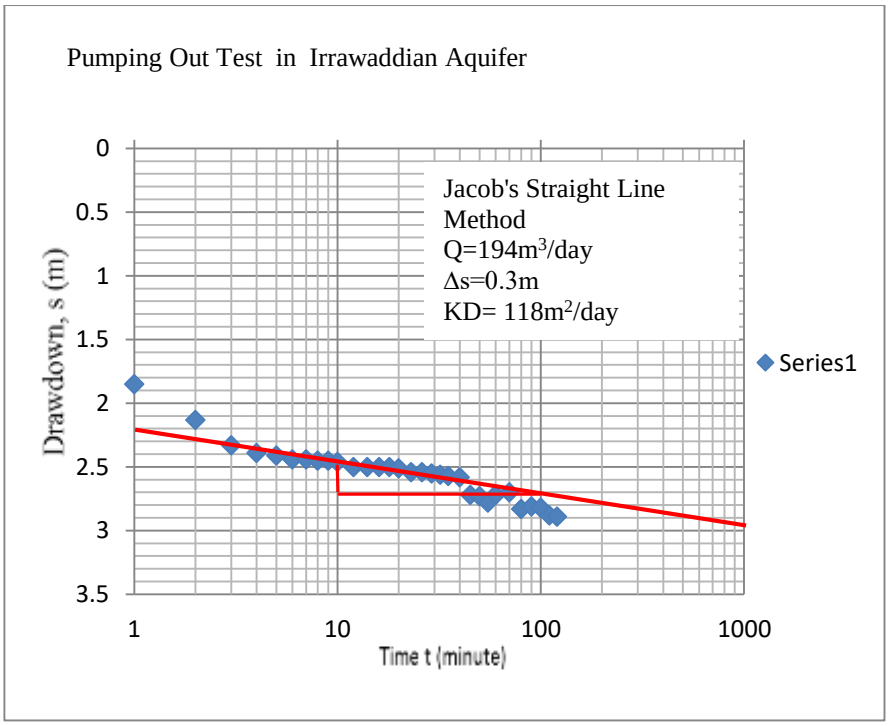


Figure 4. Pumping Out test in Irrawaddian Aquifer at Yon Daw Village

Recovery Test in Irrawaddian Aquifer

The objectives of the recovery test are to measure the residual drawdown, to calculate the transmissivity of the aquifer and check the result of pumping test data. Residual drawdown is the difference between the original water level before the start of pumping and water level measurement at a time after the cessation of pumping in a well.

Method of Recovery Test

Theis's recovery method is widely used for analysis of recovery test. Theis's recovery equation (1935) is described as follows:

$$KD = (2.3\ Q)/4\pi\Delta s' \text{ (Theis's Recovery Method)}$$

Q = 194.5 m3/day

Δ s' = 0.4 m

$KD = (2.3\ Q)/(4\pi\Delta s')$

$= (2.3\times194.5)/ (4\times3.14\times0.4)$

$= (447.35)/ (5.02) = 89.47\ m^2/day$

The constant well discharge is 194.5 m^3/day and the drawdown difference is (0.4) m and the transmissivity of the aquifer is 89.47 m2/day.

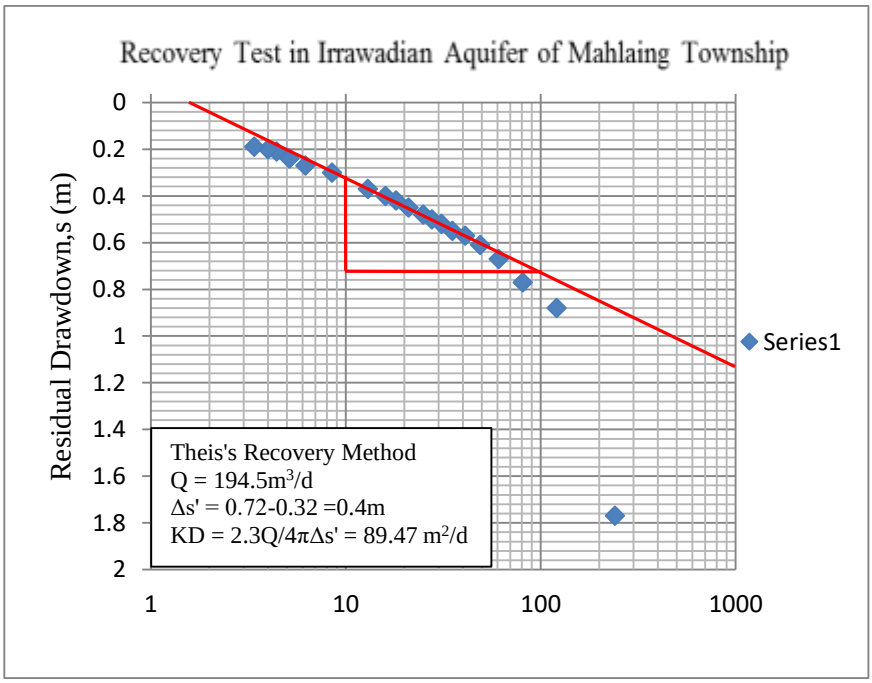


Figure 5. Recovery Test in Irrawaddian Aquifer at Yon Daw Village

Recovery Test in Peguan Aquifer

Unfortunately, piezometer was absent and only recovery analysis was done. The objectives of the recovery test are to measure the residual drawdown, to calculate the transmissivity of the aquifer and check the result of pumping test data. Residual drawdown is the difference between the original water level before the start of pumping and water level measurement at a time after the cessation of pumping in a well.

Discharge rate of the well was measured by container method. In study area, pumping out test for 1 hours and 25 minutes and recovery test for 3 hours duration were performed at 6 inches diameter tube well in Peguan aquifer at Kan Pyar quarter, Ma Hlaing Township, Fig. (6).

Method of Recovery Test

Theis's recovery method is widely used for analysis of recovery test. Theis's recovery equation (1935) is described as follows:

$$KD = (2.3\ Q)/4\pi\Delta s' \text{ (Theis's Recovery Method)}$$

Q = 217 m^3/day

Δ s' = 14 m

KD = (2.3 Q)/ (4πΔs')

= (2.3×217)/ (4×3.14×14)

= (499.1)/ (175.84) = 2.8 m^2/day

The constant well discharge is 217 m^3/day and the drawdown difference is (14) m and the transmissivity of the aquifer is 2.8 m^2/day.

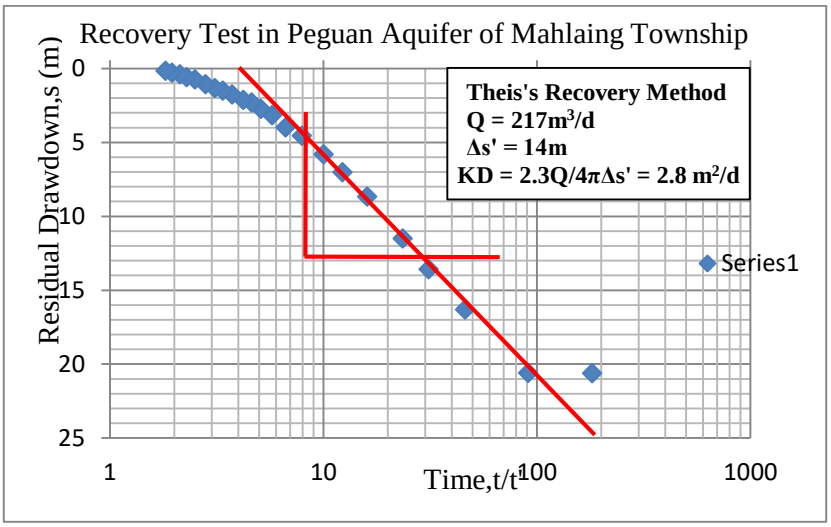


Figure 6. Recovery Test in Irrawaddian Aquifer at Kan Pyar quarter

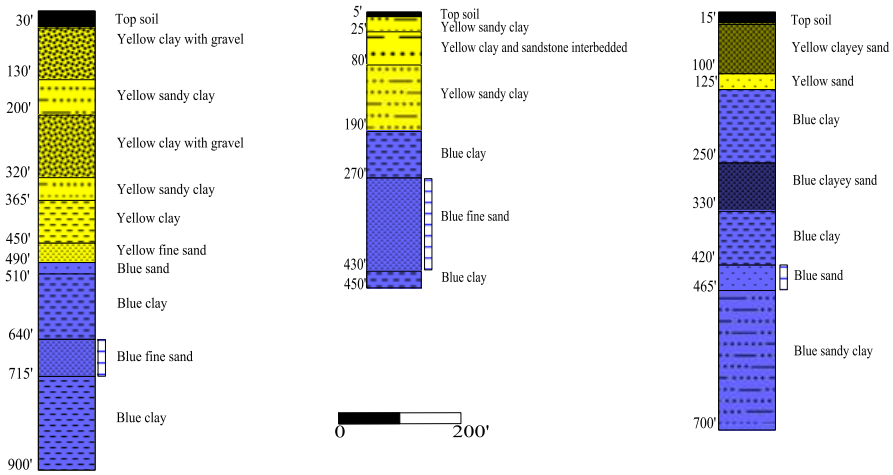


Figure 7. comparison of well logs data in the study area

Summary and conclusion

1. The study area is located in Mahlaing Township, Mandalay Region. It lies between North Latitude 20 55′00 "and 21˚ 00′00" and East Longitude 95˚ 25′00 " and 95˚ 50′00 ". It can be referred to 84 O/12, 13, 14, 15, 16 of one-inch topographic maps.
2. On the basis of lithologic character and stratigraphic position, the aquifer recognized in this area are classified generally into (2) major types such as, Irrawaddian Aquifer and Peguan Aquifer.
3. The Irrawaddian Aquifer are composed of unconsolidated blue to ash-grey coloured gravel and sand with thin blue silty clay layers are intercalation. Most of the aquifer materials are well rounded to sub-angular, medium to coarse sand and gravels. Irrawaddian aquifer is found at the depth range from 180 ft to 260 ft below the surface and it yields 1314 gph from 4" diameter tube well. Static water level is noted at 20.6 feet depth.
4. The Peguan aquifer is covered by yellow to brownish clayey soil. It is mainly composed of sandstone interbedded with shale. Water bearing layer of Peguan aquifer is very thin and about 20' to 30'. Peguan aquifer is found at the depth range from 455 ft to 485 ft below the surface and it yields 1998 gph from 6" diameter tube well. Static water level is noted at 21.83 feet depth.

Acknowledgements

This paper is part of my PhD dissertation and the author would like to express her sincerely thanks to my supervisor Dr Day Wa Aung, Professor and Head of Geology Department, University of Yangon for his kind guidance, instruction, valuable discussions. I am greatly thanking to Dr Myat Thuzar Soe, Professor and Head of Applied Geology Department for her permission and valuable administrative supports.

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GROUNDWATER QUALITY ANALYSIS OF THE PHYU TOWNSHIP, BAGO REGION

Nay Nwe Myo[1], Min Han Nyein[2], Hnin Ei Hlaing[3]

Abstract

The study area is located between in the west of the Sittaung River and eastern parts of the Bago Yoma. It is lying between North Latitude 18º 24´ to 18º 30´ and East Longitude 96º 27´ to 96º 30´. The total coverage of the area is approximately 2322.42 square kilometers. Total population of the research area is about 257,273 (2019 Cencus). The drainage pattern of the study area is dendritic and parallel pattern. According to the pattern, the bedrock of the study area is shale and sandstone. Alluvial aquifer mainly composed of the research area. Alluvial aquifer mainly composed of the blue clay, blue sand, blue sandy clay and sand with gravel in some place. According to the well log data, its thickness is varying from 90-160 feet in one place to another. At well no.16, the groundwater yield from this unit is about 500 to 800 gallons per hour (gph) from 2" diameter tube well. The collected samples are analyzed at the Water Resources and Utilization Department (W.R.U.D) and Ministry of Agriculture and Irrigation Department, the cations and anions and TDS, EC, pH, total alkalinity and total hardness. According to the Piper method, water types can be classified by 3 types. There are Sodium Chloride, Calcium Chloride and Mix types. The pH values of water samples are ranging from 7.95 to 8.6. The electrical conductivity (EC) of groundwater samples is ranging from 210 to 800 µmhos/cm. TDS concentration is ranging from 120 ppm to 520 ppm. Sodium concentration is ranging from 5 ppm to 45 ppm. Calcium concentration is ranging from 9 ppm to 48 ppm and does not exceed the W.H.O standard (2011) of 200 mg/L. The value of iron in the research area is ranging from 0.0 ppm to 4.5 ppm. Bicarbonate concentration in the research area is ranging from 20 ppm to 92 ppm. The concentration of sulfate in the research area is ranging from 8 ppm to 70 ppm. The results of chemical data analyzed by the SSP% method, the SAR method and the MAR method show that some tube wells can be assessed suitable for use as irrigation water. According to the above methods and the WHO Drinking Water Standard, the data can assess whether the groundwater of the research area is suitable for drinking water, domestic use, and irrigation water, except that some tube wells are not suitable.

Keyword: SSP%, SAR and MAR

Location, Size and Accessibility

The study area is located between in the west of the Sittaung River and eastern parts of the Bago Yoma. The location map of the study area is shown in Figure (1). Geographically, the area is bounded by Oktwin Township in the north, Htantabin and Kyaukkyi Townships in the east, Kyauktaga Township in the South and Nattalin Township in the west. It is lying between North Latitude 18º 24´ to 18º 30´ and East Longitude 96º 27´ to 96º 30´. This area refers to the UTM Map and Map Sheet No. 1896-1, 5, 9 in UTM. The total coverage of the area is approximately 2322.42 square kilometers. Total population of the research area is about 257,273 (2019 Cencus).

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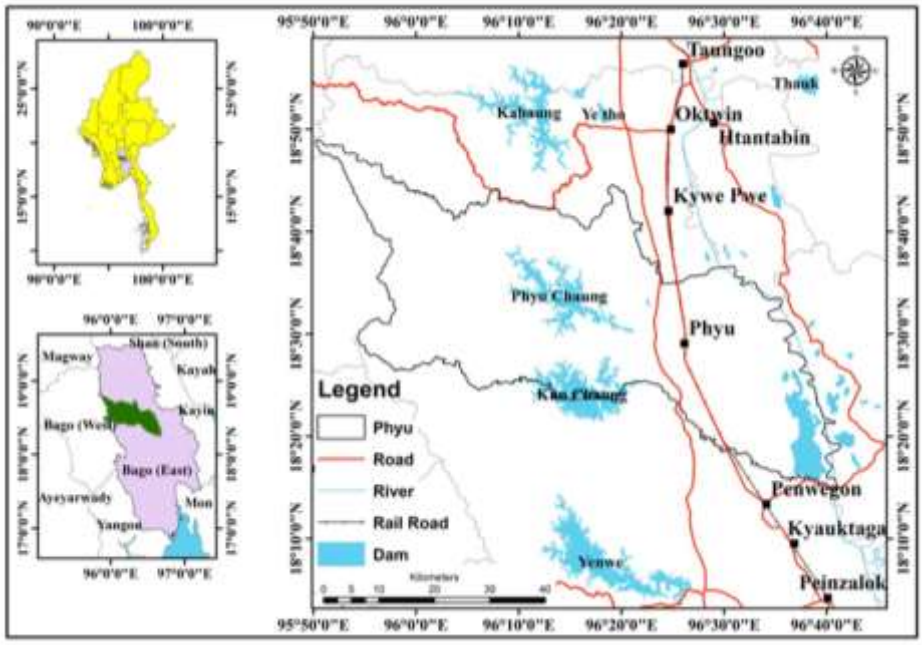


Figure 1. Location Map of the Study area

Purpose of the study

The purposes of this research are described as follows;

- To determine the chemical composition, quality and types of groundwater
- To access the groundwater quality for suitable sources of irrigation, domestic uses and industry use

Method of Study

Before commencement of this field work, the author tried to get information and literatures from available sources. Brittle plastic bottles have been prepared to bring back the water samples from the field for the analysis of their quality. The present research work consists of literature review, Geographic Information System (GIS) analysis, field investigation, and laboratory work. Method of study has two methods. There are Field Methods and Laboratory Methods.

Field Methods

During the fieldwork, the location of the well by G.P.S (Global Positioning System), the measurement of water level, well depth, well logging and collection of rocks and water samples, to set the information from the local people who need and well water were taken into the recorded.

Laboratory Methods

In the laboratory of Utilization of Water Resources Department, measurement of cations (Ca^{2+}, Mg^{2+}, Na^{+}, $K^{+,}$ and Fe^{2+}) and anions (HCO_3^-, SO_4^- and Cl^-), total dissolved solids (TDS), total hardness (TH), p^H and electrical conductivity (EC), smell, salinity, color, were made.

Previous Investigation

Most previous works in this study area emphasized on geology, hydrogeology and others. They are listed as follows:

Aye Aye Myint made “Environmental Geological Study of Taungoo and its environs” for Ph.D dissertation in 2009.

Bender, F., (1983) placed the study area into the Back Arc area of sedimentation in the Pegu Yoma and in the Sittaung Basin.

Kyaw Aung studied groundwater chemistry and water flow direction in the study area and made a study of the "Hydrogeological investigation of the groundwater of Taungoo-Oktwin area and Taungoo Town" for D.A.G. dissertation in 1979.

Kyaw Ye Aung made "Assessment of Groundwater Quality of the Oktwin Township, Bago Region" for M.Sc degree in 2016.

Thandar Aung made "Assessment of Groundwater Quality in Phyu Township, Bago Region" for M.Sc degree in 2016.

Thin Thin Khaing made "Analysis of poor drainage system and flood frequency during the rainy season in Taungoo" for M.Res.degree in 2005.

Wint Wint Htun studied Environmental Aspects of Kabaung Dam Project Area, Oktwin Township, Bago Division (East) "for M.Rse degree in 2005.

Zin Nwe Khaing studied "Assessment of Groundwater Quality in Taungoo Township, Bago Region" for M.Sc degree in 2016.

Drainage Pattern

Phyu of the main river is Sittaung. Phyu has many streams. All streams are freshwater type. The drainage pattern of the study area is dendritic and parallel pattern. According to the pattern, the bedrock of the study area is shale and sandstone. The drainage map of the study area is shown in Figure (2).

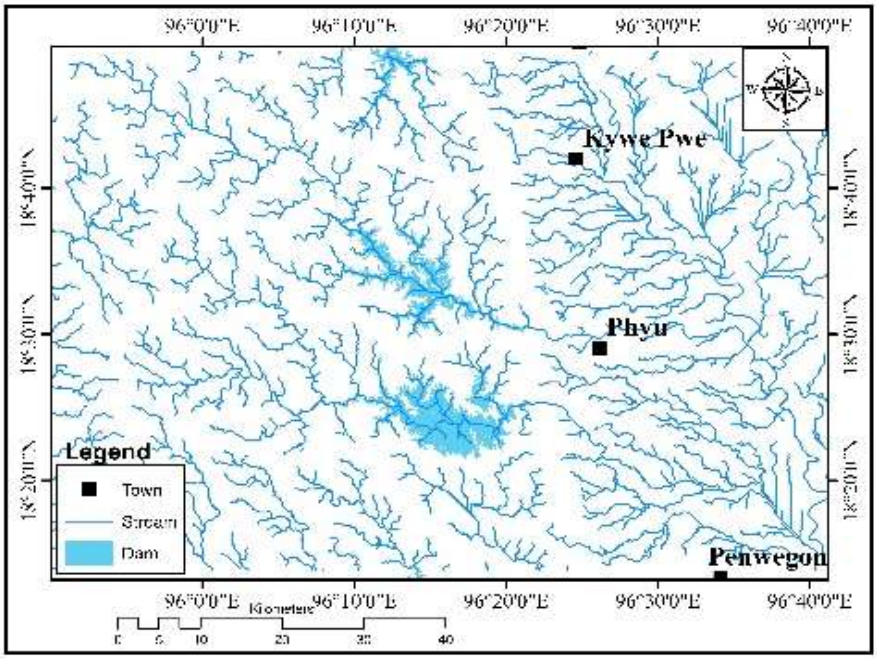


Figure 2. Drainage Map of the Study Area

Climatological Features

The climate data are based on the record of (Sources: The Department of Meteorology and Hydrology Kaba-Aye) for the period of 2011-2019. The monthly maximum temperature is high in April with 38.4 Cº and minimum temperature in January is only 15.9 Cº. The average monthly rainfall is 210 (mm). The monthly rainfall is high in Aug with 642 (mm).The relative humidity is

high in Aug with 96 %. The climatological condition of the study area is shown in Figures (3, 4, 5 and 6) respectively.

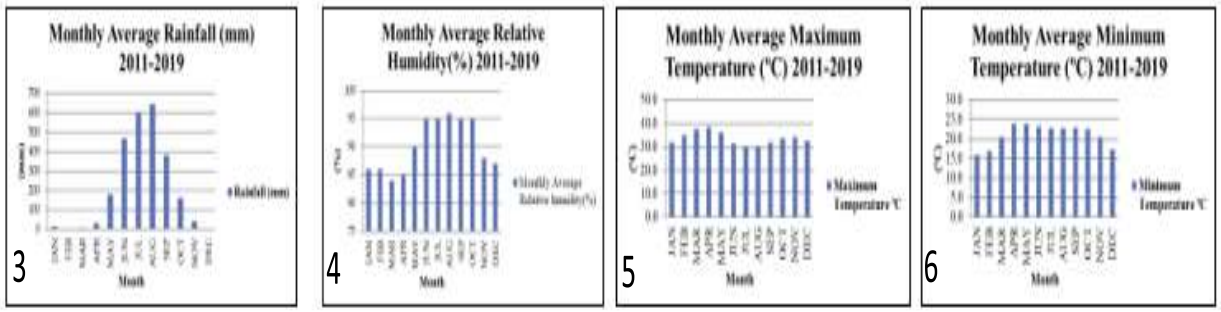


Figure 3. Monthly Average Rainfall, **Figure 4**. Monthly Average Relative Humidity, **Figure 5**. Monthly Average Maximum Temperature and **Figure 6**. Monthly Average Minimum Temperature

Regional Geologic Setting

The investigated area is located in the Pegu region, and the Bago Yoma area is situated in the eastern part of the Central Myanmar Tertiary Basin. Pegu Yoma is a morphological and geological unit about 400 miles (644 km) long and 40 miles (64.4 km) wide that generally strikes NNW-SSE lying between the Mogok Belt (Searle and Ba than Haq, 1964) and Shan Plateau (Eastern Highlands) to the east and Central Volcanic Line to the west. The study area lies between the right lateral Sagaing fault and the Papun fault (Soe Thura Tun, 2007).

In the study area, the Irrawaddy Formation is well exposed on both sides of the Yangon-Mandalay Highway, and the Pegu Group is widely distributed in the western part of the study areas. The regional geologic map of the study area is shown in Figure (7) and Table No. (1) Stratigraphic succession of the study area.

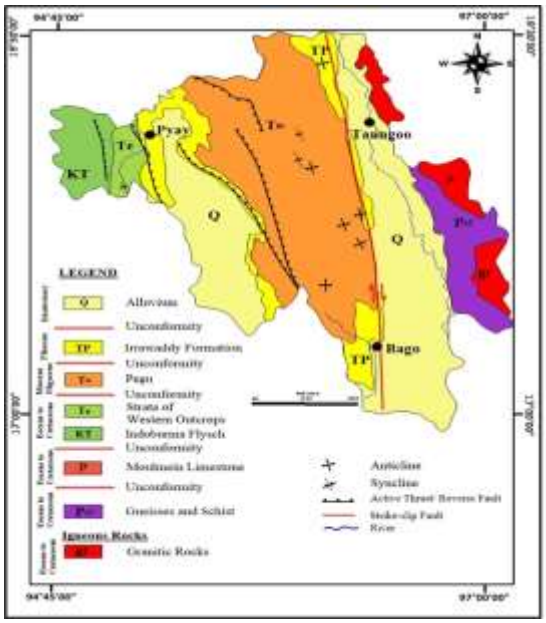


Figure 7. Regional Geologic Map of the Study Area, Source; (MGS 2014)

Table 1. Stratigraphic Succession of the Study Area

Geologic Age	Rock units	Lithology
Recent	Alluvium	clay loam and sandy clay, overburden soil, bluish brown, and yellowish brown in colour, moderately plastic and soft
Pliocene	Irrawaddy Formation	coarse current bedded and poorly consolidated buff color sandstone interbedded with gritty, pebbly beds, minor sandy clay and shale
Miocene	Pegu Group	light grey colour massive sandstones intercalated with grey shale and buff colour sandstones and siltstone
Late Cretaceous to Early Eocene	Igneous Rocks	Pegmatite dykes and quartzofeldspathic veins, Microgranite, Biotite granite, Porphyritic biotite granite

Hydrogeologic Characteristics of the Research Area

Collection of Data

One-inch topographic map was used in the collection of the water samples of the area under investigation. All water sample data are collected from the Water Resources and Utilization Department (W.R.U.D) and the Ministry of Agriculture and Irrigation Department.

Table 2 Chemical analysis of the research area

Tube Well No.	TDS ppm	EC µmho/cm	pH	TH ppm	TA ppm	Na ppm	k+ ppm	Ca2+ ppm	Mg2+ ppm	Fe2+ ppm	Cl- ppm	SO4= ppm	HCO3- ppm	Aquifer Types
P1	120	210	8.32	52	60	15	13	9	7	2	20	12	44	Alluvial
P2	400	640	8.58	166	112	45	38	36	18	1	120	34	92	Alluvial
P3	200	320	8.36	74	98	25	21	20	6	2.5	33	12	74	Alluvial
P4	200	320	8.7	68	72	26	22	14	8	1	45	14	52	Alluvial
P5	220	340	8.3	76	80	26	22	14	10	0.5	42	19	20	Alluvial
P6	160	270	8.32	90	86	11	9	20	10	3	22	17	52	Alluvial
P7	520	800	8.43	286	96	35	30	48	40	0.5	156	70	64	Alluvial
P8	300	470	8.39	112	96	35	29	22	14	3	74	28	76	Alluvial
P9	240	380	8.58	76	92	30	26	18	7	0	52	12	76	Alluvial
P10	160	250	8.34	104	70	5	4	21	13	2	22	23	58	Alluvial
P11	260	420	8.54	168	90	20	17	26	25	4.5	43	48	66	Alluvial
P12	220	340	7.95	74	84	21	23	12	11	2.5	43	18	64	Alluvial
P13	160	250	8.37	98	60	8.5	7	20	12	3	30	21	40	Alluvial
P14	240	380	8.34	92	92	29	24	16	12	0.5	45	23	64	Alluvial
P15	200	320	8.37	42	86	35	29	10	4	0	42	8	66	Alluvial
P16	220	350	8.37	118	78	15	13	15	19	2.5	43	34	66	Alluvial
P17	160	265	8.3	100	72	12	10	14	15	0	26	29	60	Alluvial

Aquifers

In the research area, alluvial aquifer mainly composed of the blue clay, blue sand, blue sandy clay and sand with gravel in some place. According to the well log data, its thickness is varying from 90-160 feet in one place to another. At well no.16, the groundwater yield from this unit is about 500 to 800 gallons per hour (gph) from 2" diameter tube well.

Classification of Piper Diagram (Piper 1944 and Hill 1940)

This method was proposed by Piper (1944) and Hill (1940). This method of the tri-linear diagram is widely used to depict chemical data and show the relative concentrations of the major cations (Ca^{+2}, Mg^{++} and K^{+}) and anions (CO_3^{-},HCO_3^{-},Cl^{-} and $SO_4^{=}$) . Cations are plotted on the left triangle and anions on the right triangle. Piper diagrams are shown in Figure (8). According to the Piper method, water types can be classified by 3 types. There are Sodium Chloride, Calcium Chloride and Mix types.

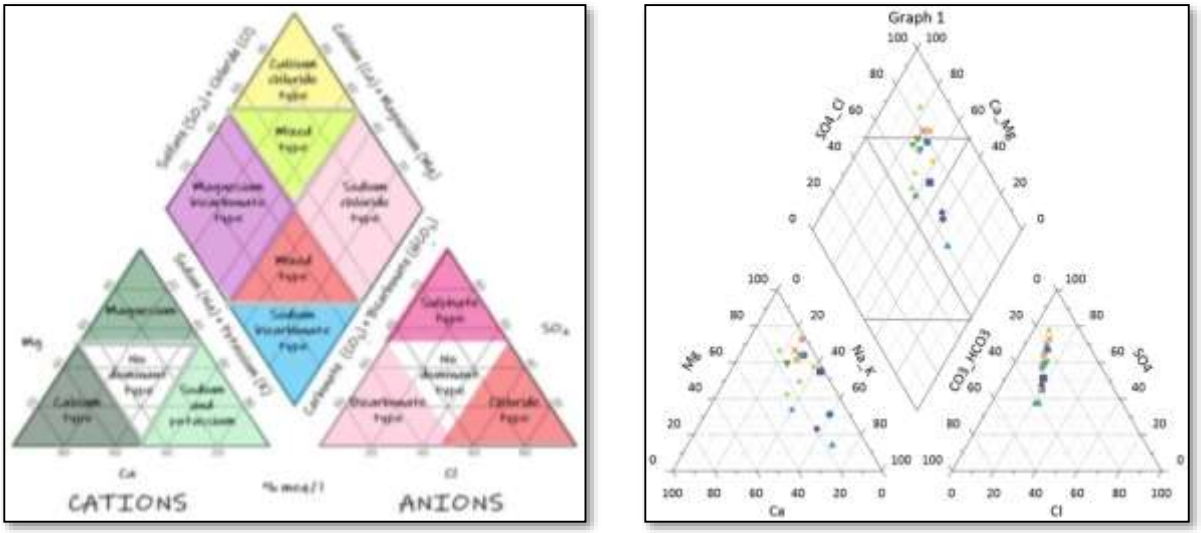


Figure 8. Classification of the Piper diagram, after Piper (1944) and by Hill (1940)

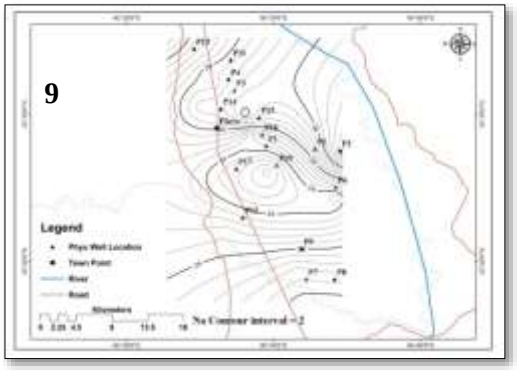
Chemical Analysis of the Groundwater

Chemical Analysis is important to specify the actual characteristics of groundwater. Determination of pH, total dissolved solids, T.D.S, electric conductivity E.C, dissolved cations of Na^{+}, K^{+}, Ca^{++}, Mg^{++} and Fe^{++} and dissolved anions of are made in the laboratory.

In groundwater resources evaluation, the quality of groundwater is as important as its quantity. The chemical and physical constituents of groundwater determine its usefulness for municipal, commercial, industrial, agricultural and domestic water supplies.

Detail description of chemical composition of the study area

All water samples are collected from water Resources Department Yangon. The collected samples are analyzed for the cations (calcium Ca^{++} , Magnesium Mg^{++} , Iron Fe^{++} , Sodium Na^{+}, Potassium K^{+}) and anions Bicarbonate (HCO_3^{-}), Sulphate ($SO_4^{=}$) and Chloride (Cl^{-}) are represented shown in Figure. (9, 10, 11, 12, 13, 14, 15 and 16).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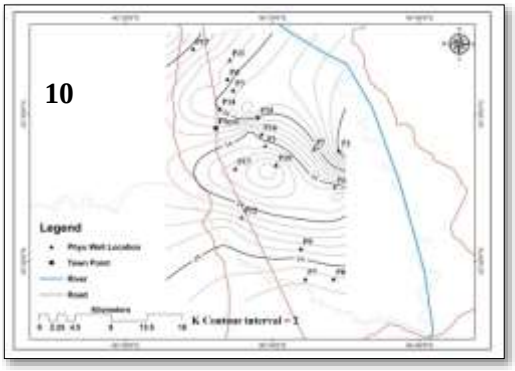
Na Contour interval = 2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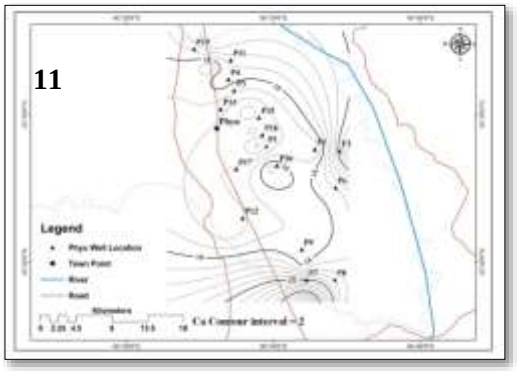
K Contour interval = 1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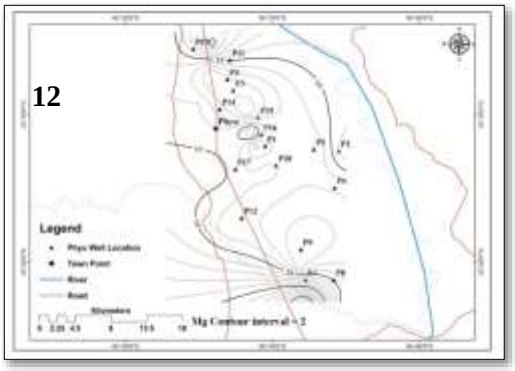
Ca Contour interval = 2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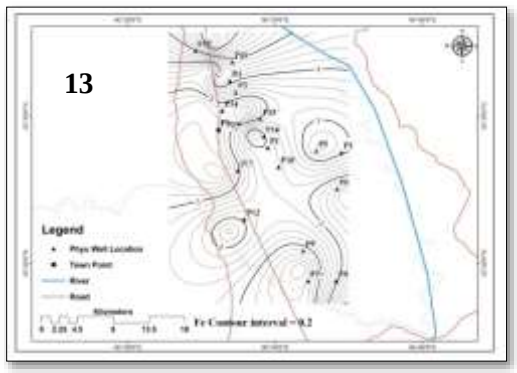
Mg Contour interval = 1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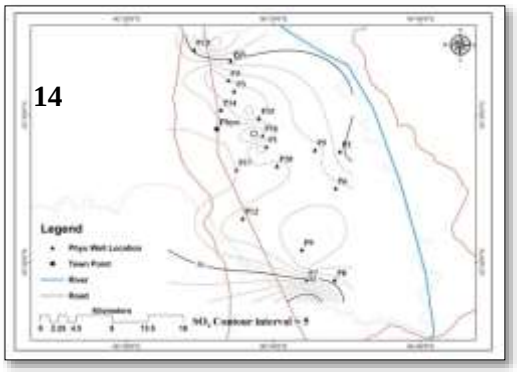
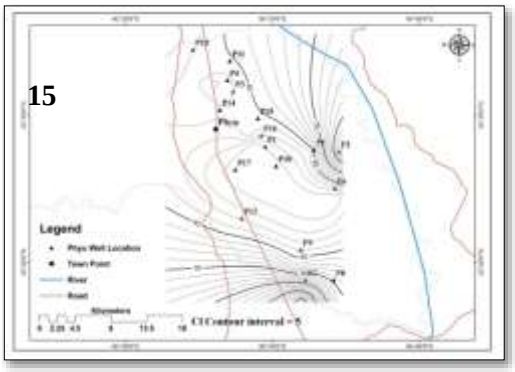
Legend
Fe Contour interval = 0.2


14
Legend
SO4 Contour interval = 5


15
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Cl Contour interval = 5

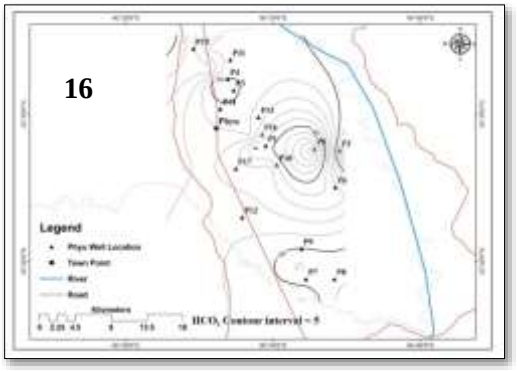

Figure 9. 10, 11, 12, 13, 14, 15 and 16. Distribution Map of the Major Cations and Anions in research area.

Domestic Purposes for Water Quality

The pH values of water samples are ranging from 7.95 to 8.6. The electrical conductivity (EC) of groundwater samples is ranging from 210 to 800 µmhos/cm. TDS concentration is ranging from 120 ppm to 520 ppm. Sodium concentration is ranging from 5 ppm to 45 ppm. Calcium concentration is ranging from 9 ppm to 48 ppm and does not exceed the W.H.O standard (2011) of 200 mg/L. The value of iron in the research area is ranging from 0.0 ppm to 4.5 ppm. Bicarbonate concentration in the research area is ranging from 20 ppm to 92 ppm. The concentration of sulfate in the research area is ranging from 8 ppm to 70 ppm. The water quality shows the domestic uses and drinking water of water quality in Table No. (3).

Table 3 Comparison with the study area and World Health Organization (W.H.O) Guideline Values for Drinking Water Quality Standard (2011)

Characteristics	Guideline value (2011)		The range obtained from groundwater	Remark
	Desirable	Max Permissible		
Calcium (mg/l)	75	200	9-48	Suitable
Magnesium (mg/l)	30	150	4-40	Suitable
Sodium (mg/l)	0	200	5-45	Suitable
Potassium (mg/l)	0	200	4–38	Suitable
Sulphate (mg/l)	0	250	8 – 70	Suitable
Chloride(mg/l)	200	250	20-156	Suitable
Iron (mg/l)	0.3	1	0 – 4.5	Unsuitable
TDS (mg/l)	0	1000	120-520	Suitable
pH	6.5	8.5	7.95 – 8.6	Potable
EC (micro mhos/ cm)	0	1500	210–800	Suitable

Agriculture purposes for Groundwater Quality

Agriculture is the basis of the Myanmar economy. The quality of water for irrigation is classified by Sodium Adsorption Ratio (SAR), Magnesium Adsorption Ratio (MAR) and Soluble Sodium Percentage (SSP or Na %). The respective values of all water quality parameters are summarized in each table.

Sodium Adsorption Ratio (SAR) (Richardson, 1954)

Sodium Adsorption Ratio (SAR) is most commonly used to assess the suitability of irrigation water and classification based on the SAR values is expressed in Table No. (4). The SAR measures sodicity in terms of the relative concentration of sodium ions to the sum of calcium and magnesium ions in a water sample. Sodium concentration in water affects the deterioration of the soil properties reducing permeability. SAR is calculated using the following formula:

$$S.A.R = \frac{Na}{\sqrt{\frac{Ca+Mg}{2}}}$$

Table 4. Classification of Water based on the SAR

Type of water	SAR value	Classification
Low Sodium water	<10	Excellent
Medium Sodium water	10-18	Good
High Sodium water	18-26	Doubtful
Very High Sodium water	>26	Unsuitable

Where the ionic concentrations are expressed in meq /L. The result of the Sodium Adsorption Ration (SAR) is shown in the Figure. (17) and Table No. (6).

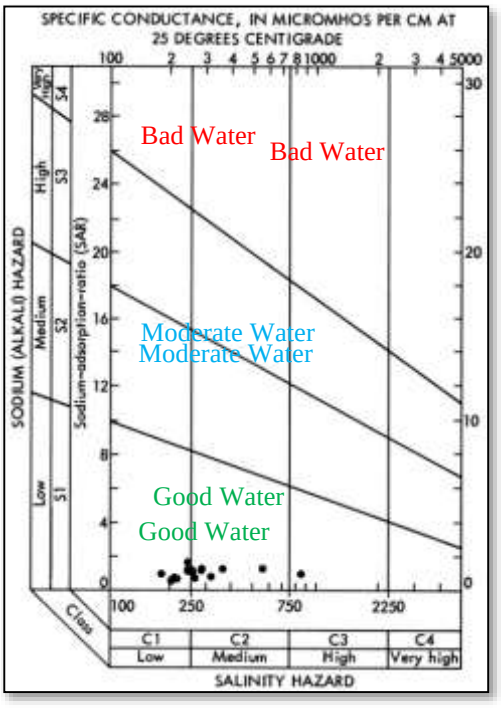

Figure 17. Sodium Adsorption Ration (SAR)

Magnesium Adsorption Ratio (MAR) (Raghunath, 1987)

The magnesium content of water is considered one of the most important qualitative criteria in determining the quality of water for irrigation. Generally, calcium and magnesium maintain a state of equilibrium in most water. More magnesium in water will adversely affect crop yield as the soil becomes more saline, Raghunath, H. M., (1987). The values of the magnesium adsorption ratio of each aquifer are tabulated in Table No. (6).

$$\text{M. A. R.} = \frac{\text{Mg2+}}{\text{Ca2+ + Mg2+}} \times 100$$

The value of the magnesium adsorption ratio of the research area ranges from 33 to 68 %. The acceptable limit of the magnesium adsorption ratio is 50 %.

Soluble Sodium Percentage (%) (Wilcox, 1955)

Sodium concentration plays an important role in the evaluation of groundwater quality for irrigation because sodium causes an increase in the hardness of the soil as well as a reduction in its permeability. The sodium percentage (Na %) is calculated using the formula given below:

$$\text{SSP} = \frac{(\text{Na} + + \text{K+})}{\text{Ca 2} + + \text{Mg2} + + \text{Na} + + \text{K} +} \times 100$$

The methods of classification of groundwater based on Na% (Wilcox,1955) is shown in Table No. (5). The result of the SSP% are shown in Table No. (6).

Table 5. Classification of Groundwater based on Na % (Wilcox, 1955)

(Na %) Percentage of Sodium	Classification
< 20	Excellent
20 - 40	Good
40 -60	Permissible
60 - 80	Doubtful
> 80	Unsuitable

Table 6. Summary Table for various methods of irrigation water quality in the Research Area

Tube Well No.	EC micro mhos/ cm	SAR	MAR%	SSP%
1	210	0.8	56	49
	Excellent	Excellent	Unsuitable	Permissible
2	640	1.2	45	47
	Good	Excellent	Suitable	Permissible
3	320	1	33	52
	Good	Excellent	Suitable	Permissible
4	320	1.1	49	56
	Good	Excellent	Suitable	Permissible
5	340	1.1	54	53
	Good	Excellent	Unsuitable	Permissible
6	270	0.4	45	28
	Good	Excellent	Suitable	Good

Tube Well No.	EC micro mhos/ cm	SAR	MAR%	SSP%
7	800	0.8	58	29
	Permissible	Excellent	Unsuitable	Good
8	470	1.2	51	50
	Good	Excellent	Unsuitable	Permissible
9	380	1.2	39	57
	Good	Excellent	Suitable	Permissible
10	250	0.2	51	13
	Good	Excellent	Unsuitable	Excellent
11	420	0.6	61	28
	Good	Excellent	Unsuitable	Good
12	340	0.9	60	50
	Good	Excellent	Unsuitable	Permissible
13	250	0.3	50	22
	Good	Excellent	Suitable	Good
14	380	1.1	55	51
	Good	Excellent	Unsuitable	Permissible
15	320	1.8	39	73
	Good	Excellent	Suitable	Doubtful
16	350	0.5	68	30
	Good	Excellent	Unsuitable	Good
17	265	0.5	64	29
	Good	Excellent	Unsuitable	Good

Results and Outcomes

The results of chemical data analyzed by the SSP% method, the SAR method and the MAR method show that some tube wells can be assessed suitable for use as irrigation water. PIPER method can be classified the water types, drinking water, domestic use and Irrigation water. Water Types can be analyzed into 3 water types. If high amount of Iron concentration, it can be reduced of amount with aeration methods and sand filtering methods. According to the above methods and the WHO Drinking Water Standard, the data can assess whether the groundwater of the research area is suitable for drinking water, domestic use, and irrigation water, except that some tube wells are not suitable.

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PETROGENESIS OF IGNEOUS ROCKS EXPOSED AROUND THE ROAD SECTION FROM WASHAUNG TO SADON VILLAGE, WAINGMAW TOWNSHIP, KACHIN STATE

Me Me Aung[1], Htet Htet Wint War[2]

Abstract

The Myitkyina mafic-ultramafic belt, eastern part of Kachin State, is a key to understand tectonic evolution of Tethys Ocean in SE Asia. We took a survey at the Washaung and Sadon villages about 9miles (14km) and 14miles (23km) respectively to east of Myitkyina Township where comprises magmatic rocks of pegmatite/aplite, hornblende granite, granodiorite, diorite, dolerite, peridotite and metamorphic rocks of amphibolite, hornblende-biotite gneiss, biotite gneiss and migmatite. Based on field criteria, petrographic data and previous published literature they jointly suggest that as the boundary between magmatic and metamorphic rocks is intrusive contact. The magmatic rocks have typical SSZ-type (supra-subduction zone) features, suggesting products of partial melting of subduction oceanic plate. The subsequent partial melting of this underplated mafic rock may be caused by heat carried upward by basaltic magma. Fractional crystallization and contamination occurred when it passes through the crust. Some granite and pegmatite were probably formed by partial melting of lower or middle crust. The igneous are estimated to be Mid Jurassic to Cretaceous in age. All facts indicate the igneous rocks from the study area may be formed as continental arc which related to subduction of Neotethys Ocean during the Mesozoic and Early Cenozoic time.

Keywords: Myitkyina, continental arc, mafic-ultramafic rocks

Introduction

In Myanmar, mafic and ultramafic rocks are mainly exposed in two belts: the western belt occurring along the eastern margin of Indo-Burma Range (IBR), and the eastern belt outcropping along the Tagaung-Myitkyina-Mogok. The western belt has been regarded as remnant of the Neotethys Ocean. While the eastern belt is debated on: (a) relict of the Mesotethys Ocean (Liu et al., 2016); (b) the northern extension of the IBR ophiolite (Mitchell et al., 2015); (c) a continental margin arc of the Neotethys ocean (Zhang et al., 2018). Based on the field results and microscopic features, we will describe which model is more appropriate for the study area. The first model is based on Jurassic ages of magmatic rocks along the Myitkyina belt that can compare with ages of the ophiolite from the Mesotethys Ocean in the Tibet region (Liu et al., 2016). The second model is supposed that the Myitkyina belt locates to north of the IBR ophiolite after ca. 300 km restoration of right-lateral strike-slip movement (Mitchell et al., 2015). While the third model is based on rock assemblages in the Myitkyina mafic-ultramafic belt and their SSZ-type geochemical features (Zhang et al., 2018). Each model seems to be supported by their mentioned evidence. However, recent years studies show that remnants along the Neotethys Ocean also contains many Jurassic components, such as the Middle Jurassic chert in ophiolites of the Naga Hills and Yazagyo (Myanmar), which are coeval with the ages of the Myitkyina mafic rocks. Therefore, only the ages of the mafic rocks cannot constrain whether they belong to Mesotethys or Neotethys Ocean. As mentioned in the third model, there are outcrops andesite, hornblende gabbro and diorite along the Myitkyina belt, rock assemblages of continental margin, which would not be component of ophiolite.

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Above introduction shows that the controversial issue between these models is what kind of rock assemblages along the Myitkyina mafic-ultramafic belt and what kind of relationship between their contact boundaries, and even the magmatic rocks and their host metamorphic rocks. Therefore, this study will be conducted at the northern segment of Eastern Ophiolite belt falls within the Kachin State.

The study area is situated in the eastern part of Kachin State and is located about 5miles (8km) from east of Myitkyina Township. The location of the study area is shown in figure (1) Latitude 25°21' to 25°25' N, Longitude 97°35' to 97°54′ E.

The rock units exposed in the study area are mainly hornblende granite, granodiorite, diorite, dolerite, gabbro and peridotite for igneous, and amphibolite, gneiss and migmatite for metamorphic. We first focus on field relationship of these rocks from the Washaung to Sadon villages and combined with other published lectures to solve above discuss debate. Then we further imply their tectonic affinities and possible regional evolution.

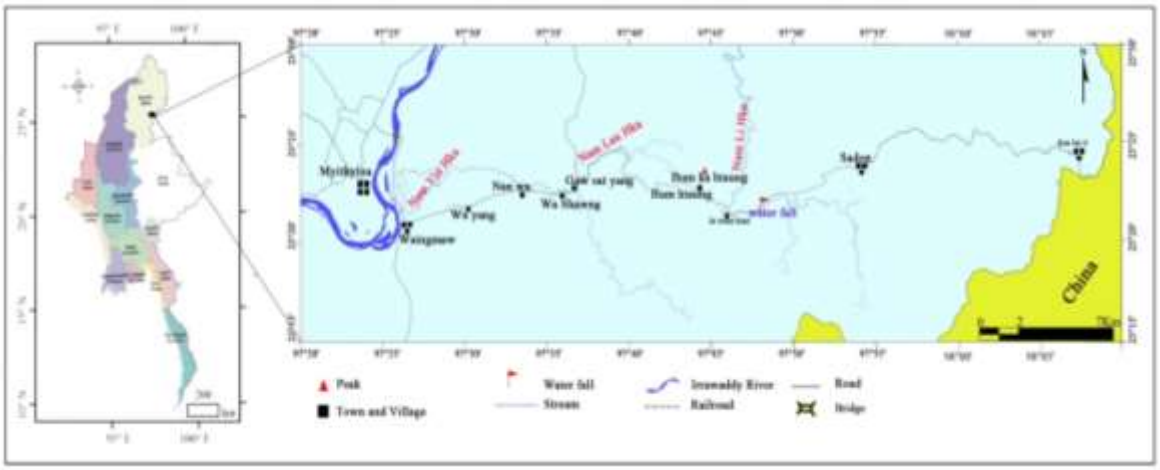


Figure 1. Location map of the study area.

Purpose and method of study

The main purposes of this research are (a) to give a map view of distribution and types of the rock units, and (b) to study petrogenesis of igneous rocks.

The locations of the outcrops were recorded by GPS (Global Positioning System) navigator. Structures such as lithologic contacts and structural trends were measured by aiding Brunton transit and checked with the Landsat images. The field data are plotted on Google Earth and UTM map linked with GPS data to illustrate the distribution of rock units. In the laboratory, more than (40) thin sections are observed to understand their constituent minerals and their texture. Major and minor oxides composition of rocks was analyzed by X-ray Fluorescence Method which was made at Mandalay University Research Centre.

Regional Geologic Setting

The Kachin State is one of the best places to search the trace for tectonic evolution of SE Asia. Geologically, it contains six main places from west to east; Jade Mine Uplift (JMU), Nanyaseik diopside-phlogopite-ruby marble, granite (Na), Kumon Range (KR), Katha-Gangaw Range (KGR), Tagaung-Myitkyina Belt (TMB) and Mogok Metamorphic Belt (MMB) (Mitchell

et al., 2007). Jade Mines Belt and Tagaung-Myitkyina Belt are parts of the Eastern Ophiolite Belt which was formed during the Middle Jurassic, i.e., ~166–176 Ma (Mitchell, 1993; Mitchell et al., 2007; Yang et al., 2012; Liu et al., 2016). In 2018, Zhang, et al. considered that the Myitkyina-Mogok ultramafic-diorite belt contains peridotite, andesite, hornblende gabbro, diorite, granodiorite and plagiogranite, all with arc geochemical signatures and ages of 177–166 Ma. Barley et al. (2003) mentioned the magmatic age of the protolith of orthogneisses from the Mandalay hill and Kyanikan was Jurassic (170 ± 1Ma) which were recrystallized during an Eocene (~43Ma) high-grade metamorphic event.

The study area, eastern part of the Kachin State, situates the northern continuation of TMB and MMB. In the west, TMB is close to the KGR. On regional scale, there is a large lineament which marked the western boundary of the KGR and is possibly one of the major splays of the Sagaing Fault. The study area extends to Yunnan (China) to the east. The main rock units exposed in the study area are undifferentiated metamorphic rocks, intrusive and extrusive igneous rocks. They are trending nearly N-S with eastern and northeastern dip. Sedimentary rocks comprised with minor amount. Regional geologic setting of Myanmar including the study area is shown in figure (2).

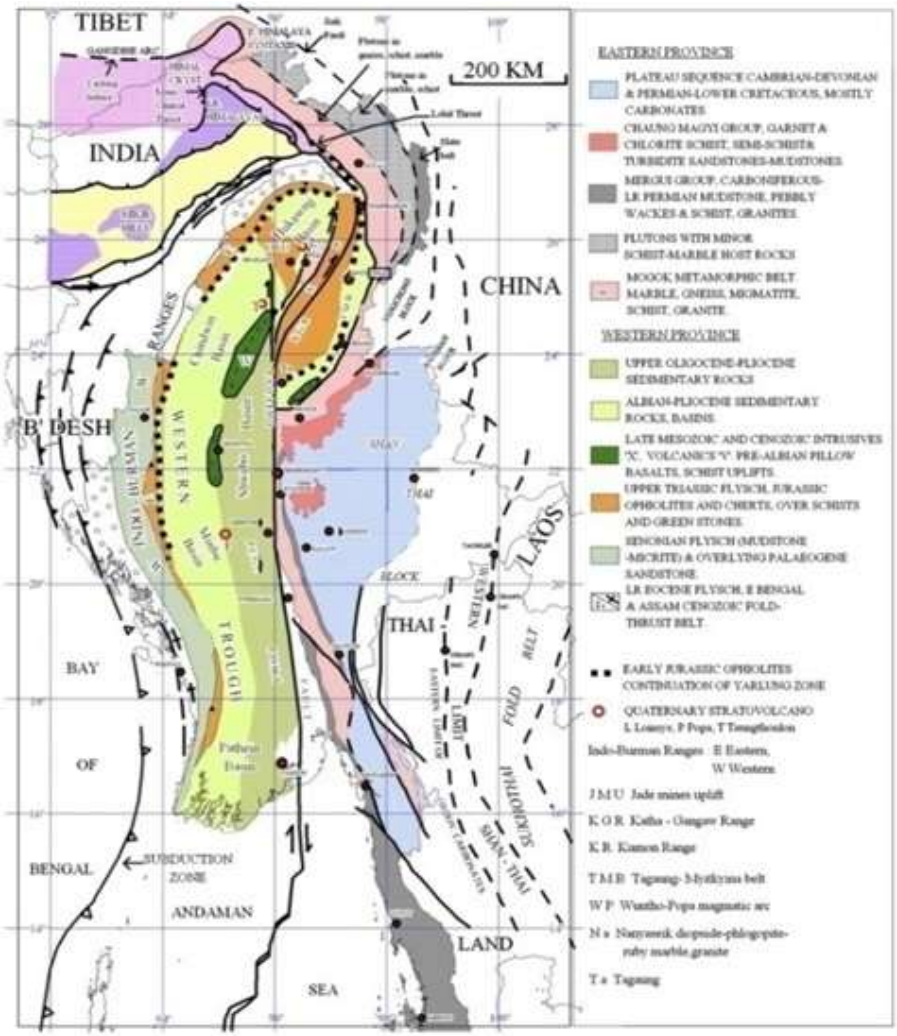


Figure 2. Regional geologic setting of Myanmar including the study area.
(***Source: Mitchell et al., 2007***)

Rock sequence

The rock units comprise within the study area are intrusive and extrusive igneous rocks, along with various metamorphic rocks. Although the rocks exposed are documented as the TMB and MMB, their ages and tectonic affinities are still controversial.

Based on the field data and previous authors' radiometric dating result, the age of metasedimentary rocks of the study area can be correlated to the Lower Paleozoic sedimentary sequence of the western Shan Plateau region and Jurassic for metaigneous. The igneous are estimated to be Mid Jurassic to Cretaceous in age. The rock sequence of the area is shown in table (1).

Table 1. Rock sequence of the study area

Lithologic Unit	Age
Sedimentary Units	
Alluvium	Quaternary
Siltstone and silty sandstone	Paleocene to Eocene
Igneous Units	
Pegmatite/Aplite	Early Cenozoic
Hornblende granite	Cretaceous
Granodiorite	
Diorite	
Dolerite	to
Lherzolite	
Pyroxenite	Mid. Jurassic
Metamorphic Units	
Meta igneous: Amphibolite	Jurassic
Meta igneous: Hornblende-biotite gneiss	
Meta sedimentary: Biotite gneiss	Lower Paleozoic
Migmatite	

Results on field data

The major rock types range from felsic to ultramafic rocks and metamorphic rocks. The igneous rocks comprise pegmatite/aplite, hornblende granite, granodiorite, diorite, dolerite, gabbro, lherzolite and pyroxenite. Metamorphic rocks contain hornblende-biotite gneiss, amphibolite, biotite gneiss and migmatite. The whole area except the road site is rather difficult to access because of dense forest, highly mountainous nature, and sparse population. Therefore, good exposure can be found along the road section and some stream section. The main ridges in the study area are Bum Taung, Bumkahtaung and Nga layin Taung Figure (3).

Most of the granitoids are intruded into metasedimentary rocks. The contact between diorite and granitoids is sharp, i.e., diorite and granitoids are cropped out separately along the road from Bum Taung to Inwant Kaung villages. Ultramafic and granitoids is faulted contact. Most of the metamorphic rocks, especially amphibolite is found along the La Na Hka Fault (F_3) and Sadon Chaung fault (F_4). The distribution map of rock units for the study area is shown in Figure (4).

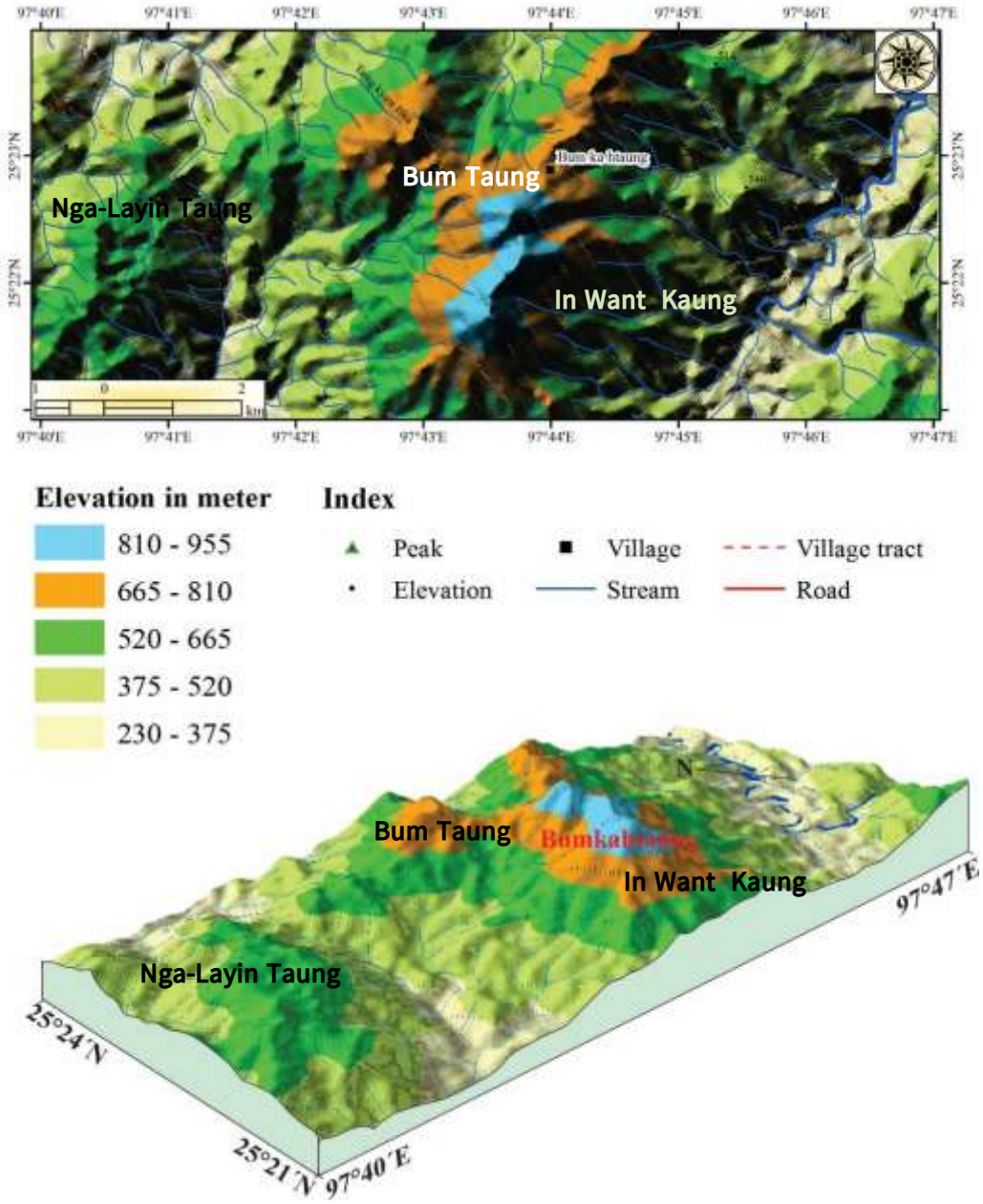


Figure 3. Three-dimensional image of ridges from the study area.

Features of Igneous Rocks

Good exposures of igneous rocks are found along the road site especially at the center and eastern parts of the study area.

Pegmatite occurs as veins and small dykes. Most of the veins are 1 inch to 10 inches in width and the main constituent minerals are quartz and feldspar. These veins are intruded into

metamorphic and all igneous units, indicating that these veins are the youngest unit of the study area.

Hornblende granite and granodiorite occupied two third of the whole area. They cropped out at the Wuyan village (N 25º 21' 36.1" and E 97º 47' 50.7"), Bum-Taung (N 25º 22' 54.8" and E 97º 44' 7.7"), In-want-Kaung (N 25º 21' 35.2" and E 97º 45' 27.1") and Sadon waterfall and around Sadon Village. They exhibit batholiths to small semi-circular bodies with exfoliation nature (Fig.5). They have complex texture, i.e., change from granitic to granodioritic composition within a batholith. They have fine to medium-grained, homogeneous texture and display light grey to whitish on fresh surface while dark grey color on weather surface Figure (6). Various sizes of mafic microgranular enclaves are found in some places and their sizes ranges from 2cm to 3ft that are located at the core and margin of pluton and are generally in sharp contact with the surrounding granitoids Figure (7). Hornblende granite composed essentially of quartz, feldspar, hornblende, and biotite. Granodiorite has more mafic minerals and plagioclase feldspar than granite. Xenolith, which are biotite gneiss, are also found in granitoid rocks that indicates granitoids are younger than metamorphic rocks.

Sporadic occurrence of medium to coarse grained texture and greenish grey colour **diorite** is found at N 25º 23' 47.7"; E 97º 37' 14.9", and N 25º 22' 28"; E 97º 44' 10". It is composed essentially of plagioclase and hornblende with biotite. It highly develops joint Figure (8) and extents is about 80 ft in width and 40 ft in thickness. Xenoliths contained in diorite is as same mineral composition as the host diorite, but the texture is different.

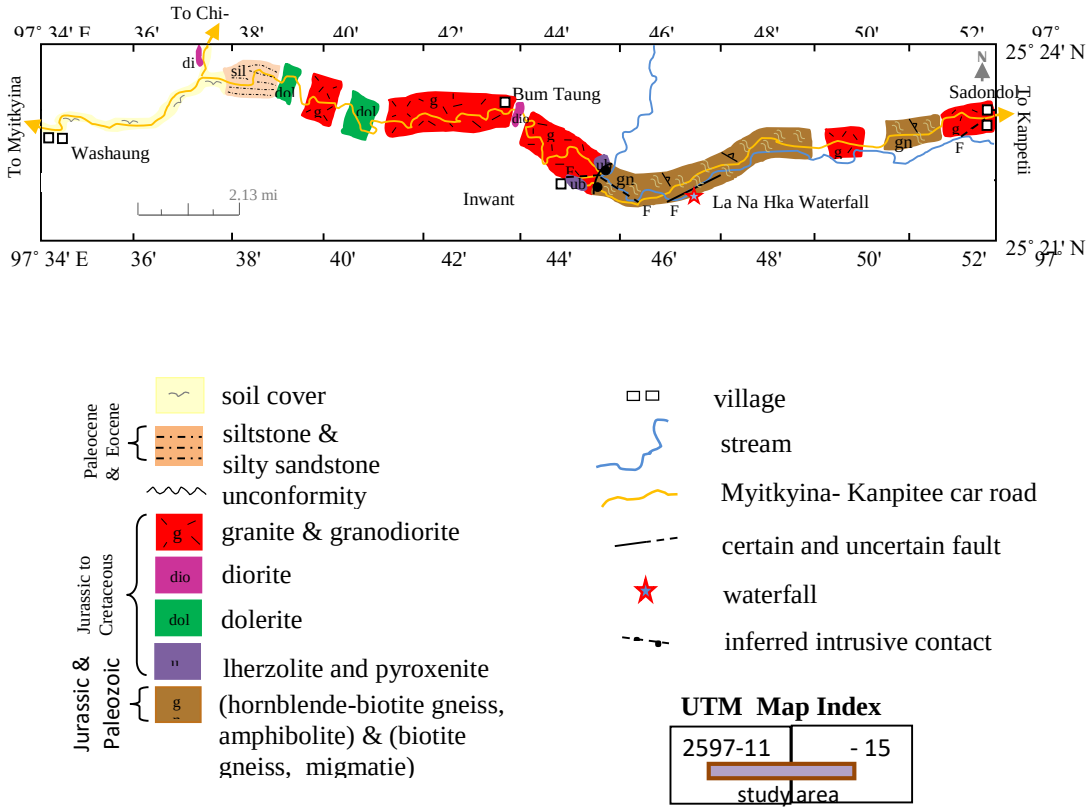


Figure 4. Distribution map for igneous and metamorphic rocks of the study area.
(Modified after Me Me Aung et al., 2019a)

Good exposure of **dolerite dyke** is exposed at N 25º 22' 37.3"; E 97º 40' 59.2", N 25º 23' 30.3"; E 97º 39' 6.4" and N 25º 22' 48.8"; E 97º 41' 34.1". It has very fine to fine-grained, hard, and compact nature and bluish dark to dark grey colour on weathered surface Figure (9). They exhibit massive boulders with highly jointed nature. Some small outcrops are 4.6m (15ft) in width and NW-SE in trend.

Lherzolite and pyroxenite are limitedly cropped out near the Inwang Kaung Bridge especially at N 25° 21' 51.5"; E 97°45' 31.8", N 25° 21' 28.8"; E 97°45' 25" and N25° 21' 51"; E 97°45' 32". They have coarse-grained texture and are composed mainly of olivine and pyroxene. They occur as rather complex nature, i.e., lherzolite to pyroxenite, within a same boulder Figure (10). They possess hard and compact nature and display as sheeted dyke. Most of the weather surface has been at least partly altered to serpentinite, a process in which pyroxene and olivine are converted to green serpentine minerals. These rocks are faulted contact with granitoids.

Figure 5. Exfoliation nature in hornblende granite.

(Loc. N 25º 22' 54.7"; E 97º 52' 54.3")

Figure 6. Dark grey color wea-ther surface in ranodiorite.

(Loc. N 25º 21' 35.2"; E 97º 45' 27.1")

Figure 7. Oval shape with chill margin mafic microgranular enclaves in granite.

(Loc. N 21' 35.8"; E 97º 45' 30.1'")

Figure 8. Highly jointed diorite.

(Loc. N 25º 22' 28";E 97º 44' 10.1")

Figure 9. Hard and compact nature of dolerite.

(Loc. N 25º 21' 28.8"; E 97º45' 25")

Figure 10 Small sheeted outcrop nature of lherzolite and pyroxenite.

(Loc. N 25º 21' 51"; E 97º45' 32")

Features of Metamorphic Rocks

Hornblende-biotite gneiss is exposed at N 25° 21' 19.5"; E 97°47' 9.4" and N 25° 21' 29"; E 97° 46' 44.3". It has foliated medium- to coarse-grained texture and is composed of quartz, feldspar, hornblende, and biotite. It displays hard, compact, and massive to boulder nature Figure (11). Augen texture, size ranges from 0.3 mm to 2 cm in diameter, is well recognized and it suggests

metamorphism and shearing. The contact relation between this unit and other metamorphic units is rather complex. **Amphibolite** is especially found along the Sadon Chaung fault (F_4) near the Sadon primary school. It has a highly jointed nature, fine to medium-grained texture and shows dark green to black on fresh surface. Some outcrops show rib-and-furrow nature and karst nature, i.e., pit hole and grooves on weather surface Figure(12). It is chiefly composed of amphibole minerals, plagioclase, quartz, and other accessory minerals.

Biotite gneiss unit is especially cropped out around the Sadon waterfall. It shows a well foliated, medium-grained texture and is composed of quartz, feldspar and biotite. It has medium to thick bedded nature that suggests sedimentary origin Figure (13). **Migmatite** unit is limitedly found at the La Na Hka waterfall at N25° 21' 36.1"; E 97°47' 50.7" and N25° 21' 29"; E 97°46' 44.4". It possesses medium- to coarse-grained texture and composed essentially of quartz, feldspar, hornblende, and biotite. It displays a mixture of well foliated gneiss components and igneous components within a massive. Ptygmatic folds and veins are well recognized on the weather surface. In some places, amphibolite, and biotite rich components (melanosome) intercalated with leucosome Figure (14). Biotite gneiss and migmatite display gradational contact and migmatite occupy lower rock sequence. All of the above facts suggest sedimentary protolith.

Figure 11. Massive Boulder and jointed nature orthogneiss.

(Loc. N 25º 21' 35.2"; E 97º 45' 27.1")

Figure 12. Rib-and-furrow nature on weather surface of amphibolite.

(Loc. N 25º 22' 35"; E 97º 53' 47.1")

Figure 13. Well foliated texture in biotite gneiss. (Loc. N25° 21' 19.5"; E 97°47' 9.4")

Figure 14. Component of melanosome and leucosome in migmatite.

(Loc. N 25° 21' 29"; E 97° 47' 44.3")

Petrogenesis of igneous rocks

The petrogenesis of igneous rocks is deciphered based on field data, microscopic features, geochemical results, and previous published literature. The major and trace element oxides are in table (2).

Field Criteria

1. Along the Myitkyina-Kanpitee road section, from Washaung to Sadon village, hornblende granite and granodiorite comprise **40 to 60 %**, diorite **1 to 5%**, dolerite **5 to 15%** and lherzolite and pyroxenite **1 to 5%** Figure (4). Metaigneous rocks especially amphibolite is occupied **1 to 5%** of the overall abundance. Rhyolite, dacite and gabbro are cropped out at the northeastern part of the area. The composition ranges from **basalt to rhyolite** in volcanic is corresponding by the **peridotite-gabbro-diorite-granodiorite-granite** plutonic sequence. These rock assemblages suggest continental arc, which would not be component of ophiolite. Therefore, the igneous rocks from the study area may be formed by plumbing system feeding the continental arc volcanic.

2. Mafic microgranular enclaves are found in hornblende granite, granodiorite, and diorite. They indicate the introduction of mafic magma into the magma chamber and its subsequent cooling following incomplete mixing.
3. Some enclave displays chill margin which suggests large temperature difference from the host granite. Some shows zone nature that indicates the trace of difference composition and difference cooling rate of enclaves. All these facts suggest that these granitoids rocks and enclaves are a product of partial melting and fractional crystallization of basic magma, and enclaves are trapped blobs of basic initial magma.
4. Mostly discordant and some concordant structural relations to the country rocks.
5. Temperature of country rock, metamorphic rocks, from the study area may be greater than 450˚C, i.e., the country rock belongs to amphibolite facies.

Microscopic Features

1. Under the microscope, hornblende is common mafic minerals in granite, granodiorite, and diorite. Pyroxene and olivine constitute pyroxenite and peridotite. (Figures. 15,16,17,18,19)
2. Dolerite exhibits ophitic texture that indicates the minerals crystallized out of the magma to in a certain order: first plagioclase, then pyroxene. (Figure. 20)
3. Cross-hatched twins are frequent in alkali feldspar. Zoning and twinning are common in plagioclase. (Figures. 21, 22)

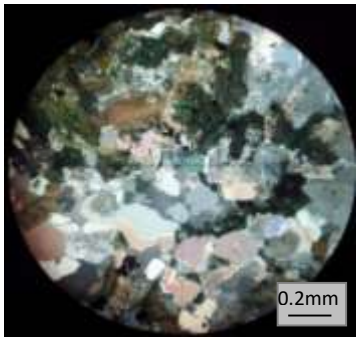


Figure 15. Orthoclase, quartz and hornblende in horn-blende granite. (X.N)

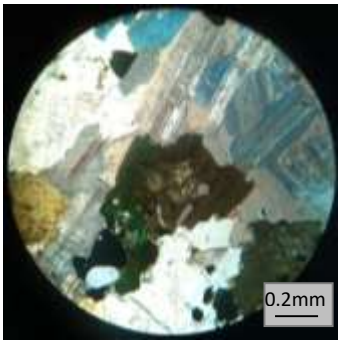


Figure 16. Polysynthetic twin-ing in oligoclase of the granodiorite. (XN)

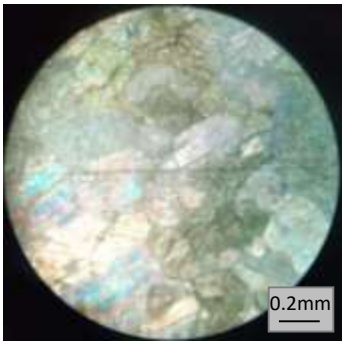


Figure 17. Hornblende in diorite. (X.N)

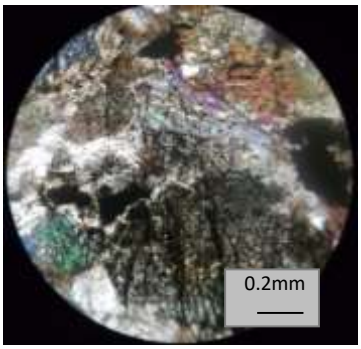


Figure 18. Orthopyroxene and clinopyroxene minerals in pyroxrnite. (X.N)

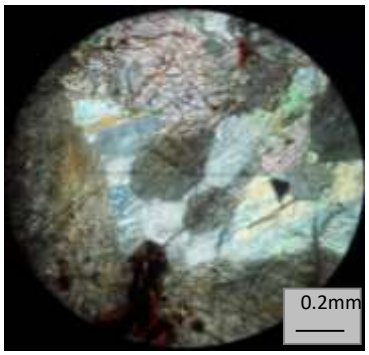


Figure 19. Olivine (Olv), diopside (Di) and enstatite (En) minerals in lherzolite. (X.N)

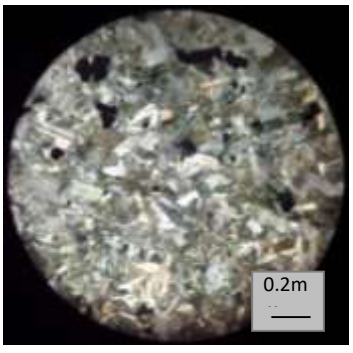


Figure 20. Ophitic texture in dolerite. (X.N)

According to the previous published literature and petrographic criteria, the granitic rocks from the study area are dominated by I-type granites rather than S-type granites. The estimated depth of emplacement may be epizone to mesozone. In addition, mafic enclaves in granites suggest the process of partial melting and fractional crystallization of basaltic magma.

Geochemical Results

From the petrochemical interpretation, the chemical description in most common use is based on silica percentage. For the study area SiO_2 content ranges from 65.7 to 67.3 % in granite, 67.3 % in diorite and 47.2% in dolerite. Based on the above criterion, the magma responsible for the igneous rocks of the study area possess acidic to basic in composition.

Concerning the mineral chemistry, the igneous rocks of the study area have the mole percent alumina is greater than the sum of lime, soda, and potash ($Al_2O3 > CaO+Na_2O+K_2O$). According to the Shand'classification (1949), these rocks belong to peraluminous suite.

In AFM diagram Figure (23), all the igneous rocks fall in the calc-alkaline field. In addition, the later stage of magmatic evolution trend showing an increase in K_2O and Na_2O with depletion of MgO.

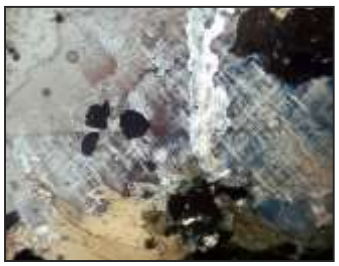


Figure 21. Cross-hatched twin in microcline of hornblende granite. (X.N)

Figure 22. Zoning in albite of hornblende granite. (X.N)

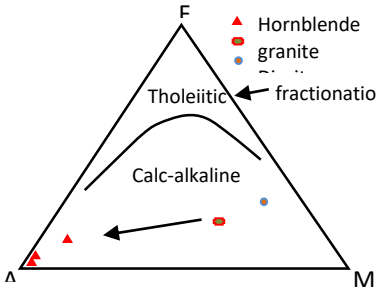
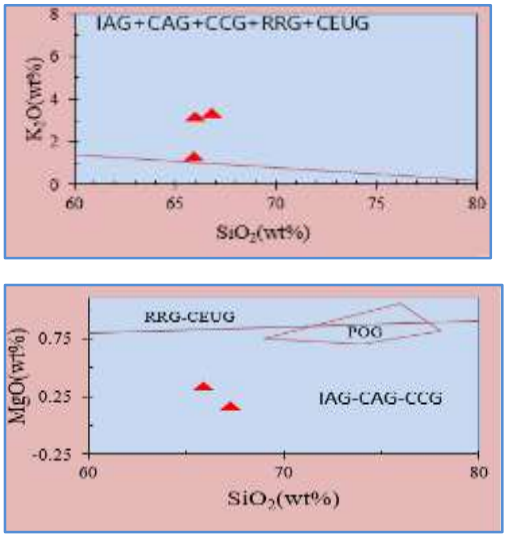


Figure 23. $FeO_{(t)}$ (Na_2O+K_2O) –MgO, AFM diagram.

Discrimination of the tectonic environment for the granites of the study area has been based on the work of Maniar and Piccoli (1989). K_2O versus SiO_2 diagram discriminates the IAG+CAG+CCG+ RRG+ CEUG and OP fields. All the granitic rocks from the study area fall within the IAG+CAG+CCG+ RRG+ CEUG field (Fig.24a). In the MgO versus SiO_2 variation diagram Figure (24b), all granitic rocks fall in the IAG+CAG+CCG field too. According to the above criterion, it can be resolved that the granitic rocks from the study area fall in the IAG+CAG+CCG field which indicates the orogenic granitoids. Hence it seems reasonable that the granite was formed in the continent in relation to the subduction of an oceanic plate beneath the continent.



Orogenic Granitoid
- POG - Post Orogenic Granitoid
- IAG - Island Arc Ganitoid
- CAG - Continental Arc Granitoid
- CCG - Continental Collision Granitoid

Anorogenic Granitoid
- OP - Oceanic Plagiogranite
- CEUG - Continental Epiorogenic Uplift Granitoid
- RRG - Rift-Related Granitoid

Figure 24. (a) K_2O versus SiO_2 diagram for granitic rocks of the study area.
(b) K_2O versus SiO_2 diagram for granitic rocks of the study area.
(based on Maniar and Piccoli, 1989)

Zhang, J.E. et al., 2018 explained the Myitkyina-Mogok ultramafic-diorite belt, located along the eastern margin of the Myanmar Central Basin that was associated with the continental sliver, contains peridotite, andesite, hornblende gabbro, diorite, granodiorite and plagiogranite, all with arc geochemical signatures and ages of 177–166 Ma. Moreover, he mentioned their outcrops andesite, hornblende gabbro and diorite along the Myitkyina belt, rock assemblages of continental margin, which would not be component of ophiolite. According to the field relationships and microscopic features, his model is more appropriate for the study area.

According to the above criteria, the igneous rocks from the study area may be formed as continental arc which related to subduction of Neotethys Ocean during the Mesozoic and Early Cenozoic time. The magma source may relate to the partial melting of subduction oceanic slab generates primary magma and then at the base of the crust where assimilation and the solidification of a gabboric crustal underplate process may take place. Subsequent partial melting of this mafic underplate may result from heat carried upward by subsequent basaltic magma. Fractional crystallization and contamination of continental crust materials occurred when it passed through the crust. Some granite and pegmatite were probably formed by partial melting of lower or middle crust.

Table 2. Chemical composition of igneous rocks from the study area. (in wt %)

Rock Types	**Hornblende Granite**			**Diorite**	**Dolerite**
Sample. ID	**H-8**	**T-11**	**S-4**	**W-10**	**T-29**
SiO_2	65.70	67.3	66.00	54.80	47.20
Al_2O_3	19.70	21.1	21.6	19.00	22.60
Na_2O	9.75	6.90	7.20	2.40	7.51
K_2O	0.55	3.31	3.10	0.06	0.09

Rock Types	**Hornblende Granite**			**Diorite**	**Dolerite**
Sample. ID	**H-8**	**T-11**	**S-4**	**W-10**	**T-29**
CaO	1.79	0.72	0.94	8.97	5.81
MgO	0.93	0.21	0.34	9.79	11.7
Fe_2O_3	1.38	0.24	0.55	3.75	4.23
MnO	0.04	0.01	0.02	0.05	0.08
P_2O_5	0.07	0.02	0.04	0.65	0.06
Cr_2O_3	0.00	0.00	0.00	0.00	0.05
SrO	0.004	0.02	0.02	0.01	0.004
TiO_2	0.13	0.04	0.07	0.46	0.24
Total	**100.04**	**99.87**	**99.88**	**99.94**	**99.57**

Summary and conclusion

The study area mainly comprises intrusive and extrusive igneous and metamorphic rocks created at differing geological episodes. The igneous rocks include pegmatite/aplite, hornblende granite, granodiorite, diorite, dolerite, lherzolite and pyroxenite. Metamorphic rocks are metaigneous rocks (amphibolite and hornblende-biotite gneiss) and metasedimentary rocks (biotite gneiss and migmatite). The Kachin State is one important segment to trace tectonic evolution of Tethys Ocean in SE Asia. Field data, microscopic features and previous published literature indicate that the igneous rock assemblages point out continental arc, which would not be component of ophiolite. The igneous rocks of the study area may be formed by the partial melting of subduction oceanic plate which generates primary magma first. And then, it would be contaminated by the continental crust materials when it travels through the crust. Some granite and pegmatite probably formed by partial melting of lower or middle crust. Therefore, they may be formed as continental arc which related to subduction of Neotethys Ocean during the Mesozoic and Early Cenozoic time. The full age range of the rocks is unknown, but intrusive activity probably extended from the Jurassic into the early Cenozoic.

In the present study, we described petrogenesis of rocks based on field relationship combined with microscopic features, but many data need to support to solve the tectonic evolution of Tethys Ocean in SE Asia. Therefore, many researchers should make researches respect to geochemistry and petrology to get the reliable tectonic history.

Acknowledgement

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