Dhaka Univ. J. Biol. Sci. **29**(1): 75-86, 2020 (January)

MORPHOLOGICAL CHARACTERIZATION OF SEED-BORNE FUNGI ASSOCIATED WITH BRRI RICE VARIETIES IN BANGLADESH

TANIA SULTANA, MA BASHAR AND SHAMIM SHAMSI*

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Morphological characterization, Seed-borne fungi, BRRI rice varieties

Abstract

Twenty five fungal species, representing 13 genera were found to be associated with seeds of 20 varieties of rice (BRRI dhan 56 to BRRI dhan 75). Fungi were isolated following "Tissue planting" method and "Blotter method". The isolated fungi were *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, A. terreus, Bipolaris multiformis, B. oryzae, B. sorokiniana, Chaetomium globosum, Curvularia lunata, Fusarium equiseti, F. fugikuroi, F. oxysporum, F. proliferatum, Microdochium fisheri, Nigrospora oryzae, Penicillium* sp., *Pestalotiopsis oxyanthi, Phanerochaete chrysosporium, Rhizopus stolonifer, Sarocladium oryzae, Syncephalastrum racemosum* and *Trichoderma viride.*

Introduction

Rice (*Oryza sativa* L.) is the second largest crop grown in the world in terms of both area and production. It is the staple food for more than half of world's population. The majority of the rice produced comes from China, India, Indonesia, Pakistan, Bangladesh, Vietnam, Thailand, Myanmar, Philippines and Japan[1].

Rice suffers from more than 60 different diseases of which 43 are known to occur in Bangladesh. Among these diseases, 27 are seed-borne of which 14 are of major importance[2]. Different seed-borne diseases of rice are bakanae, stackburn, brown spots, black kernel, blast, sheath blight, sheath rot, stem rot and leaf scald and they cause yield reduction, quality deterioration and germination failure[3-5]. In Bangladesh, approximately 2.5 million tons of rice worth more than Tk.12000 million is lost annually due to diseases caused by seed-borne pathogens(6).

Gopalakrishnan *et al.*[7] recorded eight genera of fungi *viz., Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma* associated with rice seed. Ora *et al.*[8] detected 12 seed-borne pathogens of rice. In India Archana and Prakash[9] found 16 genera of fungi comprising 27 species associated with the rice seed samples. Irshad *et al.*[10] reported 18 fungal species belonging to eight genera in local and imported paddy seed lots. Habib *et al*.[11] also isolated 10 seed-borne fungi from 15 varieties of rice in Pakistan.

*Author for correspondence: <prof.shamsi@gmail.com>.

The information about morphological features of seed-borne fungi with newly released BRRI rice varieties is inadequate in Bangladesh. Therefore, the present investigation has been undertaken to investigate morphological characteristics of seed-borne fungi associated with BRRI dhan 56 to BRRI dhan 75.

Materials and Methods

Seeds of 20 BRRI released rice varieties (BRRI dhan56 to BRRI dhan75) were collected from Genetic Resources and Seed Division of Bangladesh Rice Research Institute (BRRI) during January, 2016 to July, 2017. The samples were kept in brown paper bag, labeled properly and stored immediately in a dry safe place in the laboratory until used for the experiments and preserved at 4ºC in refrigerator for further studies. The experiment was conducted in Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka, Bangladesh.

The fungi were isolated from the seed samples following Tissue Planting method on PDA medium[12] and Blotter method[13]. For Tissue Planting method, 400 seeds were washed in sterile water and then surface sterilized by dipping in 10% chlorox for 5 minutes. Then they were transferred into a sterile Petri plate containing sterile blotting paper to remove the excess surface water. The seed inocula were placed in Petri plates containing sterilized PDA medium. Each Petri plate contained 15 ml of PDA medium with an additional of 1 drop (ca. 0.03 ml) of lactic acid.

Then the inoculated plates were incubated at room temperature (25 ± 2°C) for 5 - 7 days. For Blotter method, 400 surface sterilized seeds of each sample were placed on two layers of moist blotting paper (Whatman No.1) in sterilized Petri plates and incubated at 25 ± 2°C for 5 - 7 days. A total number of 100 inocula were transferred in 10 Petri plates. After 7 days of incubation the fungi were observed and recorded.

Morphological studies of the fungal isolates were done in order to determine their identification. The microscopic structural characters of the isolated fungi were recorded under Nikon D 5000 digital camera. Identification of the isolates were determined based on morphological characteristics observed under a compound microscope following the standard literature[14-19]. All the specimens, were preserved in Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh.

Results and Discussion

Twenty-five fungal species, representing 13 genera were found to be associated with seeds of 20 different BRRI rice varieties. The fungi were *Alternaria alternata* Keissler, *A. tenuissima* Samuel Paul Wiltshire, *Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *A. ochraceus* K. Wilh., *A. terreus* Thom., *Bipolaris multiformis* (Jooste) Alcorn, *B. oryzae* (Breda de Haan) Shoemaker, *B. sorokiniana* (Sacc.) Shoemaker, *Chaetomium globosum*

Kunze ex Fr., *Curvularia lunata* (Wakker) Boedijn, *Fusarium equiseti,* (Corda) Saccardo., *F. fugikuroi* Nirenberg, *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg, *Microdochium fisheri* Hern.-Restr. & Crous., *Nigrospora oryzae* (Berk. & Br.), *Penicillium* Link., *Pestalotiopsis oxyanthi* Thum., *Phanerochaete chrysosporium* Burds., *Rhizopus stolonifera* (Ehrenb.) Vuill, *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw, *Syncephalastrum racemosum* Cohn ex J. Schrot. and *Trichoderma viride* Pers.

Taxonomic enumeration of fungi

Alternaria alternata (Fr.) Keissler, Beih. Bot., Zbl. **29**: 434 (1972) **(Fig. 1A)**

Colonies usually black or olivaceous black, sometimes grey. Conidiophores golden brown, smooth up to 28 - 99 μm long and 3 - 5 μm thick with one or several conidial scars. Conidia formed in long, often branched chains, obclavate with a short conidial or cylindrical beak, sometimes up to but not more than one third the length of the conidium, pale to mid golden brown, smooth or verruculose, overall length 22.5 - 52.2 μm, 4.5 - 16.3 μm thick in the broadest part; beak pale 2.5 - 5 μm thick.

Specimen examined: Isolated from BRRI dhan 57 variety, BRRI, Joydebpur, Gazipur, T Sultana 82, 14 January, 2016.

Alternaria tenuissima (Kunze ex Pers.) Wiltshire, Trans. Br. mycol. Soc. **18**:157 (1933) **(Fig. 1B)**

Colony dark blackish brown to black. Conidiophores solitary up to 30 × 3 - 6 μm, simple or branched, straight or flexuous, more or less cylindrical, septate, pale or mid pale brown. Conidia solitary, straight or curved, obclavate, conidium ellipsoidal tapering gradually to the beak, usually shorter, pale to mid clear golden brown, usually smooth, 22.2 - 70.3 × 10.2 - 25.4 μm body generally with 4 - 7 transverse and several longitudinal or oblique septa, beak shorter than or the same length as the body, cylindrical, 3 - 5 μm thick.

Specimen examined: Isolated from BRRI dhan 72 variety, BRRI, Joydebpur, Gazipur, T Sultana 97, 13 March, 2016.

Aspergillus flavus Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin **3**(1): 16 (1809) **(Fig. 1C)**

Colony on PDA was grayish powdery and fast growing. Conidial heads were yellow to green became brownish in edge. Conidiophores were less than 1 mm length and 10 - 20 μm diameter, vesicle was glubose to subglubose. Conidia were glubose minutely accumulate and measured 2.5 - 3.5 μm.

Specimen examined: Isolated from BRRI Dhan 65 variety, BRRI, Joydebpur, Gazipur, T Sultana 11, 25 October, 2015.

Aspergillus fumigatus Fresenius. Beitragezur Mykologie **3**:81 (1863) **(Fig. 1D)**

Colonies greenish, mycelia well developed, septate. Cells are multinucleate. Conidiophores are long, often with a foot cell, straight or flexuous, swollen at the apex into a spherical vesicle. Conidia catenulate, dry, usually globose, echinulate and smooth. Colonies of the fungus produced thousands of minute pale green conidia 2 - 3 µm.

Specimen examined: Isolated from BRRI dhan 75 variety, BRRI, Joydebpur, Gazipur, T Sultana 28, 11 October, 2015.

Aspergillus niger van Tieghem Ann. Sci. Nat. Bot. Ser. 5, **8**: 240 (1867) **(Fig. 1E)**

Colonies effuse, black. Conidiophores brown 200 - 400 × 7 - 10 µm. Vesicles globose or sub globose, thick walled, commonly 20 - 50 µm, occasionally up to 100 µm in diameter. Foot cell present. Sterigmata 20 - 30 × 6 - 8 µm. Conidia black or dark brown, catenulate, dry, usually globose, one celled, globose, 2 - 4 (5) µm in diameter.

Specimen examined: Isolated from BRRI dhan 71 variety, BRRI, Joydebpur, Gazipur, T Sultana 102, 21 June, 2015.

Aspergillus ochraceus K. Wilh., Beitrage zur Kenntnis der Pilzgattung**: 66** (1877) **(Fig. 1F)**

Colonies yellow to yellow-orange, ochraceus or buff, powdery to granular. Conidial heads radiate, later splitting into several columns. Conidiophores brownish, 1 - 1.5 µm long, rough walled. Vesicles globose; phialides biseriate covering almost the entire surface of the vesicle. Conidia spherical to sub spherical, 2.5 - 3.5 µm in diameter., smooth walled to finely roughened. Pink to vinaceous-purple coloured, irregular shaped sclerotia (up to 1 mm diam.) may be formed in some isolates.

Specimen examined: Isolated from BRRI dhan 67 variety, BRRI, Joydebpur, Gazipur, T Sultana 32, 10 March, 2015.

Aspergillus terreus Thom. Amer. J. Bot. **5**(2): 85 (1918) **(Fig. 1G)**

Colonies moderately fast rapidly growing flat, velvety to slightly granular, or powdery, occasionally floccose with thin irregular margins, cinnamon-buff to brown, rarely orange-brown, consisting of a dense felt of conidiophores with reverse yellow to pale brown. Amber coloured exudate produced in some strains. An isolate with deep orange colonies with lemon yellow diffusible pigment has been described. Conidial heads pale-brown, long, densely columnar, characteristically appearing fan-shaped. Conidiophores short, 100 - 250 mm long, flexuous, smooth walled with dome-shaped vesicle, 10 - 20 mm diameter. Phialides biseriate on upper two third of the vesicle. Conidia hyaline, smooth-walled, spherical to broadly elliptical, 1.5 - 2.5 mm diameter.

Specimen examined: Isolated from BRRI dhan 57 variety, BRRI, Joydebpur, Gazipur, T Sultana 122, 20 October, 2015.

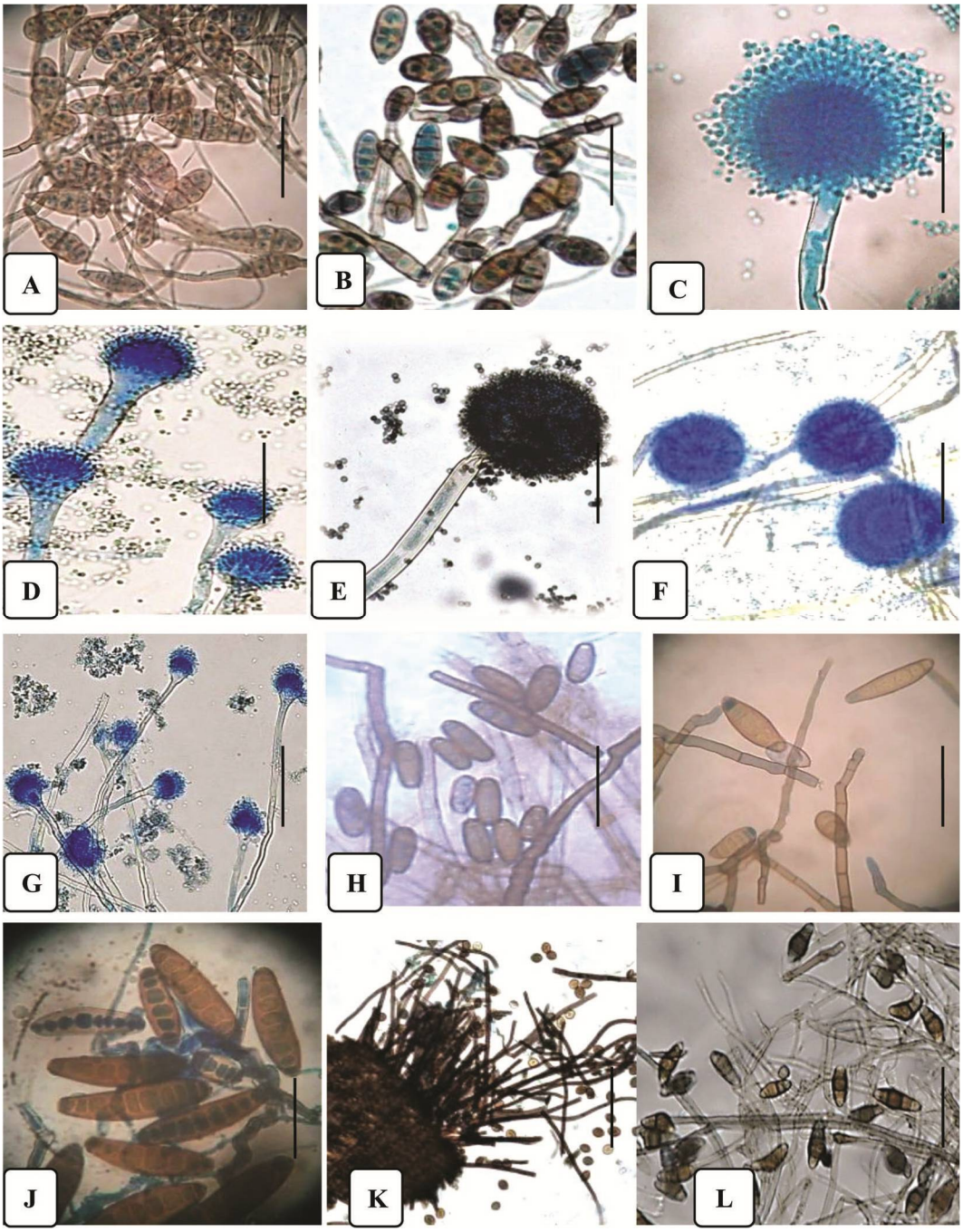

Fig. 1. Conidiophores and conidia of A. *Alternaria alternata*, B. *A. tenuissima*, C. *Aspergillus flavus*, D. *A. fumigatus*, E. *A. niger*, F. *A. ochraceus*, G. *A. terreus*, H. *Bipolaris multiformis*, I. *B. oryzae*, J. *B. sorokiniana*, K. *Chaetomium globosum* and L. *Curvularia lunata*. (Bar = 50 µm).

Bipolaris multiformis (Jooste) Alcorn, Mycotaxon **17**:68 (1983) **(Fig. 1H)**

Colonies effuse, grey, dark blackish brown or black. Conidiophores solitary flexuous or geniculate, septate, pale to mid brown. Conidia straight, ellipsoidal, oblong or cylindrical, rounded at the ends, pale to mid brown. The main axis 14 - 23 × 1.5 - 2.0 µm. The terminal branches are tapering towards the apex. Conidia hyaline, smooth, aseptate, cylindrical, 2 - 14 × 1.5 - 1.8 µm.

Specimen examined: Isolated BRRI dhan 60 variety, BRRI, Joydebpur, Gazipur, T Sultana 130, 17 August, 2017.

Bipolaris oryzae (Breda de Haan) Shoemaker **37**(5): 883 (1959) **(Fig. 1I)**

Colonies on PDA was slowly growing, dark to slightly black becoming cottony towards the margin, zonated and black on the reverse side. Conidiophores were short and long. Conidia were dark brown to oliveceous brown, obclavate, fusiform, 5 - 11 pseudo septa and measured 13.37 - 125.68 ×10.52 - 18.65 µm.

Specimen examined: Isolated from BRRI dhan 64 variety, BRRI, Joydebpur, Gazipur, T Sultana 14, 24 February, 2016.

Bipolaris sorokiniana (Sacc.) Shoemaker Canadian J. Bot. **37**(5): 883 (1959) **(Fig. 1J)**

Colonies olivaceous brown to very dark becoming generally lighter towards the periphery, margin mostly smooth. Conidiophores brown, short, erect, in most cases single, bearing 1 - 6 conidia. Conidia ellipsoid, dark brown, mostly straight or slightly curved, wall thick but less so towards the ends, broadest in the middle, ends rounded, scar clear within the basal cell. Terminal portion of the end cells sub hyaline, 6 - 9 pseudoseptate, 48.0 - 88.6 ×17.2 - 25.8 µm.

Specimen examined: Isolated from BRRI dhan 65 variety, BRRI, Joydebpur, Gazipur, T Sultana 148, 8 December, 2016.

Chaetomium globosum Kunze ex Fr. Systema Mycologicum **3**: 255 (1829) **(Fig. 1K)**

Colonies golden brown, mycelium dark. Perithecia dark brown to black and clothed, especially in the upper part, by dark brown setae. There setae may be simple or branched, straight, wavy or spirally coiled, smooth or ornamented in various ways. The lemon-shaped, brown ascosporous freed from their asci through the ostiole, 5.2 - 6 × 2.8 - 4 µm.

Specimen examined: Isolated from BRRI dhan 56, BRRI, Joydebpur, Gazipur, T Sultana 79, 29 October, 2015.

Curvularia lunata (Wakker) Boedijin, Mycol. Pap. **106**: 2-43 (1966) (**Fig. 1L)**

Colonies effuse, brown, grey or black, hairy, cottony or velvety. Conidiophores solitary, mostly unbranched, straight, mostly flexuous geniculate, mid brown, septate up

to 250 µm. Conidia mostly 3-septate, dark brown, mostly curved, smooth 25.2 - 14.4 × 7.2 - 13.5 µm. *C. lunata* from rice seed along with other pathogens.

Specimen examined: Isolated from BRRI dhan 60 variety, BRRI, Joydebpur, Gazipur, T Sultana 61, 27 March, 2016.

Fusarium equiseti E.J Butler & Hafiz Khan) W. Gams, (1971) (**Fig. 2A)**

Colony white and slightly dark towards the periphery of the Petri dish. Mycelia were hyaline, conidiophores were single, and conidia were hyaline 3 - 4 septa, measuring 68.6 - 165.5 × 10.8 - 16.9 µm.

Specimen examined: Isolated from BRRI dhan 70 variety, BRRI, Joydebpur, Gazipur, T Sultana 55, 25 June, 2017.

Fusarium fujikuroi Gibberellafujikuroi (Sawada) Wollenw., (1931) (**Fig. 2B)**

Colony white, floccus to slightly felt. Conidia were hyaline, fusiform, ovate or clavate; one or two celled, measured 26.7-73.6 × 8.1-17.0 µm. Mycelium sparse to densely floccose or felted. Conidiophores hyaline, 0-2 septate.

Specimen examined: Isolated from BRRI dhan 58 variety, BRRI, Joydebpur, Gazipur, T Sultana 126, 26 October, 2017.

Fusarium oxysporum Schlecht, Flora berol. **2**: 139, (1824) (**Fig. 2C)**

Mycelium delicate white in color in culture plate. Microconidia borne on simple phialides arising laterally on the hyphae. Microconidia generally abundant, variable, oval-ellipsoid, cylindrical, straight, 5 - 12 × 2.2 - 3.5 µm. Macroconidia thin walled, generally 3 - 5 septate, fusoid-subulate and pointed at both ends; 3 septate 27 - 46 × 3 - 5 µm, 5 septate 35 - 60 × 3 - 5 µm.

Specimen examined: Isolated from BRRI dhan 75 variety, BRRI, Joydebpur, Gazipur, T Sultana 10, 02 June, 2016.

Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach& Nirenberg (1976) (**Fig. 2D)**

Colony white to light pinkish, floccose aerial mycelium on PDA. Pigments produced on PDA varied from white, light yellowish-brown to reddish-brown, light pink, light to deep purple brown with or without concentric rings and light violet or deep violet with concentric rings. Macroconidia were hyaline, delicate, slightly sickle-shaped or almost straight, 3-5 septate and produced in sporodochia. The size of macroconidia averaged 17.39 - 38.1 × 1.9 - 3.1 µm. Microconidia were hyaline, 1 - 2 celled, fusiform to oval. The microconidia were agglutinated in short to long chains and or in false heads. Microconidia produced mostly from polyphialides.

Specimen examined: Isolated from BRRI dhan 71 variety, BRRI, Joydebpur, Gazipur, T Sultana 133, 30 October, 2017.

Microdochium fisheri Hern.-Restr. &Crous, Persoonia **36**:68, (2016) (**Fig. 2E)**

Colonies were flat, margin entire, slightly raised to umbonate centre, white with reverse greyish orange. Mycelium was superficial and immersed. Hyphae smooth-walled, septate, branched, hyaline. Conidia solitary, simple, smooth-walled, 1 septate (rarely 2 septate), fusiform, subpyriform to clavate, hyaline, 4.8 - 12 × 1.6 - 3.6 µm apex rounded, base tapering towards a subtruncate and unthickened hilum. Conidia sometimes form a floret appearance on conidiogenous cells. Conidiogenous cells mainly terminal, mono and polyblastic, denticulate, straight or curved, cylindrical to slightly inflated in the median region, 7 - 31.5 × 1.5 - 3 µm, hyaline, smooth. Conidiophores micronematous, arising as lateral, branches from superficial mycelium, smooth-walled, simple to branched, hyaline 12.5 - 90 × 1.4 - 3 µm.

Specimen examined: Isolated from BRRI dhan 71 variety, BRRI, Joydebpur, Gazipur, T Sultana 212, 23 August, 2017.

Nigrospora oryzae (Berkeley & Broome) Petch, J. Indian bot. Soc. 24 (1924) (**Fig. 2F)**

Colonies at first white with small shining black conidia easily visible under a low power dissecting microscope, later brown or black when sporulation is abundant. Conidia solitary, with a violent discharge mechanism, acrogenous, simple, spherical. Conidiophores micronematous or semi macronematous, branched, flexuous, colorless to brown, smooth. Conidiogenous cells 6 - 9 µm diameter.

Specimen examined: Isolated from BRRI dhan 67 variety, BRRI, Joydebpur, Gazipur, T Sultana 141, 20 September, 2017.

Penicillium Link. (Fr.) Sacc. Bur. Anim. Ind., Bul. **118**: 31-33 (1910) (**Fig. 2G)**

Colony on PDA was velvety with areal mycelium and very fast growing and ashy colour. The reverse colour of the plate was yellow to brownish. Conidiophores were smooth, vesiculate, containing phialides, conidia were glubose, 3 - 3.5 µm diameter. Conidia hyaline or brightly colored in mass, one celled, mostly globes or ovoid, produced basipetally.

Specimen examined: Isolated from BRRI dhan 70 variety, BRRI, Joydebpur, Gazipur, T Sultana 196, 11 July, 2015.

Pestalotiopsis oxyanthi (Thum.) Steyaert, Bulletin du Jardin Botanique de l'Etat a Bruxelles **19**(3): 329 (1949) (**Fig. 2H)**

Colonies white, cottony, reverse white. Hyphae septate, branched, hyaline. Acervuli black, small, shining. Conidiophores septate, branched, dark brown, cylindrical or lageniform, formed from the upper cells of the pseudoparenchymata. Conidia fusiform, straight or slightly curved, mostly 3 euseptate: basal cells hyaline, truncate, with an endogenous, cellular appendage: apical cell conic, hyaline, with 2 or more apical, simple

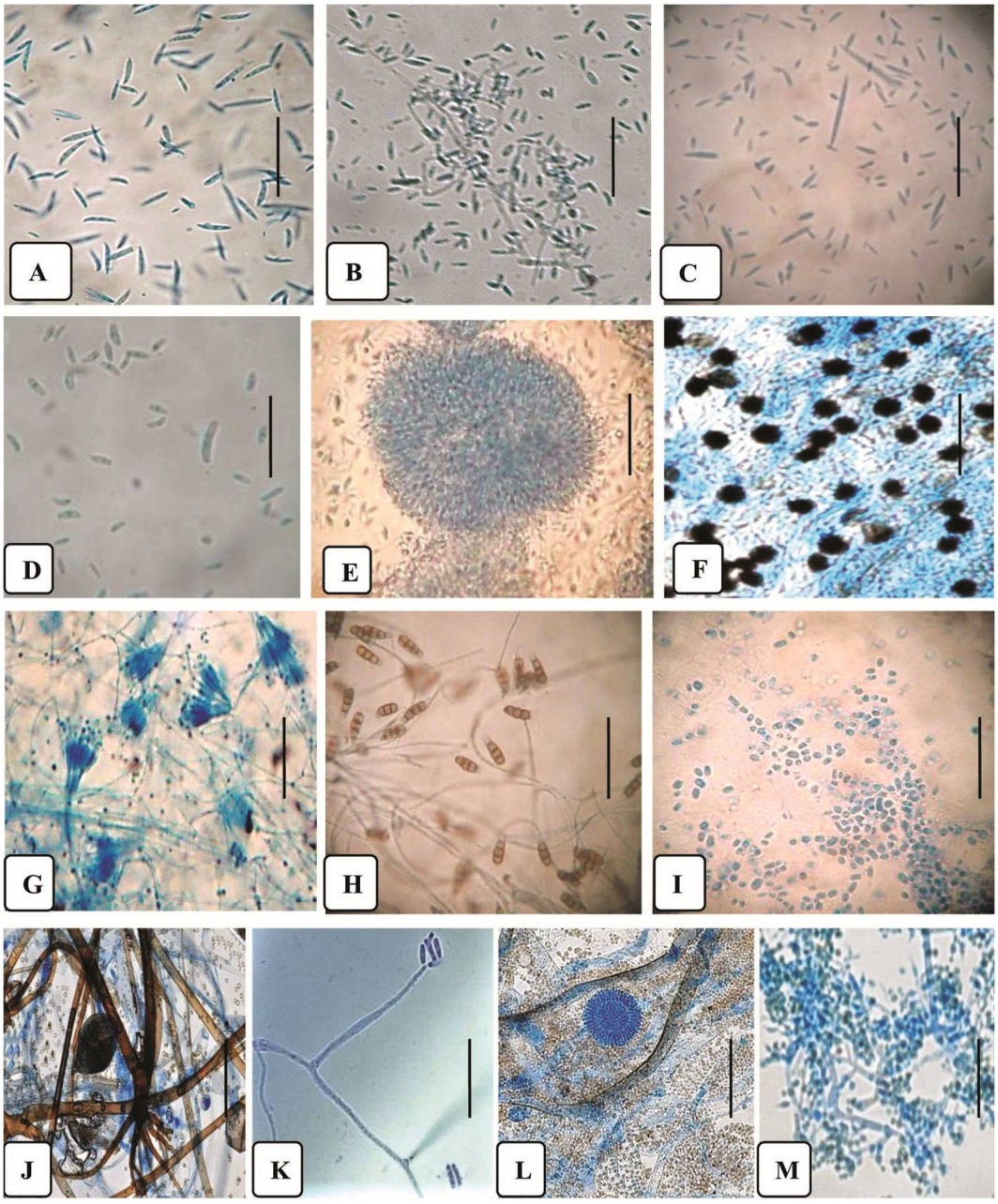

Fig. 2. Conidiophores and conidia of A. *Fusarium equiseti,* B. *F. fugikuroi,* C. *F. oxysporum,* D. *F. proliferatum,* E. *Microdochium fisheri,* F. *Nigrospora oryzae,* G. *Penicillium* sp. H. *Pestalotiopsis oxyanthi,* I. *Phanerochaete chrysosporium,* J. *Rhizopus stolonifera,* K. *Sarocladium oryzae,* L. *Syncephalastrum racemosum* and M. *Trichoderma viride.* (Bar = 50 µm).

or branched, spathulate or espathulate appendages: mediam cells brown, sometimes versicoloured, thicker-walled, smooth, 14 - 23 × 5 - 7.5 µm.

Specimen examined: Isolated from BRRI dhan 73 variety, BRRI, Joydebpur, Gazipur, T Sultana 210, 11 September, 2017.

Phanerochaete chrysosporium Burds., Mycotaxon **1**(2):124 (1974) (**Fig. 2I)**

Mycelium white to gray, abundant in culture; conidia gray or tan in mass, 1 celled, short cylindric to rounded, catenulate, formed acropetally; conidiophores branched, its cell differing little from the older conidia.

Specimen examined: Isolated from BRRI dhan 72 variety, BRRI, Joydebpur, Gazipur, T Sultana 182, 29 December, 2015.

Rhizopus stolonifer (Ehrenb.:Fr.) Vuillemin. Toney Bot.Clup. **69**:592-616. (1902) (**Fig. 2J)**

Colony on PDA was initially white, cottony, mycelium hyaline, aseptate, rhizoids well developed at nodes, sporangiophores arised in clusters, irate, aseptate, light brown, 629.5 - 1002.5 × 5.5 - 11.7 µm. Spores were round to ovule, hyaline or grayish brown, one celled smooth, 3.8 to 6.4 µm in diameter. Columella present. Sporangium produces nonmotile, brownish sporangiospores, 4 - 6 µm in diameter.

Specimen examined: Isolated from BRRI dhan 56 variety, BRRI, Joydebpur, Gazipur, T Sultana 160, 04 April, 2016.

Sarocladium oryzae (Sawada) W. Gams & D. Hawksworth (1976) (**Fig. 2K)**

Colony appears white, compact or cottony, reverse yellowish pink. The fungus produces whitish, sparsely branched, septate mycelia. Conidiophores arising from mycelia slightly thickened from hyphae, branched once or twice, each times with 3 - 4 branches in a whorl. The main axis 14 - 23 × 1.5 - 2.0 µm. The terminal branches are tapering towards the apex. Conidia hyaline, smooth, aseptate, cylindrical 2.0 - 14 × 1.5 - 1.8 µm. Chlamydospores absent.

Specimen examined: Isolated from BRRI dhan 60 variety, BRRI, Joydebpur, Gazipur, T Sultana 309, 21 July, 2017.

Syncephalastrum racemosum Cohn ex J. Schrot. Kryptogamen-Flora von Schlesien 3-1(2): 217 (1886) (**Fig. 2L)**

Colonies transparent, fluffy, grow very rapidly and fill the Petri plate on PDA medium in 48 hours. Mycellium grow rapidly, abundantly branched. Sporangiophores are frequently branched and rather short. Conidiophores erect, branched tips enlarged bearing a head of rod-shaped merosporangia (4 - 6 × 9 - 60 µm) each producing a row of nearly spherical spores, resembling a chain of conidia. Each merosporangium contains a single row of 3 - 18 merosporangiospores.

Specimen examined: Isolated from BRRI dhan 68 variety, BRRI, Joydebpur, Gazipur, T Sultana 110, 21 June, 2016.

Trichoderma viride Pers. Neues Magazin fur die Botanik **1**:92 (1794) (**Fig. 2M)**

Colony effuse, light green. Conidiophores hyaline, much branched, bearing phialides single or in groups. Conidia hyaline, powdery mass, 1 celled, ovoid, borne in small terminal clusters 3.5 - 5 µm, usually easily recognized by its rapid growth and green patches or cushions of conidia. It is used in the commercial production of enzyme cellulase

Specimen examined: Isolated from BRRI dhan 61 variety, BRRI, Joydebpur, Gazipur, T Sultana 218, 21 April, 2016.

Acknowledgement

The first author (TS) expresses her heartfelt gratitude to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for providing financial assistance to this research work through Bangobandhu Science and Technology Fellowship Trust.

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(Manuscript received on 28 April, 2019; revised on 15 September, 2019)