STUDY ON PALYNOLOGICAL, NUTRITIONAL VALUES AND ANTIMICROBIAL ACTIVITY OF BEE POLLEN FROM DIVISION OF APICULTURE, NAYPYITAW

Win Theingi Aung[1], Nay Zin Myint[2], Myat Myat Moe[3]

Abstract

Bee pollens of kapok and sunflower field sites were collected from Division of Apiculture, Naypyitaw during June-February, 2017. Bee pollens come from the pollen baskets of returning foragers of bee were gathered by bee house. Bee pollen grains were examined by light microscope and photomicrographs. The characters of each pollen grain were identified and classified by using literatures. Pollen grains of 12 species belonging to 10 genera of 8 families were found in the kapok and sunflower field sites. They are *Albizia*, *Mimosa*, *Ceiba*,*Celosia*, *Physalis* and *Vernonia*. And then, the pollen species of sunflower field sites were obtained *Albizia*, *Brassica*, *Richardsonia*, *Ocimum*, *Helianthus* and *Vernonia*. Among them, *Vernonia* pollen grains are found in both two sites. The pollen grains are monads, tetrads and polyads. Tetrad grain is observed in only one species *Mimosa*; polyads as in 2 species of *Albizia* and the remaining of 9 species are monads. The aperture type, number, position, size, sculptures of each grain were presented and varied. There are colpate, colporate and porate. The aperture types are 3 species of colpate (*Brassica*, *Richardsonia* and *Ocimum*), 3 species of colporate (*Ceiba*, *Physalis* and *Helianthus*) and 3 species of porate (*Mimosa*, *Celosia, Vernonia* and 2 species of *Albizia*). The smallest pollen (15.5 – 17.5 × 16.5 – 18.0 μm) is *Mimosa pudica* L. and the largest pollen (43.8 – 102.5 × 75.5 – 120.0 μm) is *Albizia saman* (Jacq.) Merr. The nutritional values were carried out at Department of Research and Innovation Analysis Department. According to the analysis of D.R.I, nutritional values of bee pollens were found carbohydrate (sugar) as major constituents and followed by protein. And then, antimicrobial activity was performed in the microbiological laboratory of Dagon University. The ethanolic and methanolic extracts of bee pollen had provided the best clear zone against *Escherichia coli* and *Pseudomonas aeruginosa* in microbial activity.

Key words: Pollen morphology, Bee plants, SuperFood and Medicine

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Introduction

The relationship between bee, flowers and man is one of the wonders of the universe, being a living proof that the flora, fauna and man to live in harmony. Bees need flowers to feed themselves, plants need bees to be pollinated and to produce seed to ensure the perpetuation of plant species.

Palynology is the science of studying pollen collected from air, water, sediment deposits and not least the study of bee pollen. Palynology is an interdisciplinary science, biological sciences and particularly botany (Bhojwanii & Bhatnagar, 2005).

Pollen is a fine powder-like material of male reproductive cells. Pollen grains can be distinguished by different shape and color, by different content in nutrients, vitamins and biologically active substances of each plant (Bogdanov, 2012).

Bee pollen is a collection of pollen grains from various botanical sources, bees collected on the tibia of their hind legs by mix with nectar and secretion from the hypopharyngeal glands such as β-glycosidase enzymes (Carpes *et al*., 2009).

Bee pollen are natural raw materials include pollen and nectar. Pollen is the bee's major source of proteins. Nectar is a bee's source of carbohydrates. Nectar provides carbohydrates and pollen supplies the remaining dietary requirements such as protein, lipids, vitamins and minerals (Campos *et al*., 2008).

Bee pollen has also been used for many centuries in traditional medicine and supplementary nutrients, primarily because bee pollen has complete food and health benefits (Cheng *et al*., 2013).

Antimicrobial properties can protect the human body against both cellular oxidation reactions and pathogens (Senguel *et al*., 2009). In addition, bee pollen has antimicrobial effects (Haas, 1992).

The aims and objectives of this research work are to study pollen morphology for identifying the various pollen types of bee pollen present in field sites, to provide information that natural energizer can be obtained by taking bee pollen and to investigate antimicrobial activity in order to know what types of bacteria can be killed by taking bee pollen.

Materials and Methods

The research was conducted on two samples of bee pollen (Figure 1) harvested from different sites in Division of Apiculture, Pyinmana Township, Naypyitaw. The bee pollen specimens were kapok and sunflower field sites. These samples were obtained directly beekeepers in June-February, 2017 and were collected by using pollen collectors.

Pollen collection by bees

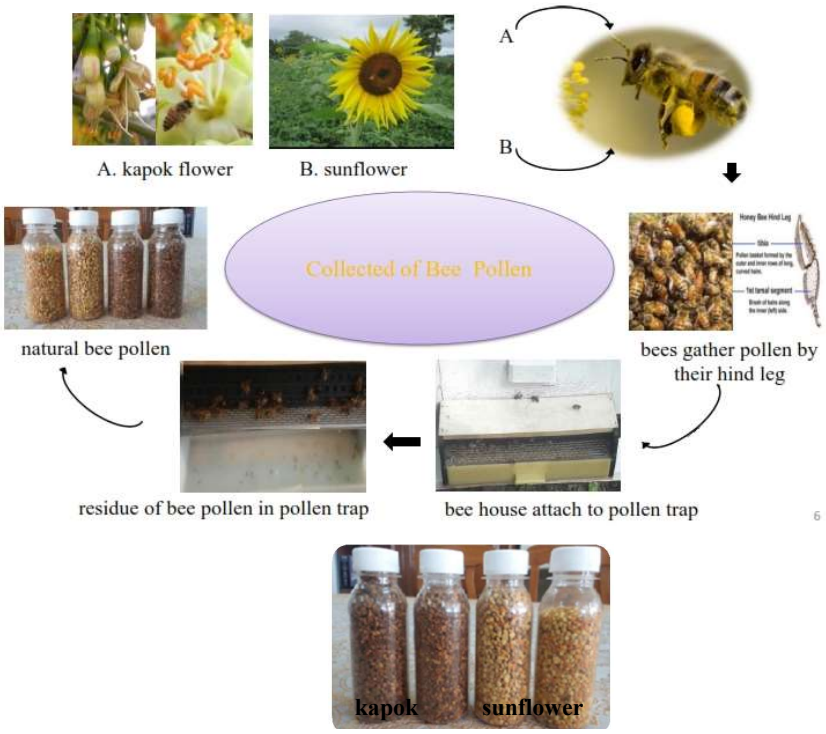
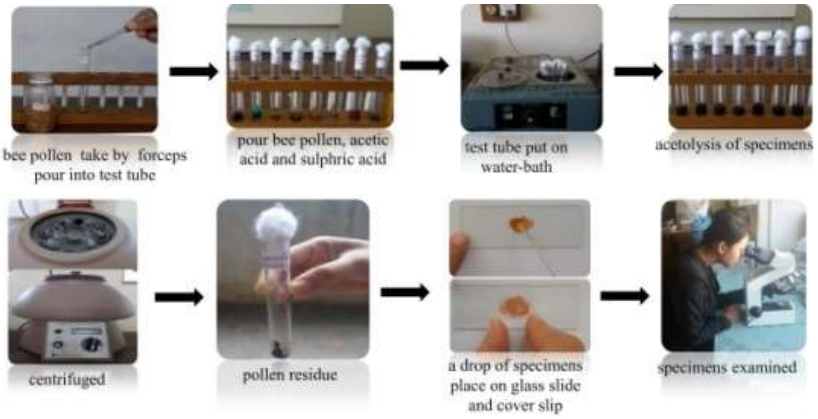


Figure 1. kapok and sunflower bee pollen

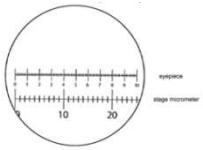
For studies of bee pollen morphology, acetolysis of pollen grains (Erdtman, 1952) and glycerine jelly of acetolysis pollen grains by Kisser's formula (Erdtman, 1952) were prepared. Pollens counting method were used by using ocular microscope grains (Brookes and Thomas, 1967).

Acetolysis of pollen grains (Erdtman, 1952) for studies of pollen morphology



Glycerine jelly of acetolysis pollen grains by Kisser's formula (Erdtman, 1952)

Pollen counting method by using ocular the microscope



Ocular

$$\text{Percentage of particular pollen grains} = \frac{\text{No. of particular pollen type}}{\text{Total No. of trapped pollen grains}} \times 100$$

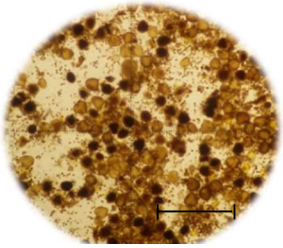
In nurtitional values, the analysis of bee pollens (kapok and sunflower) were determined by using A.O.A.C (Horwitz, 1997) method carried out at Department of Research and Innovation Analysis Department (D.R.I).

As antimicrobial activities, six solvent extracts of kapok and sunflower bee pollens were tested on seven pathogenic microorganisms by using paper disc diffusion method described by (Cruickshank, 1968).

Results

Pollen morphology of two samples of bee pollen (kapok and sunflower) has been studied from Division of Apiculture at Pyinmana Township, Naypyitaw. In pollen morphology, 12 species that belong to 10 genera of 8 families have been observed. Distribution of bee pollen sample in kapok field site is presented in Figure 2; Table 1 and Figure 3; Table 3 is sunflower field site. And then, the families of pollen morphology in the samples are listed according to APG III (2009) system and the genus according to alphabetical order as follow in Tables 1 and 3. Finally, the nutritional values of two bee pollens were included (Table 5 and Figure 7) and antimicrobial activity of diameter of each solvent extracts of two bee pollens were tested in Table 6 and Figure 9.

Microscopical images of kapok bee pollen field sites



Outline of pollen morphology (X10, Scale bar = 10 μ)

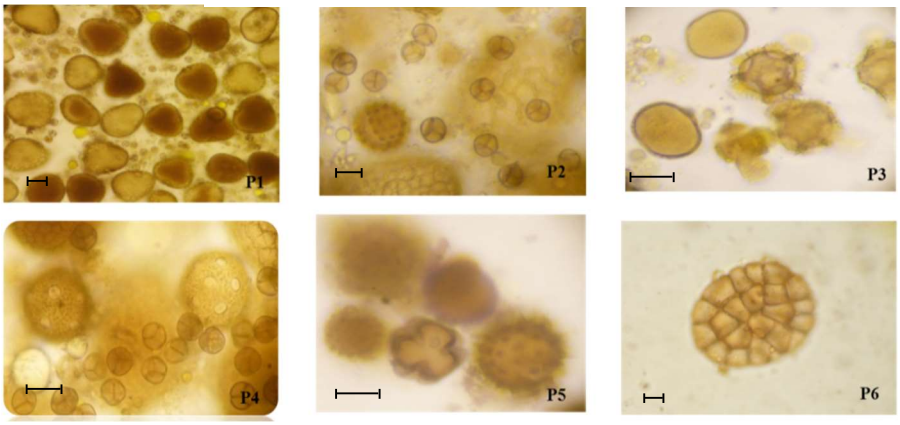
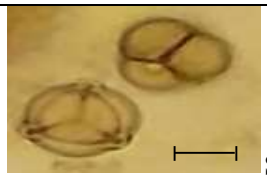
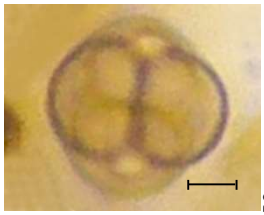


Figure 2. Pollen morphology of bee pollen P1-P6 in kapok bee pollen field sites

Table 1. Pollen type and its morphology of bee pollen in kapok field sites

Scale bar = 10 µ

No.	Pollen type	Plant name; Family; Myanmar name; English name and Pollen Morphology
P6.	 Surface view	-*Albizia saman* (Jacq.)Merr. -Fabaceae (Mimosoideae) -Myanmarkokko -Rain tree -Polyad, more than 16-celled, very large, 43.8 – 102.5 x 75.5 – 120.0 µm in length and breadth; amb subglobose; each grain triporate, very small, 2.2 x 1.5 – 2.5 µm in length and breadth; exine 1.5 – 1.8 µm thick, sexine as thicker as nexine; sculpturing faintly reticulate.
P2.	 Surface view  Surface view	-*Mimosa pudica* L. -Fabaceae (Mimosoideae) -Htikayon -Senistive plant -Tetrad, tetragonal, very small, 15.5 – 17.5 x 16.5 – 18.0 µm in length and breadth; amb rounded; each grain triporate, very small, 10.0 – 12.5 x 11.5 – 14.0 µm in length and breadth; exine 1.0 – 1.5 µm thick, sexine thicker than nexine; sculpturing psilate.

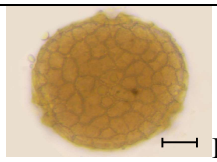
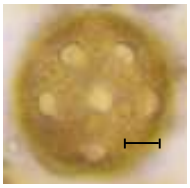
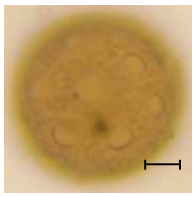
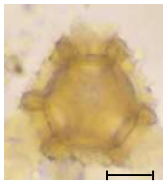
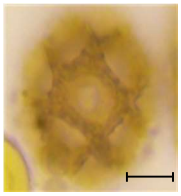
No.	Pollen type	Plant name; Family; Myanmar name; English name and Pollen Morphology
P1.	 Polar view  Equatorial view	-*Ceiba pentandra* (L.) Gaertn. -Malvaceae (Bombacoideae) -Thinbawletpan -Kapok -Tricolporate, oblate spheroidal, large, 39.5 – 41.5 x 52.5 – 55.0 µm in length and breadth; amb rounded; colpi longicolpate; pori lalongate; exine 1.5 – 2.0 µm thick, sexine thicker than nexine; sculpturing retipilate.
P4.	  Surface view	-*Celosia argentea*L. -Amaranthaceae -Tawkyetmauk -Wildcock's comb -Polyporate (about 10), spheroidal, medium,26.5–30.5µm in diameter; amb circular; pori circular; exine 1.5 – 2.0 µm thick, sexine thicker than nexine; sulpturing reticulate.
P5.	 Polar view  Equatorial view	-*Physalis minima*L. -Solanaceae -Baungpan -Wild gooseberry -Tricolporate, subprolate, medium, 23.5 – 26.2 x 22.5 – 25.0 µm in length and breadth; amb rounded triangular; colpi longicolpate; pori lalongate; exine 1.3 – 1.8 µm thick, sexine thicker than nexine; sculpturing reticulate.
P3.	  Surface view	-*Vernonia cinerea* (L.) Lees. -Asteraceae -Kadupyan -Purple ironweed -Triporate, spheroidal, medium, 21.5 – 29.5 µm in diameter; amb rounded; pori circular; exine 2.5 – 5.5 µm thick, sexine thicker than nexine; sculpturing echinolophate (lophoreticulate).

Table 2. Distribution of pollen morphology in Kapok bee pollen field sites

Code No.	Kapok field sites	Total No. of grains counted	Percentage
P1.	*Ceiba pentandra* (L.) Gaertn.	495	55.5%
P2.	*Mimosa pudica* L.	251	28.1%
P3.	*Vernonia cinerea* (L.) Lees.	80	9.0%
P4.	*Celosia argentea* L.	32	3.6%
P5.	*Physalis minima* L.	26	2.9%
P6.	*Albizia saman* (Jacq.) Merr.	8	0.9%

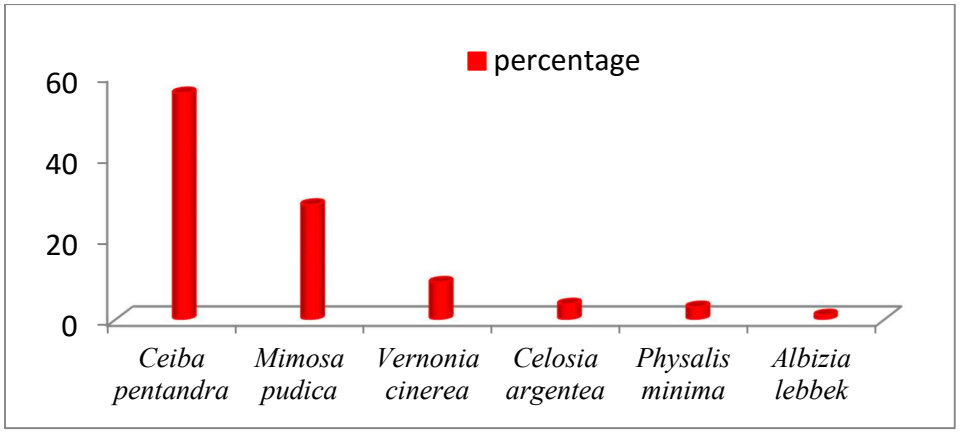
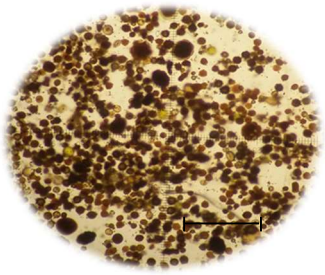


Figure 3. Distribution of pollen morphology in Kapok bee pollen field sites

Microscopical images of sunflower bee pollen field sites



Outline of pollen morphology (X10, Scale bar = 10 μ)

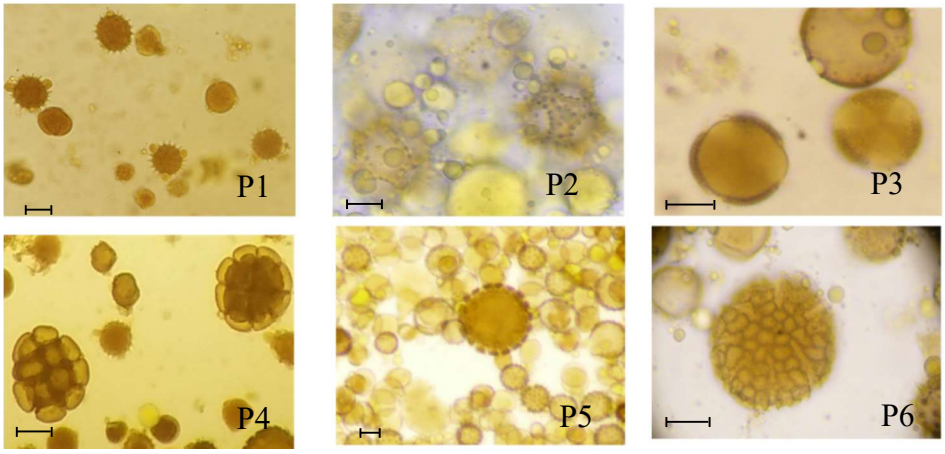
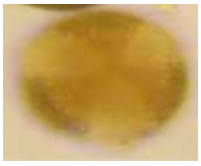
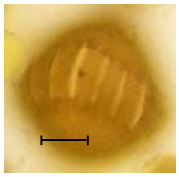
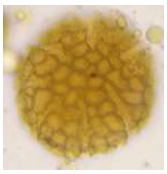


Figure 4. Pollen morphology of bee pollen P1-P6 in sunflower bee pollen field sites

Table 3. Pollen type and its morphology in bee pollen in sunflower field sites

Scale bar = 10 µ

No.	**Pollen type**	**Plant name; Family; Myanmar name;English name and Pollen Morphology**
P4.	 Polar view	-*Albizia lebbek* Benth. -Fabaceae (Mimosoideae) -Anyakokko -Sristree -Polyad, 16-celled, medium, 28.5 – 32.5 x 30.0 – 35.0 µm in length and breadth; amb subglobose; each grain triporate, small, 10.0 – 12.5 x 11.0 –13.0 µm in length and breadth; exine 1.5 – 1.8 µm thick, sexine thicker than nexine; sculpturing reticulate.

No.	Pollen type	Plant name; Family; Myanmar name;English name and Pollen Morphology
P3.	 Polar view  Equatorial view	-*Brassica campestris* L. -Brassicaceae -Monnyin -Mustard -Tricolpate, oblate spheroidal, small, 21.5 – 22.5 x 23.0 – 25.0 µm in length and breadth; amb rounded; colpi longicolpate; exine 1.3 – 2.0 µm thick, sexine thicker than nexine; sculpturing reticulate.
P5.	 Polar view  Equatorial view	-*Richardsonia brasiliensis* Gomes. -Rubiaceae -Notknown -Mexican clover -Polycolpate (about 18), oblate, large, 55.0 – 60.0 x 70.0 – 85.0 µm in length and breadth; amb circular; colpi brevicolpate; exine 2.3 – 3.0 µm thick, sexine thicker than nexine; sculpturing reticulate.
P6.	 Polar view  Equatorial view	-*Ocimum americanum* L. -Lamiaceae -Pinseinyaing -Wild ocimum -Hexacolpate, prolate spheroidal, medium, 32.5 – 42.5 x 30.0 – 37.5 µm in length and breadth; amb rounded; colpi longicolpate; exine 1.7 – 2.3 µm thick, sexine thicker than nexine; sculpturing reticulate.

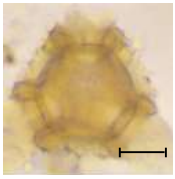
No.	Pollen type	Plant name; Family; Myanmar name;English name and Pollen Morphology
P1.	 Polar view  Equatorial view	-*Helianthus annuus* L. -Asteraceae -Neykyapan -Sunflower -Tricolporate, oblate spheroidal, small, 17.5 – 22.5 x 18.0– 23.0 µm in length and breadth; amb rounded; colpi brevicolpate; pori lalongate; exine 2.0–2.5 µm thick, sexine thicker than nexine; sculpturing echinate.
P2.	  Surface view	-*Vernonia cinerea*(L.) Lees. -Asteraceae -Kadupyan -Purple ironweed -Triporate, spheroidal, medium, 22.5 – 30.0 µm in diameter; amb rounded; pori circular; exine 2.5 – 5.5 µm thick, sexine thicker than nexine; sculpturing echinolophate (lophoreticulate).

Table 4. Distribution of pollen morphology in sunflower bee pollen field sites

Code No.	Sunflower field sites	Total No. of grains counted	Percentage
P1.	*Helianthus annuus* L.	630	49.9%
P2.	*Vernonia cinerea* (L.) Lees.	414	32.8%
P3.	*Brassica campestris* L.	96	7.6%
P4.	*Albizia lebbek* Benth.	63	5.0%
P5.	*Richardsonia brasiliensis* Gomes.	53	4.2%
P6.	*Ocimum americanum* L.	6	0.5%

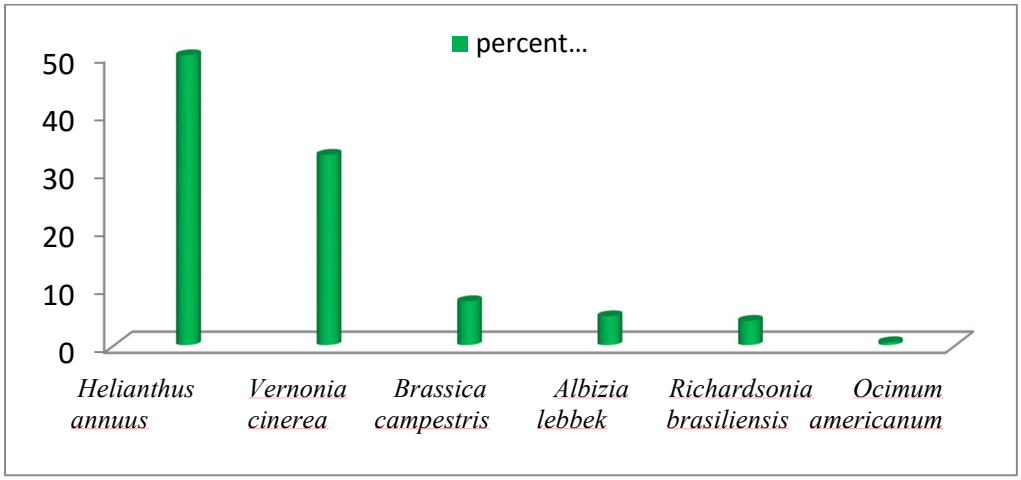


Figure 5. Distribution of pollen morphology in sunflower bee pollen field sites

The estimation of nutritional values of two bee pollen samples (kapok; sunflower)

The estimation of data was shown in Table 5 and Figure 7. The nutritional values of A.O.A.C methods contained carbohydrate, sugar, protein and fat for food and medicine.

Table 5. Nutritional values of two bee pollen samples

No.	Types of elements	Concentration (%)	
		Sunflower field site	Kapok field site
5.	Carbohydrate	41.71	45.95
6.	Sugar	37.70	28.04
1.	Protein	16.40	23.29
4.	Fat	2.55	4.02

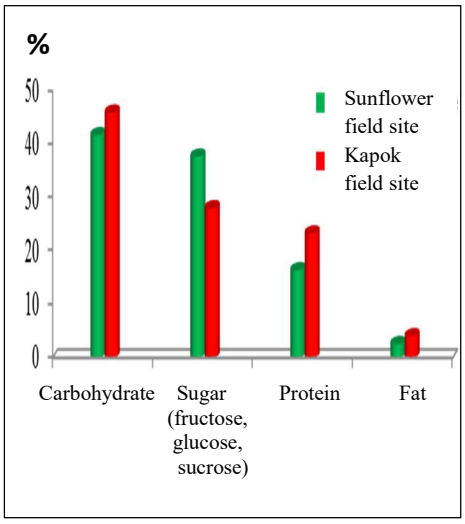
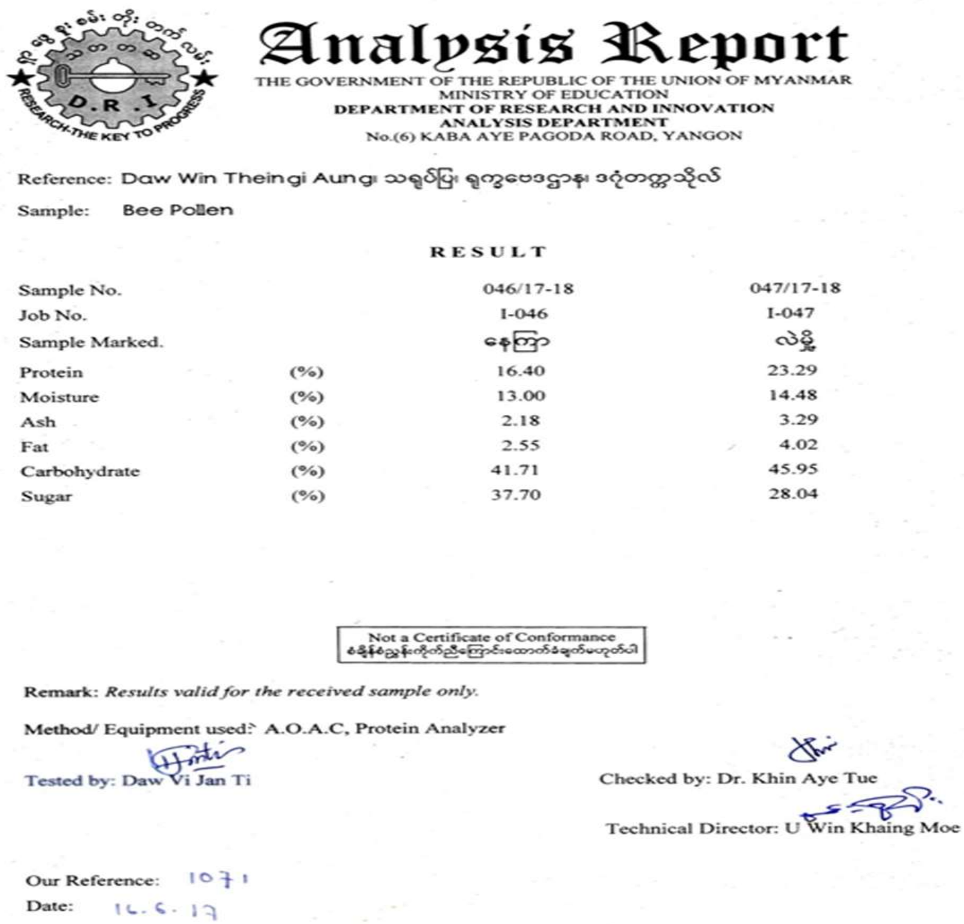


Figure 6. Nutritional values of two bee pollen samples



Analysis Report

THE GOVERNMENT OF THE REPUBLIC OF THE UNION OF MYANMAR
MINISTRY OF EDUCATION
DEPARTMENT OF RESEARCH AND INNOVATION
ANALYSIS DEPARTMENT
No.(6) KABA AYE PAGODA ROAD, YANGON

Reference: Daw Win Theingi Aung၊ သရုပ်ပြ၊ ရုက္ခဗေဒဌာန၊ ဒဂုံတက္ကသိုလ်

Sample: Bee Pollen

RESULT

Sample No.		046/17-18	047/17-18
Job No.		I-046	I-047
Sample Marked.		နေကြာ	လဲမှို့
Protein	(%)	16.40	23.29
Moisture	(%)	13.00	14.48
Ash	(%)	2.18	3.29
Fat	(%)	2.55	4.02
Carbohydrate	(%)	41.71	45.95
Sugar	(%)	37.70	28.04

Not a Certificate of Conformance
စံချိန်စံညွှန်းကိုက်ညီကြောင်းထောက်ခံချက်မဟုတ်ပါ

Remark: *Results valid for the received sample only.*

Method/ Equipment used: A.O.A.C, Protein Analyzer

Tested by: Daw Yi Jan Ti

Checked by: Dr. Khin Aye Tue

Technical Director: U Win Khaing Moe

Our Reference: 1071
Date: 16.6.17

Figure 7. The nutritional values of two bee pollen samples (kapok;sunflower) in D.R.I

The determination of antimicrobial activity of bee pollen (kapok; sunflower)

Table 6. Antimicrobial activity of different solvent extracts of bee pollen samples

Paper disc=10 mm; -= negative

Bee pollen samples	Test organisms	Diameter of zone inhibition					
		Acetone extract	Ethanol extract	Ethyl-acetate extract	Methanol extract	Pet-ether extract	Water extract
kapok	*Bacillus subtilis*	23mm	25mm	12mm	33mm	-	-
	Candida albican	12mm	25mm	12mm	20mm	-	-
	Escherichia coli	13mm	**32mm**	12mm	**35mm**	-	-
	Pseudomonas aeruginosa	13mm	**33mm**	12mm	**36mm**	-	-
	Saccharomyces cerevisiae	12mm	26mm	-	20mm	-	-
	Staphylococcus aureus	25mm	30mm	12mm	27mm	-	-
	Micrococcus luteus	21mm	27mm	12mm	20mm	-	-
sunflower	*Bacillus subtilis*	21mm	27mm	12mm	37mm	-	-
	Candida albican	12mm	13mm	12mm	22mm	-	-
	Escherichia coli	13mm	**34mm**	12mm	**40mm**	-	-
	Pseudomonas aeruginosa	12mm	**35mm**	12mm	**40mm**	-	-
	Saccharomyces cerevisiae	12mm	26mm	16mm	23mm	-	-
	Staphylococcus aureus	16mm	27mm	12mm	30mm	-	-
	Micrococcus luteus	-	20mm	12mm	24mm	-	-

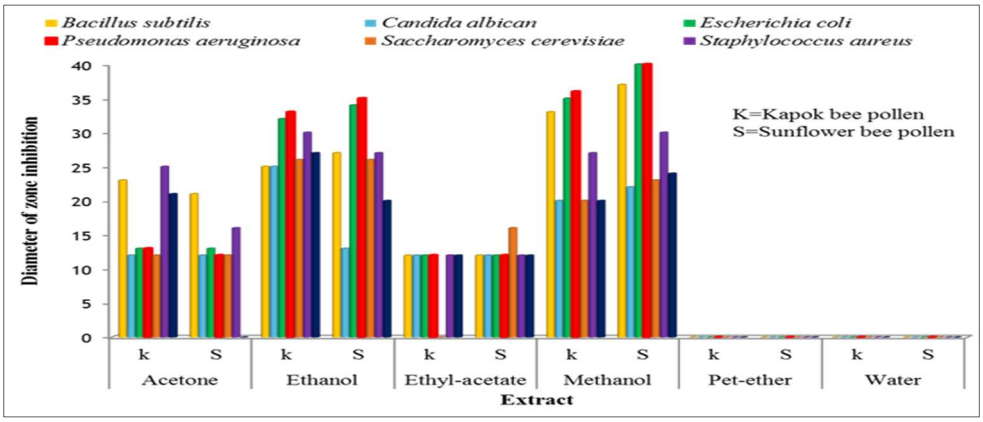
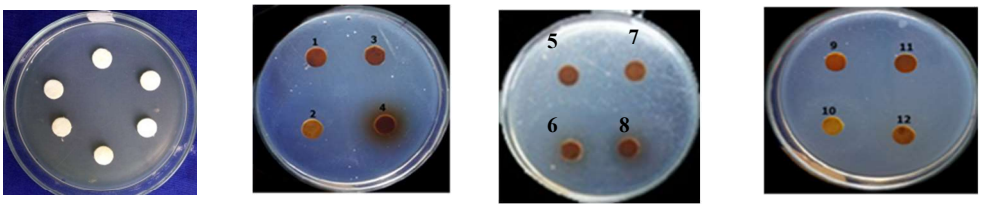
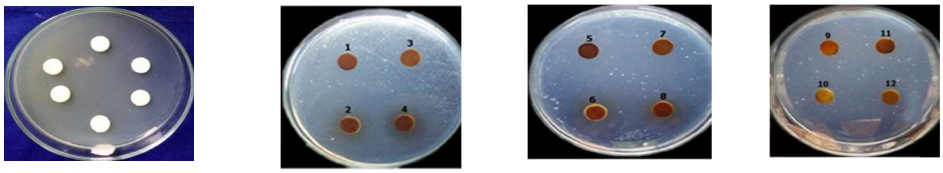


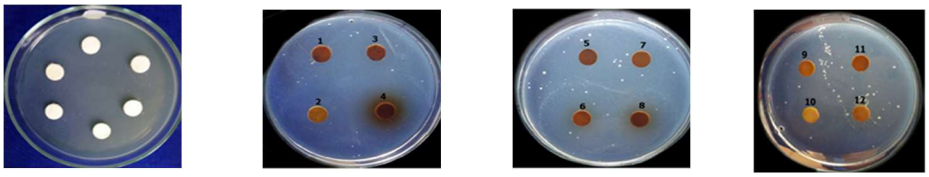
Figure 8. Antimicrobial activity of different solvent extracts of bee pollen samples



Control ***Bacillus subtilis***



Control ***Candida albican***



Control ***Escherichia coil***

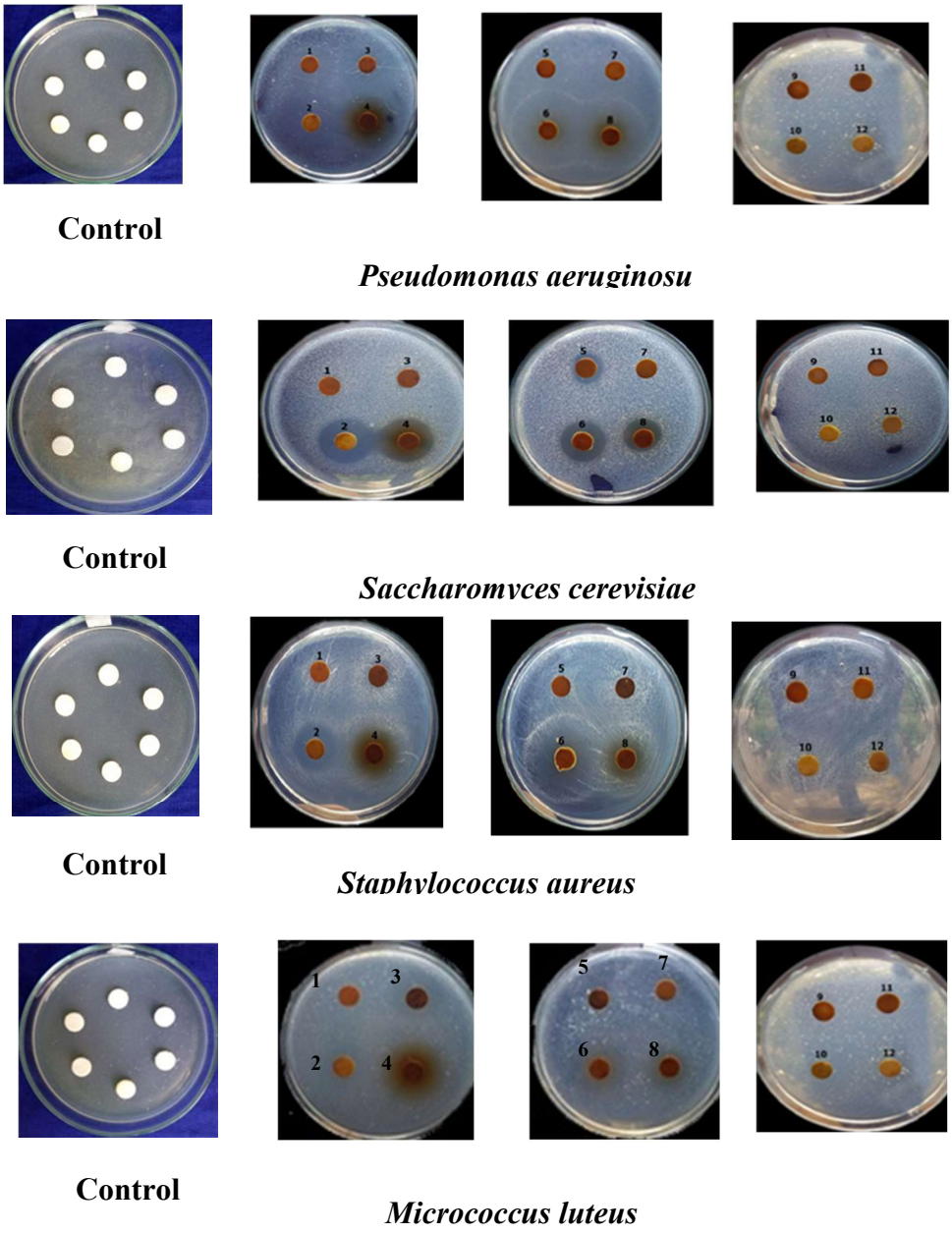


Figure 9. Diameter of zone inhibition of antimicrobial activity of two bee pollens

1, 2, 5, 6, 9, 10= sunflower bee pollen sample
3, 4, 7, 8, 11, 12= kapok bee pollen sample
1, 3 = acetone
2, 4= ethanol
5, 7= ethyl-acetate
6, 8= methanol
9, 11= pet-ether
10, 12= water

Discussion and Conclusion

This research, two samples of kapok and sunflower bee pollen collected from Division of Apiculture, Naypyitaw. According to palynological point of view, 12 species that belong to 10 genera of 8 families have been obtained in this study area. The types of pollen grains are monad, tetrad and polyads. The pollen type of *Mimosa* is tetrad and *Albizia* is polyads. The rest grains are monad.

Erdtman (1952) reported that, tetrad in *Mimosa* and polyads in *Albizia,* porate aperture in Mimosoideae. In this study, the pollen type of *Mimosa* and *Albizia* are agreed with Erdtman, 1952.

According to Sharma (1986), *Ceiba* pollen grain of Bombacoideae is tricolporate, retipilate and nexine thinner than sexine. In the study, pollen aperture of *Ceiba* species is in agreement with those given by Sharma, 1986.

Sharma *et al*. (1973) described that tricolpate and reticulate grain of *Brassica* species. In this research, *Brassica* pollen grain is agreed with Sharma *et al*., 1973.

On the basic of Chaturvedi *et al.* (1990), the pollen grains of *Celosia* are pantoporate; exine 1.5 - 5.0 μm thick. In this study, pollen character of *Celosia*is in agreement with those described by Chaturvedi *et al*., 1990.

Erdtman (1971) stated that Rubiaceae is eurpalynous family; *Richardsonia* pollen grain is 3 - polycolpate (about 12 - 18). In the study, pollen morphology of *Richardsonia*is agreed with Erdtman, 1971.

According to Ramakrishna *et al*. (2014), *Physalis* pollen grain is tricolporate and reticulates. In present work, pollen character of *Physalis* is in agreement with those given by Ramakrishna *et al*., 2014.

Solomon (1989) described that pollen grains of *Ocimum* are 3-6 colpate and reticulate. In this studied, pollen character of *Ocimum* is agreed with Solomon, 1989.

According to Paldat (2016), *Helianthus* grain is tricolporate and echinate. *Vernonia* grain is triporate and lophae by Stix, 1960. In this study, pollen morphology of *Helianthus* and *Vernonia* are agreed with Paldat, 2016 and Stix, 1960.

Hence, the studied of the pollen grain including bee pollen of two field sites are identical with these pollen characters of literatures as above.

As present in Figure 2, Kapok bee pollen field sites have 6 species. They are *Ceiba*, *Mimosa*, *Vernonia*, *Celosia*, *Physalis* and *Albizia*. In this site, *Ceiba* pollen grain is highest and *Albizia* is lowest. And then, *Vernonia*, *Celosia*, *Physalis* and *Albizia* are followed by moderate. So, *Ceiba* pollen grain was found to be the main sources in the kapok bee pollen field sites. Similarly, sunflower bee pollen field sites were found 6 species in Figure 3. They are *Helianthus*, *Vernonia*, *Brassica*, *Albizia*, *Richardsonia* and *Ocimum*. In these sites, highest pollen grain is *Helianthus* and *Ocimum* is lowest. *Vernonia*, *Brassica*, *Albizia* and *Richardsonia* are included by moderate. So, *Helianthus* pollen grain was found to be the main sources in the kapok bee pollen field sites. It can be inferred that asteraceae families with (*Vernonia*) were presented in two bee pollen of kapok and sunflower.

In this study, the nutritional values of bee pollen (sunflower; kapok) contains 41.71 and 45.95 percent of digestible carbohydrate, 37.70 and 28.04 percent of sugar (mainly fructose, glucose and sucrose), 16.40 and 23.29 percent of protein (including essential amino acids) and 2.55 and 4.02 percent of fat in bee pollen. It could be concluded that the percentage of rest constituents except sugar in kapok is more than in sunflower. But, the percentage of sugar in sunflower of bee pollen is distinctly more than in kapok. So, bee pollen of sunflower can be used as natural energizer for the body because of its sugar percentage (Table 5 and Figure 7).

As antimicrobial activities, the microorganism of *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Micrococcus luteus* were studied with acetone, ethanol, ethyl-acetate, methanol, petroleum-ether and aqueous extracts by agar disc diffusion method. Ethanol and methanol extracts were more effective than acetone and ethyl-acetate. Among them, petroleum ether and aqueous extract provided no antimicrobial activity in present results.

In this experiment, the best antimicrobial effect of bee pollen (kapok; sunflower) extracts were found at ethanolic and methanolic extracts against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The highest antimicrobial activity was shown on *Escherichia coli* and *Pseudomonas aeruginosa* with ethanolic and methanolic extracts and displayed in Table 6 and Figure 9. It was concluded in this study, the ethanolic and methanolic extracts of sunflower bee pollen were more than that of kapok in the diameter of zone inhibition of microbial activity.

According to the reports of Carpes *et al*. (2007) also reported that ethanolic and methanolic extracts of bee pollen had provided the higher antimicrobial properties against *Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antimicrobial activities of different solvent showed such as acetone, ethanol, ethyl-acetate, methanol, pet-ether and water. Ethanol and methanol extracts were more effective than acetone and ethyl-acetate. Among them, petroleum ether and aqueous extract provided no antimicrobial activity. These results are also the same as recorded in present research work.

Haggstrom (2014) suggested that disease can be caused by *Escherichia coli* such as urinary bladder, intestines, kidneys and food poisoning activity and *Pseudomonas aeruginosa* such as mucous membrane, skin and urinary tract infection. Bee pollen can be effective in treatment of various allergic diseases, immune system for various body, asthma attack and urinary infection by Talbott, 2015.

As the conclusion, this research work was carried out to prove the origin of bee pollen (kapok; sunflower) by studying pollen morphology and to suggest that bee pollen should be taken as a superfood because it has nutritious foods such as carbohydrate, sugar, protein and fat. Bee pollen is an effective medicinal treatment product according to antimicrobial activity such as *Escherichia coli* of urinary bladder etc. *and Pseudomonas aeruginosa* of mucous membrane etc. In addition, it is necessary to research for the further study what benefits it will be effective for health by how much dose used daily and what cause will happen if people take bee pollen as overdose daily diet.

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