ISOLATION AND CHARACTERIZATION OF PATHOGENIC FUNGI INFECTED ON *ORYZA SATIVA* L. VAR. PAW SAN BAYKYAR IN MONYWA DISTRICT

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Abstract

The study on the isolation and morphological characterization of pathogenic fungi infected plants of *Oryza sativa* L. (Paw San Baykyar rice variety) was carried out at the Microbiology Laboratory, Department of Botany in Monywa University, Myanmar from June to November, 2018. The disease-infected plant samples were collected from the rice fields in Monywa District. The different cultural media of Low Carbon Agar (LCA), Potato Dextrose Agar (PDA) and Water Glucose Agar (WGA) were used for the isolation of pathogenic fungal strains. The morphological characterization was studied on their colony morphology, macroscopical and microscopical characters. Total of 12 genera (*Alternaria* sp., *Aspergillus* sp., *Cercospora* sp., *Fusarium* spp., *Nigrospora* sp., *Sarocladium* sp., *Curvularia* sp., *Ustilaginoidea* sp., *Penicillium* sp., *Rhizopus* sp., *Cladosporium* sp., *Bipolaris* sp.) and 13 pathogenic fungal strains (HH 01 - HH 13) were isolated and identified from leaves, leaf sheaths, grains and seeds of the disease-infected rice plants. The HH 01 strain as *Alternaria* sp., HH 02 strain as *Aspergillus* sp., HH 03 strain as *Cercospora* sp., HH 04 strain as *Fusarium* sp., HH 05 strain as *Nigrospora* sp., HH 06 strain as *Sarocladium* sp., HH 07 strain as *Curvularia* sp., HH 08 strain as *Fusarium* sp., HH 09 strain as *Ustilaginoidea* sp., HH 10 strain as *Penicillium* sp., HH 11 strain as *Rhizopus* sp., HH 12 strain as *Cladosporium* sp. and HH 13 strain as *Bipolaris* sp. were identified.

Keywords: Pathogenic fungi, *Oryza sativa* L., Paw San Baykyar rice variety, isolation

Introduction

Oryza sativa L. is one of the most important cereal crop of family Poaceae. It is a staple food crop of 60 percent of the world's population. The edible uses of rice include namely, rice flakes, puffed rice, rice wafers and canned rice. It is also used in starch and brewing industries. The byproduct by rice milling that is rice husk and bran are used as a cattle and poultry feed. Rice is one of the diverse crop grown in different agro-climatic conditions (Ramakrishnan 1971). Rice has been the focus in the history of Myanmar economic development. Myanmar' Paw San rice is one of the world's most recognized high quality rice, it was awarded the world's best rice at the Rice Trader's World Rice Conference in 2011 (Myint & Napasintuwong 2016).

Microorganisms play an important role in affecting the quality of seed of which fungi are the largest group. These pathogens are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages (Uma & Wesely 2013). Rice suffers from many diseases caused by fungi, bacteria, viruses, nematodes and other non-parasitic disorders. Fungal disease is considered as the principal disease of rice because of its wide distribution and its destructiveness under favorable conditions for yield loss. The biotic pathogens can infect the crop at any time from seed germination to harvest (Devi & Pushpalatha 2013).

Fungi are a major cause of reduction in the quality of rice due to high moisture and temperature conditions before its harvest (Uma & Wesely 2013). More than 100 species of fungi have been identified on rice seeds so far. However, their severity depends on the time of sampling, location and varieties are different (Monajjem *et al*. 2014).

Rice is affected by as many as 36 seed borne diseases of which 31 were caused by fungi which are mostly namely, *Pyricularia oryzae*, *Alternaria padwickii*, *Helminthosporium* sp.,

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Gibberella fujikuroi, *Gibberella rosa*, *Fusarium cereali*, *Nigrospora* sp., *Epicoccum* sp., *Phyllosticta glumarum*, *Alternaria* sp. and *Helicoceras oryzae*. The most common storage fungi are *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, *Chaetomium*, *Dematium*, *Monilia*, *Oidium*, *Streptomyces*, *Syncephalastrum* and *Verticillium* (Sharma & Kapoor 2017).

The aim of present study deals with the isolation and morphological characteristics of pathogenic fungi from infected *Oryza sativa* L. var. Paw San Baykyar from Monywa District. The specific objectives were to isolate the pathogenic fungi which infected on the plants of Paw San Baykyar rice variety, to identify the micro- and macroscopical characters of cells and colony morphology of isolated pathogenic fungal strains, and to record the pathogenic fungi which can be caused diseases on different plant parts of those rice variety under field conditions in Monywa District.

Materials and Methods

Collection of the Infected Plant Parts

The plant samples from the infected plant parts of *Oryza sativa* L. (Paw San Baykyar rice variety) were collected from the rice fields of Monywa District from June to November 2018. Isolation of pathogenic fungi was done as soon as possible after the infected plant samples were brought to the Microbiology Laboratory of Botany Department in Monywa University. Plant pathogenic fungi were isolated by direct isolation method according to Ando (2015), using PDA medium. Low Carbon Agar (LCA) and Water Glucose Agar (WGA) were used for the isolation and identification of pathogenic fungi (Motlagh 2010).

Isolation and Identification of Microorganisms

The disease-infected parts of *Oryza sativa* L. var. Paw San Baykyar were used for the isolation of pathogenic microorganisms. Fungi identification was followed by the methods of Barnett (1955), Larone (1995), Mew & Gonzales (2002), Tanaka (2008), Ladhalakshmi *et al.* (2011), Abass & Mohammed (2014), Kidd *et al.* (2016).

Measuring of Microorganisms

The fungal spores were measured according to the methods of Kokate (2000). These isolated fungal strains were examined under light microscope, XSZ – 107BN and the results were recorded.

Results

Total of 13 pathogenic fungal strains (HH 01 - HH 13) were isolated and identified from the disease-infected parts of *Oryza sativa* L. var. Paw San Baykyar. The macroscopical and microscopical characters of those isolated pathogenic fungal strains were also shown in Table 1 and Figure 1-13.

The isolated pathogenic fungal strains were found in different plant parts of those rice variety with the pathogenic disease-symptoms. The pathogenic fungal strains of HH 01 and HH 02 were found in stackburn symptom infected leaves which caused large oval or circular spots with a pale brown margin. Color of center eventually becomes white and bear dots in leaves (Fig 1 A and Fig 2).

The HH 03, HH 04 and HH 05 strains were found in leaf spot symptom infected leaves which caused short, linear, brown lesions mainly on the leaves (Fig 3 A, Fig 4 and Fig 5). The HH

06, HH 07 and HH 08 strains were found in leaf sheath rot symptom infected leaf sheaths which caused irregular spots, with gray to light brown centers surrounded by distinct dark reddish brown margins (Fig 6 A, Fig 7 and Fig 8). HH 09 strain was found in false smut symptom infected grains which caused greenish spore balls that have velvety appearance. The color of the ball become orange and later yellowish green or greenish black (Fig 9 A). HH 10 and HH 11 strains were found in seed blight symptom infected on grains which caused initially small, oblong, and brown then gradually enlarge and coalesce, becoming whitish with small black dots (Fig 10 A and Fig 11). HH 12 and HH 13 strains were found in black kernel symptom infected seeds which caused black spots, discoloration and empty (Fig 12 A and Fig 13).

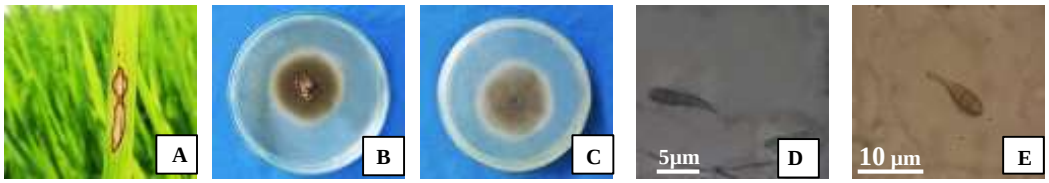

Figure 1 *Alternaria* sp. (HH 01 strain) isolated from stackburn infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. stackburn infected leaves; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. conidia.

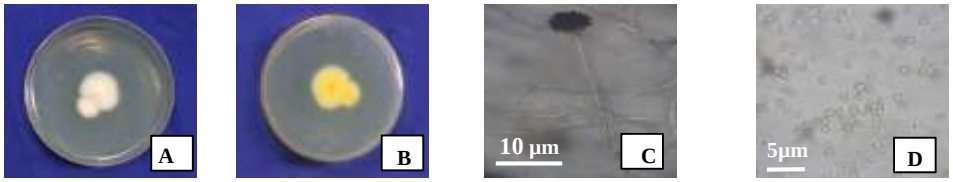

Figure 2 *Aspergillus* sp. (HH 02 strain) isolated from stackburn infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

Figure 3 *Cercospora* sp. (HH 03 strain) isolated from leaf spot infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. leaf spot infected leaves; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

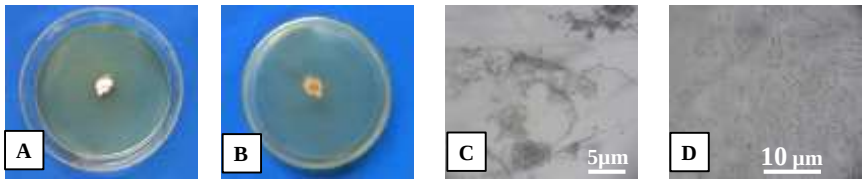

Figure 4 *Fusarium* sp. (HH 04 strain) isolated from leaf spot infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

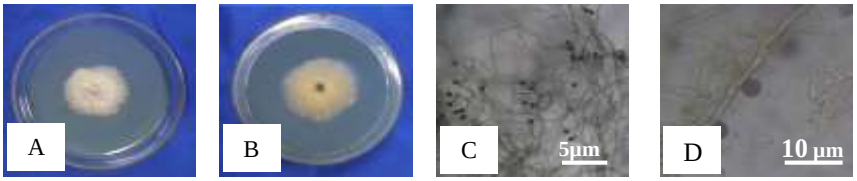

Figure 5 *Nigrospora* sp. (HH 05 strain) isolated from leaf spot infected leaves of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

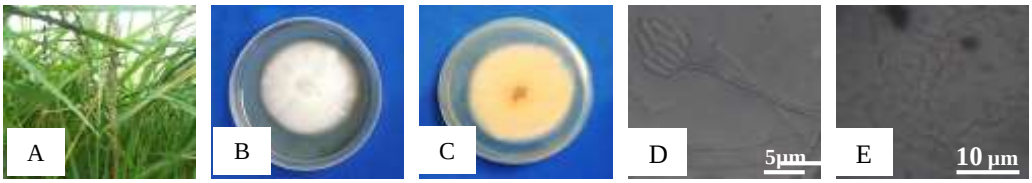

Figure 6 *Sarocladium* sp. (HH 06 strain) isolated from leaf sheath rot infected leaf sheaths of *Oryza sativa* L. var. Paw San Baykyar. A. leaf sheath rot infected leaf sheaths; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

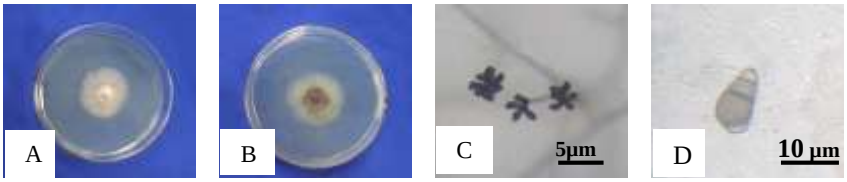

Figure 7 *Curvularia* sp. (HH 07 strain) isolated from leaf sheath rot infeceted leaf sheaths of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

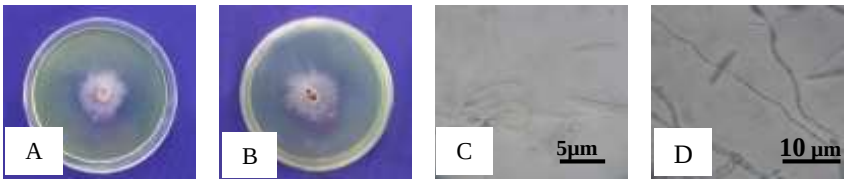

Figure 8 *Fusarium* sp. (HH 08 strain) isolated from leaf sheath rot infected leaf sheaths of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

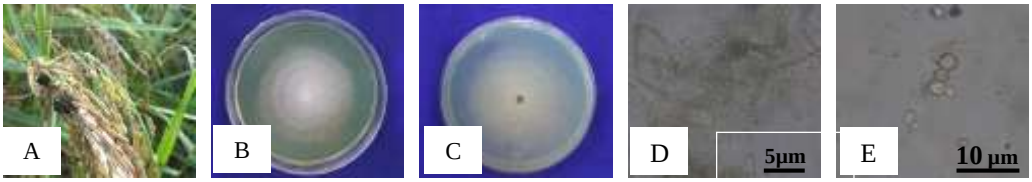

Figure 9 *Ustilaginoidea* sp. (HH 09 strain) isolated from false smut infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. false smut infected seeds; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

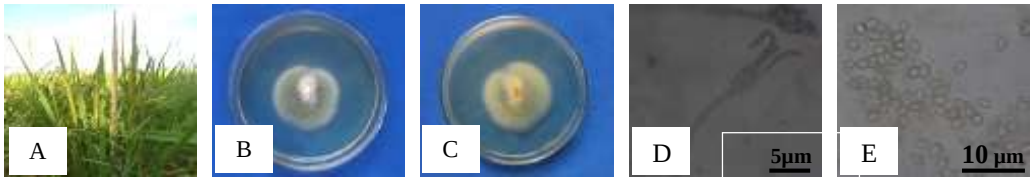

Figure 10 *Penicillium* sp. (HH 10 strain) isolated from seed blight infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. seed blight infected seeds; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

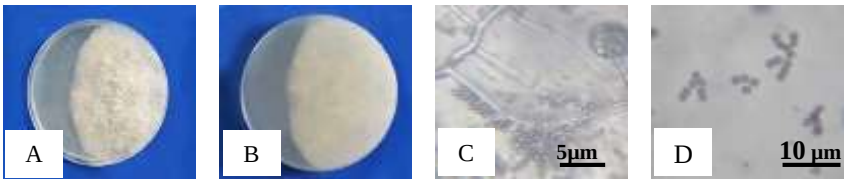

Figure 11 *Rhizopus* sp. (HH 11 strain) isolated from seed blight infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

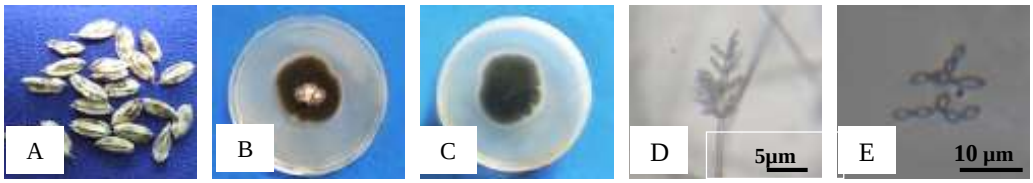

Figure 12 *Cladosporium* sp. (HH 12 strain) isolated from black kernel infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. black kernel infected grains; B. surface colony characters on PDA medium (3 days); C. reversed colony characters; D. hypha; E. Conidia.

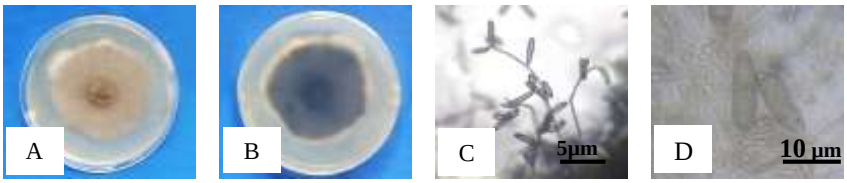

Figure 13 *Bipolaris* sp. (HH 13 strain) isolated from black kernel infected seeds of *Oryza sativa* L. var. Paw San Baykyar. A. surface colony characters on PDA medium (3 days); B. reversed colony characters; C. hypha; D. Conidia.

Table 1 Macroscopical and microscopical characters of isolated pathogenic fungal strains from the disease infected parts of *Oryza sativa* L. var. Paw San Baykyar

Isolated Strains	Pathogenic Fungi	Macroscopical Characters	Microscopical Characters	Diseases
HH 01	*Alternaria* sp.	Greenish black or brown and 5.5 cm. Reverse remained black.	Septate hyphae. Conidiophores were dark and short. Conidia were dark, fusiform to obclavate, thick-walled, 3-5 septate, second cell from the base larger than the rest of the cells and 35.5 - 45.5 µm.	Stackburn
HH 02	*Aspergillus* sp.	Suede-like and cinnamon-buff, white and 2.1 cm. Reverse remained yellow.	Septate hyphae. Conidiophores stipes were usually short, brownish or smooth-walled, terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia were globose to ellipsoidal, slightly yellow, hyaline and 1.5 - 2.5 µm.	Stackburn
HH 03	*Cercospora* sp.	White, with sinuate margins and 2.0 cm. Reverse remained pale red.	Hyphae were swollen. Conidiophores were brown, 3 or more septate. Conidia were hyaline or light olive, 3-10 septate, cylindrical to clavate and 18.1 - 28.0 µm.	Leaf spot
HH 04	*Fusarium* sp.	White and 3.8 cm. Reverse remained white with a dark center.	Septate hyphae. Conidiophores were hyaline, short, simple to multibranched. Conidia were hyaline, fusiform, ovate or clavate, single-celled to 4-celled and 10.5 - 16.0 µm.	Leaf spot
HH05	*Nigrospora sp.*	White to gray and 4.5 cm. Reverse remained gray.	Septate hyphae. Conidiophores were short, simple, inflated below the tip. Conidia were black, spherical, globose or subglobose, single and 4.5 - 8.0 µm.	Leaf spot
HH 06	*Sarocladium* sp.	White and 6.7 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were simple and hyaline. Conidia were grouped in slimy head, hyaline, cylindrical or ellipsoidal and 35.0 - 40.5 µm.	Leaf sheath rot
HH 07	*Curvularia* sp.	Gray and 3.8 cm. Reverse remained black.	Septate hyphae. Conidiophores were dark brown, unbranched or branched typically bent. Conidia were dark brown, boat-shaped, 3-5 celled, end cells lighter; one or two of the central cells enlarged and 12.0 - 18.0 µm.	Leaf sheath rot

Isolated Strains	Pathogenic Fungi	Macroscopical Characters	Microscopical Characters	Diseases
HH 08	*Fusarium* sp.	White and 1.6 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were hyaline, single, lateral. Conidia were hyaline, fusiform, slightly flattened on both end, one or two celled and 4.0 - 8.0 µm.	Leaf sheath rot
HH 09	*Ustilaginoidea* sp.	White and 5.6 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were forming and bearing conidia at the tapering apex. Conidia were yellow to dark color, ovoid and 5.0 - 7.5 µm.	False smut
HH 10	*Penicillium* sp.	Green shade, sometime white and 4.9 cm. Reverse remained pale yellow.	Septate hyphae. Conidiophores were arising singly, frequently branches, near the apex to form a brush-like, conidia-bearing apparatus, ending in phialides which pinch off conidia in dry chains. Conidia were greenish, ovoid, 1-celled and 5.0 - 8.5 µm.	Seed blight
HH 11	*Rhizopus* sp.	Cottony at first white and then gray or yellowish brown and 6.8 cm. Reverse remained white.	Non septate hyphae. Sporangiophores were simple or branched, arising from stolons opposite rhizoids usually in groups of three or more. Sporangia were greyish black, globose and 2.5 - 5.0 µm.	Seed blight
HH 12	*Cladosporium* sp.	Blackish-brown and 5.0 cm. Reverse remained olivaceous black.	Septate hyphae. Conidiophores were dark, branched variously near the apex or middle portion, clustered or single. Conidia were dark, hilum, 1- or 2-celled, ovoid to cylindrical and 5.0 - 12.0 µm.	Black kernel
HH 13	*Bipolaris* sp.	Grey to dark grey and 6.8 cm. Reverse remained black.	Septate hyphae. Conidiophore were single or in small group, straight to flexuous, pale to mid brown. Conidia were pale to mid golden brown, curved, navicular, fucoid of obclavate, occasionally almost cylindrical, 5-12 distoseptate and 26.0 - 48.0 µm.	Black kernel

Discussion

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Diseases are considered major constraints in rice production. According to the present study, twelve genera of fungal pathogens concerning with the six kinds of diseases symptoms were observed in the disease-infected parts of *Oryza sativa* L. var. Paw San Baykyar. In the present study, 12 genera of 13 pathogenic fungal strains were found in

the disease-infected plant parts of *Oryza sativa* L. var. Paw San Baykyar. The 13 isolated pathogenic fungal strains (HH 01 - HH 13) were identified.

In this study, HH 01 fungal strain, *Alternaria* sp. found that colonies were greenish black or brown with a light border and 5.5 cm. Reverse remained black. They were septate hyphae. Conidiophores were dark and short. Conidia were dark, fusiform to obclavate, thick-walled, 3-5 septate, second cell from the base larger than the rest of the cells and 35.5 - 45.5 µm. The macroscopical and microscopical characters of *Alternaria* sp. was similar with the statements of Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

The HH 02 fungal strain, *Aspergillus* sp. revealed that colonies were typically suede-like and cinnamon-buff, white and 2.1 cm. Reverse remained yellow. They were septate hyphae. Conidiophore stipes were usually short, brownish or smooth-walled, terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia were globose to ellipsoidal, slightly yellow, hyaline and 1.5 - 2.5 µm. These findings were agreed with Barnett (1955) and Larone (1995).

The HH 03 fungal strain, *Cercospora* sp. found that colonies were white with sinuate margins and 2.0 cm. Reverse remained pale red. They were swollen hyphae. Conidiophores were brown, 3 or more septate. Conidia were hyaline or light olive, 3 to 10 septate, cylindrical to clavate and 18.1 - 28.0 µm. These isolated *Cercospora* sp. was similar with the macroscopical and microscopical characters to the statements of Barnett (1955) and Mew & Gonzales (2002).

In the present study, HH 04 fungal strain, *Fusarium* sp. revealed that colonies were white and 3.8 cm. Reverse remained white with a dark center. They were septate hyphae. Conidiophores were hyaline, short, simple to multi branched. Conidia were hyaline, fusiform, ovate or clavate, 1-4 celled and 10.5 - 16.0 µm. These findings were agreed with Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

HH 05 fungal strain, *Nigrospora* sp. found that colonies were white to gray and 4.5 cm. Reverse remained gray. They were septate hyphae. Conidiophores were short, simple, inflated below the tip. Conidia were black, globose or subglobose, single and 4.5 - 8.0 µm. These findings were agreed with Abass & Mohammed (2014).

In the present study, HH 06 fungal strain, *Sarocladium* sp. revealed that colonies were white and 6.7 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were simple and hyaline. Conidia were grouped in slimy head, hyaline, cylindrical or ellipsoidal and 35.0 - 40.5 µm. HH 07 fungal strain, *Curvularia* sp. found that colonies were gray and 3.8 cm. Reverse remained black. They were septate hyphae. Conidiophores were dark brown, unbranched or branched typically bent. Conidia were dark brown, boat-shaped, 3-5 celled, end cells lighter; one or two of the central cells enlarged and 12.0 - 18.0 µm. These findings were agreed with Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

HH 08 fungal strain, *Fusarium* sp. revealed that colonies were white and 1.6 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were hyaline, single, lateral. Conidia were hyaline, fusiform, slightly flattened on the both ends, one or two celled and 4.0 - 8.0 µm. These findings were agreed with Barnett (1955), Larone (1995), Mew & Gonzales (2002) and Kidd *et al*. (2016).

HH 09 fungal strain, *Ustilaginoidea* sp. found that colonies were white and 5.6 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were forming and bearing conidia at the tapering apex. Conidia were ovoid, yellow to dark color and 5.0 - 7.5 µm. These isolated *Ustilaginoidea* sp. was similar with the macroscopical and microscopical characters to the statements of Tanaka (2008) and Ladhalakshmi *et al*. (2011).

In the present study, HH 10 fungal strain, *Penicillium* sp. found that colonies were in shades of green, sometime white and 4.9 cm. Reverse remained pale yellow. They were septate hyphae. Conidiophores were arising singly, frequently branches, near the apex to form a brush-like, conidia-bearing apparatus, ending in phialides which pinch off conidia in dry chains. Conidia were greenish, ovoid, 1-celled and 5.0 - 8.5 µm. These findings were agreed with Barnett (1955), Larone (1995) and Kidd *et al*. (2016).

In this study, HH 11 fungal strain, *Rhizopus* sp. revealed that colonies were cottony, at first white and then gray or yellowish brown and 6.8 cm. Reverse remained white. They were non septate hyphae. Sporangiophores were simple or branched, arising from stolons opposite rhizoids usually in groups of three or more. Sporangia were globose, greyish black and 2.5 - 5.0 µm. HH 12 fungal strain, *Cladosporium* sp. found that colonies were blackish-brown and 5.0 cm. Reverse remained olivaceous black. They were septate hyphae. Conidiophores were dark, branched variously near the apex or middle portion, clustered or single. Conidia were dark, hilum, 1- or 2-celled, ovoid to cylindrical and 5.0 - 12.0 µm. These findings were agreed with Larone (1995) and Kidd *et al*. (2016).

In the present study, HH 13 fungal strain, *Bipolaris* sp. revealed that colonies were grey to dark grey and 6.8 cm. Reverse remained black. They were septate hyphae. Conidiophore were single or in small group, straight to flexuous, pale to mid brown. Conidia were curved, navicular, fucoid of obclavate, occasionally almost cylindrical, pale to mid golden brown, 5-12 distoseptate and 26.0 - 48.0 µm. These findings were agreed with Larone (1995) and Mew & Gonzale (2002).

The stackburn symptoms included large oval or circular spots with a pale brown margin. Color of center eventually becomes white and bear minute black dots on leaves. Those findings were agreed with Lau and Sheridan (2012) and Asghfaq *et al*. (2017). Lau and Sheridan (2012) also stated that *Alternaria* sp., causal agent of stackburn disease on rice. Asghfaq *et al*. (2017) stated that *Alternaria* sp. was isolated from different rice varieties.

The leaf spot symptoms are short, linear, brown lesions mainly on the leaves. Those findings were in agreement with Elazegui and Islam (2003) who stated that although it may also occur on leaf sheath, pedicels and glume. In this study, *Sarocladium* sp. occurs on rotting leaf sheath enclosing the young panicles. Lesions consist of diffuse reddish brown discoloration in the leaf sheath. These observations are in agreement with Elazegui & Islam (2003) and Rawte (2007).

Ustilaginoidea sp., the fungus transforms individual seeds of the panicles into greenish spore balls that have velvety appearance. The color of the spore balls becomes orange and later yellowish green, or greenish black on grains. These findings were also agreed with the findings of Elazegui & Islam (2003) and Tripathi *et al.* (2011).

In *Bipolaris* sp., brown spot may be manifested as seed blight disease symptom in mature seeds. This fungus may also infected the glumes, causing dark brown to black oval spots, and may also infected the seed, causing a black discoloration. These observations were in agreement with Elazegui & Islam (2003), Tripathi *et al*. (2011) and Lau & Sheridan (2012). In this study, *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. were discoloration in seeds. These findings were agreed with Imolehin (1983).

Conclusion

This study was identified as 12 genera of pathogenic fungi including 13 fungal strains identified from *Oryza sativa* L. var. Paw San Baykyar. Those isolated pathogenic fungi were found in the disease symptoms of leaves, leaf sheaths and seeds of Paw San Baykyar rice variety. Thus, the present study will be provided some information of pathogenic fungi occurring on the Paw San Baykyar in Myanmar. These pathogenic fungi that cause slower growth and loss yield of rice.

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