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Original Article

Pathogenic Potentiality of Fungi Isolated from Seeds of Twenty BRRI Released Rice Varieties (*Oryza Sativa* L.)

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ABSTRACT: Twenty-five species of fungi were isolated from the seeds of twenty rice varieties (BRRI dhan 56 to BRRI dhan 75) collected from Bangladesh Rice Research Institute (BRRI). The fungi were isolated from the samples following "Tissue Planting" method and "Blotter" methods. The isolated 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. fujikuroi* 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 stolonifer* (Ehrenb.) Vuill, *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw, *Syncephalastrum racemosum* Cohn ex J. Schrot. and *Trichoderma viride* Pers were isolated from the BRRI rice varieties. Among the isolated fungi, *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikurori, Microdochium fisheri* and *Nigrospora oryzae* showed pathogenic potentiality following seed inoculation technique. These pathogenic fungi had remarkable effect on seed germination, root shoot length and mortality of rice seedlings. The present report is the first record of *Microdochium fisheri* as a pathogenic fungus for Bangladesh.

Keywords: Fungi, Rice seeds, Pathogenic potentiality, Bangladesh

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INTRODUCTION

Rice is one of the most important staple foods for more than half of the world's population and influences the livelihoods and economics of several billion people, and is grown in almost all the tropical and subtropical regions of the world[1]. Rice is cultivated in an area of 165 million hectares. The world rice production is now touching to 744.4 million tones[2]. Rice sector contributes one-half of the agricultural GDP and one-six of the national income in Bangladesh. Almost all of the 13 million farm families of the country grow rice. Thus, rice plays a vital role in the livelihood of the people of Bangladesh. However, the population of Bangladesh is still growing by two million every year. Thus, Bangladesh will require about 27.26 million tons of rice for the year 2020. Rice yield therefore, needs to be increased from the present 2.74 to 3.74 tons/ha. Thus, quality and healthy seed of high yielding rice varieties are very important to achieve this target.

Rice suffers from more than 60 different diseases. In Bangladesh, 43 diseases are known to occur on the rice crop. Among these diseases, 27 are seed borne of which 14 are of major importance [3]. Different seed-borne diseases of rice are bakanae disease, stackburn disease, brown spots, black kernel, blast disease, sheath blight, sheath rot, stem rot and leaf scald and they cause yield reduction, quality deterioration and germination failure[4-6]. It is estimated that about 14-18% yield reduction was caused by these diseases worldwide[7]. Most seed borne diseases caused by fungi al pathogens are disastrous as they may decrease seed germination, cause seed discoloration; produce toxins that may be injurious to man and domestic animals. Several seed borne fungi associated with rice seeds have been isolated in many countries including Nigeria, Pakistan, Egypt Bangladesh, and Cameroon [8-12]. But to the best of our knowledge, no report is available on seed borne pathogenic fungal status of newly released BRRI rice varieties in Bangladesh. Therefore, the present investigation was undertaken to evaluate pathogenic

potentiality of the associated fungi as well as their effect on seed germination, seedling mortality and plant height of (selected BRRI rice varieties) BRRI dhan 56 to BRRI dhan 75 rice seeds.

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 the tenure of 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.

Detection of seed borne fungi from selected variety of rice seeds was done by "Blotter paper method" and "Tissue planting method" [13,14]. Identification of the fungal isolates was determined based on morphological characteristics observed under a compound microscope following the standard literature [15-23].

Pathogenicity test of isolated fungi were done following seed inoculation technique [24]. Four hundred seeds were selected from each variety of rice seed and soaked in distilled water in three beakers for 30 minutes separately and then surface was sterilized with 10% chlorox for 5 minutes. Spore suspension of the test fungus at 10^4 /ml concentration was prepared in a 500 ml sterilized beaker. Two hundred seeds from each variety were placed in 250 ml beakers. Hundred ml of spore suspension with individual spore were added in seeds of each beaker and left undisturbed for 2 hours. Two hundred of each healthy and inoculated seeds of twenty rice varieties were selected and single seed was placed in sterilized 6 inch cotton plugged test tubes containing 10 ml (2% agar) water agar medium. Healthy seeds served as control. Observation was made for 2 weeks at 3 days interval. Germination percentage of seeds, seed mortality, roots and shoot length of seedlings were recorded on healthy and inoculated seeds of twenty rice varieties. After 15 days of inoculation. The pathogens were re-isolated from the inoculated rice seeds and confirmed their identity following Koch's postulates.

Data were evaluated by analysis of variance (ANOVA) by using STAR statistical program and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

A total of 25 species of fungi *viz.*, *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. fujikuroi, F. oxysporum, F. proliferatum, Microdochium fisheri, Nigrospora oryzae, Penicillium* sp., *Pestalotiopsis oxyanthi, Phanerochaete chrysosporium, Rhizopus stolonifer, Sarocladium oryzae, Syncephalastrum racemosum* and *Trichoderma viride* were isolated and identified from the seeds of twenty BRRI rice varieties. The frequency percentage of the aforesaid fungi associated with different rice varieties were recorded by Sultana *et al* (2018)[25]. All the isolated fungi were selected for pathogenicity test.

Out of twenty-five isolated fungi, six fungi showed positive results during pathogenicity test. The pathogenic fungi were *Bipolaris oryzae, Curvularia lunata, Fusarium equiseti, F. fujikuroi, Microdochium fisheri* and *Nigrospora oryzae* (Fig. 1 and 2). These fungi were tested for their pathogenic effects on rice seeds and seedlings. All seedlings developed disease symptoms except control and the pathogens were re-isolated from the infected seedlings.

The effects of test fungi on the seeds of rice are presented in Table 1. All the tested fungi reduced the length of roots and shoots of rice seedling. In control seeds, the average shoot length was 82.33 mm whereas the highest shoot length 52.35 mm was recorded on *Bipolaris oryzae* inoculated seeds and lowest shoot length 31.91 mm was recorded on *curvularia lunata* inoculated seeds Table 1. In control seeds, the average root length was 42.83 mm whereas the highest root length 37 mm was observed in *Fusarium equiseti* inoculated seeds and lowest root length 20.52 was shown by *Bipolaris oryzae* inoculated seeds (Table 1).

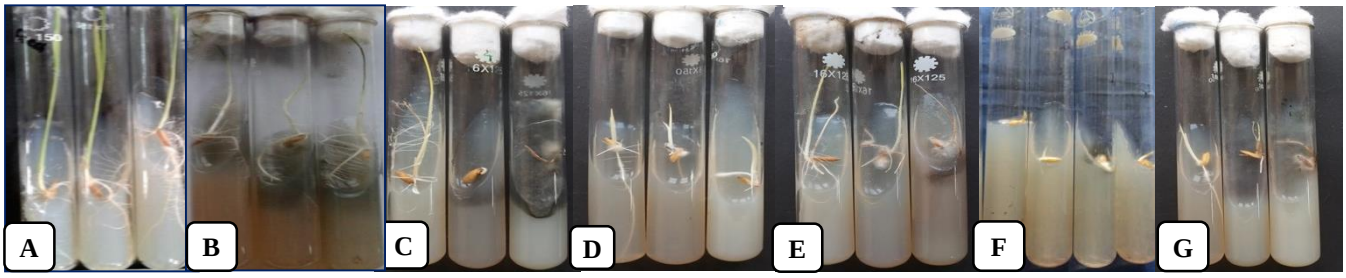

Figure 1. Pathogenicity test of six isolated fungi. A. Control healthy seeds, B-G. Inoculated seeds with *Bipolaris oryzae* (B), *Curvularia lunata* (C), *Fusarium equiseti* (D), *Fusarium fujikuroi* (E), *Microdochium fisheri* (F) and *Nigrospora oryzae* (G).

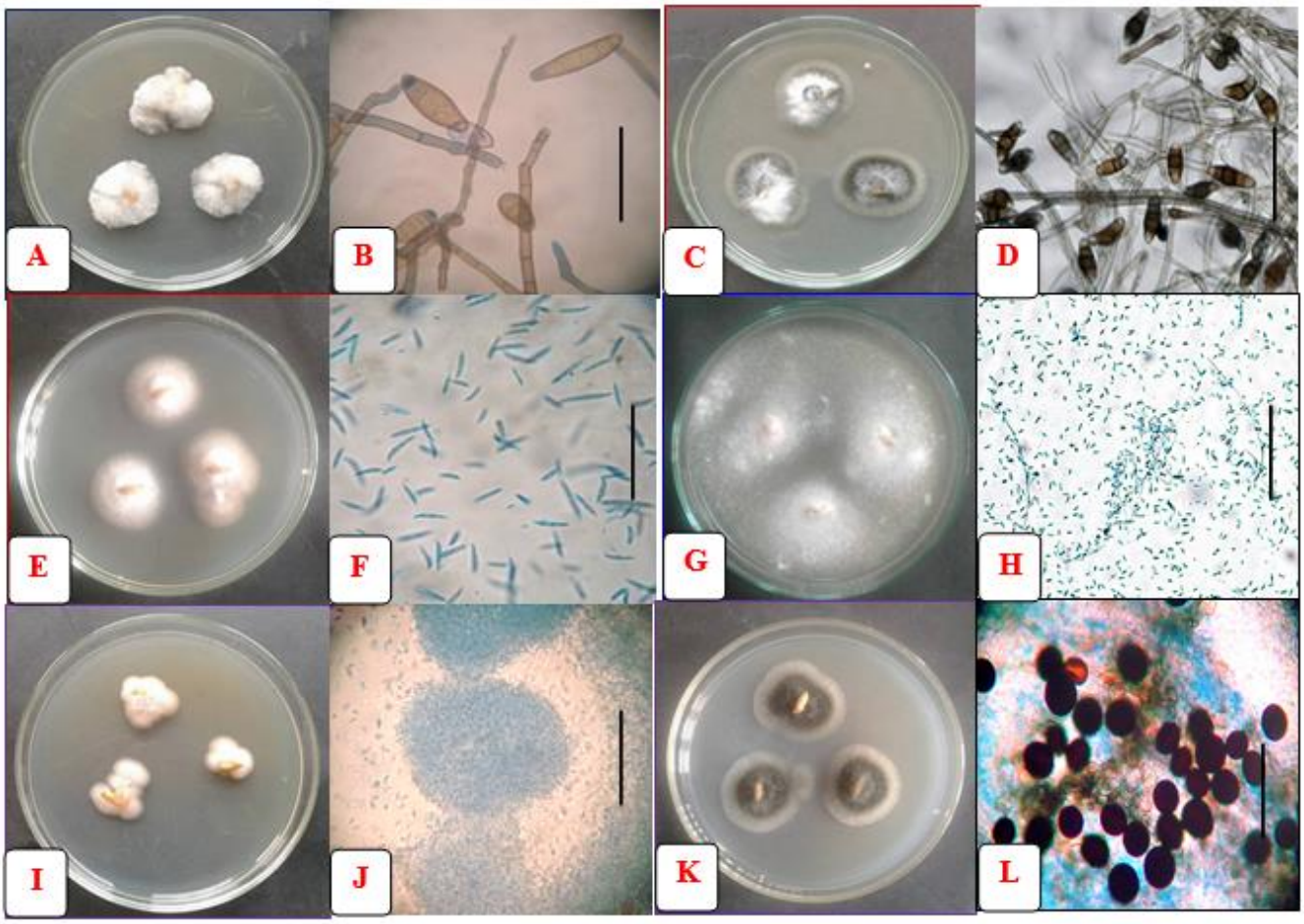

Figure 2. Re-isolated fungal colony and microscopic images of pathogens A-B. *Bipolaris oryzae*, C-D. *Curvularia lunata*, E-F. *Fusarium equiseti*, G-H. *Fusarium fujikuroii*, I-J. *Microdochium fisheri* and K-L. *Nigrospora oryzae.*(Bar = 50 µm).

Table 1. Effects of pathogenic fungi on different parameters of rice seeds.

Treatments	Germination percentage	Mortality percentage	Average root length (mm)	Average shoot length (mm)
Control	90^{a}	25.67^{d}	42.83^{a}	82.33^{a}
Bipolaris oryzae	77.67^{b}	40.83^{ab}	20.52^{f}	52.35^{b}
Curvularia lunata	60.00^{d}	48^{a}	30^{c}	31.91^{e}
Fusarium equiseti	73.67^{b}	36.33^{bc}	37^{b}	45b^{cd}
Fusarium fujikuroi	67.67^{c}	27.67^{d}	27^{cd}	39de
Microdochium fisheri	52^{e}	42.33^{ab}	21.53^{ef}	48.54^{bc}
Nigrospora oryzae	38^{f}	35^{c}	24.46^{de}	42^{cd}
CV%	2.99	6.75	4.36	5.49

*Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

In control seeds showed 90 per cent germination whereas the highest germination percentage was 77.67% in *Bipolaris Oryzae* inoculated seeds and the lowest germination percentage was 38% in *Nigrospora Oryzae* inoculated seeds (Table 1). Non inoculated control seeds showed 25.67% seedling mortality whereas the highest mortality percentage was 48% in *Curvularia lunata* inoculated seeds and the lowest mortality percentage was 27.67% in *Fusarium fujikuroi* inoculated seeds (Table 1).

An extensive study of literatures revealed that *Microdochium fisheri* has not been reported in any relevant literatures in Bangladesh. Hence, *Microdochium fisheri* Hern.-Restr. & Crous. is reported here as first time from Bangladesh [26-29].

The Taxonomic description of Microdochium fisheri is given bellow:

Microdochium fisheri Hern.-Restr. & Crous, Persoonia **36**: 68 (2016) **(Fig. 1. I-J)**

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 and hyaline. Conidia solitary, simple, smooth-walled, 1 septate (rarely 2 septate), fusiform, sub-pyriform to clavate, hyaline, 4.8-12×1.6-3.6 µm apex rounded, base tapering towards a sub-truncate and un-thickened 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.

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

Seed borne pathogens cause enormous losses to our crop. Seed borne disease causes seed rot, germination failure and seedling mortality and then reduce rice production. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants. Most pathogens causing abnormal seedling of rice are seed borne[30]. Seed borne pathogens affect seed quality[31]. The highest lethal seed infection caused by *Fusarium moniliforme*, *Trichoconis padwickii* and *Curvularia* spp. observed by Islam *et al.* (2000) [32].

This results of the present iinvestigation will be useful for designing control measure of seed borne pathogens and production of healthy seeds of rice.

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