IN VITRO CONTROL OF PATHOGENIC FUNGI ASSOCIATED WITH SELECTED BRRI RICE VARIETIES

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ABSTRACT

The present study evaluated the efficacy of five chemical fungicides, *viz.,* Capvit 50 WP, Cynil 72 WP, Kochi 80 WDG, Sinozim 50 WP and Ridomil Gold MZ 68 WG along with five plant extracts, specifically *Azadirachta indica* A. Juss., *Adhatoda vasica* Nees., *Psidium guajava* L., *Cassia alata* L. and *Citrus limon* on the radial mycelial growth of two pathogenic fungi, *Curvularia lunata* and *Drechslera oryzae* associated with newly released BRRI rice varieties at varying concentrations. Capvit 50 WP exhibited complete inhibition of *C. lunata* at 300, 400 and 500 ppm concentrations as well as *D. oryzae* at 200, 300, 400 and 500 ppm concentrations leading the selected fungicides, followed by Cynil 72 WP and Ridomil Gold MZ 68 WG. Out of the five plant extracts, *P. guajava* demonstrated maximum radial growth inhibition (72.2%) against *C. lunata* at 20% concentration followed by *A. indica*. (56%), *A. vasica*. (52.3%), *C. alata* (24%) and *C. limon* (18.1%). Capvit 50 WP and Cynil 72 WP, among the fungicides, are potential *in vivo* controls for the tested pathogens associated with rice varieties. Additionally, out of the five plant extracts, *Psidium guajava* L. can be effective against *C. lunata*, while *Azadirachta indica* A. Juss. is a promising botanical fungicide against *D. oryzae*.

KEYWORDS: Pathogenic fungi, *In vitro*, Fungicides, Plant extracts, BRRI rice varieties

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Introduction

Rice (*Oryza sativa* L.) is a staple food for thirty-three per cent of the world's population[1] and it is the most important food crop in the context of Bangladesh. In Bangladesh, approximately 2.5 million tons of rice worth more than Tk. 12000 million is damaged annually due to diseases caused by seed borne pathogens[2] and most of the major diseases of rice are seed borne[3].

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. During storage a lot of storage fungi grows in the rice seeds which causes seed health deterioration, reduction in seed germination rate, post-germination diseases and ultimately yield loss. Some of the major diseases of rice caused by fungi are brown spot (*Drechslera oryzae* and *Bipolaris sorokiniana*), bakane (*Fusarium moniliforme*), blast (*Pyricularia oryzae*), sheath rot (*Sarocladium oryzae*), sheath blight (*Rizoctonia solani*) etc.

Currently Bangladesh has achieved food security by cultivating high-yielding rice varieties. According to BRRI, there are total of 94 hybrid rice varieties and more than 78 hybrid rice varieties are grown in the field level[4]. In Bangladesh, several researches have been done separately on fungal association with BRRI rice varieties and their management up to BRRI dhan77 in different time span[5-7]. However, BRRI dhan78 to BRRI dhan89 have been released from BRRI, but so far, no remarkable work has been done on seed borne diseases associated with the newly released varieties. Therefore, with a view to identifying and finding out the management of the mycoflora associated with the newly released BRRI rice varieties in storage condition, the present research work is done.

Materials and Methods

Seed samples of ten (500 gm each) newly released varieties (BRRI dhan 78 – BRRI dhan 89, except BRRI dhan 79 and BRRI dhan 85) were collected from Bangladesh Rice Research Institute, Joydebpur, Gazipur in May 2019. The samples were then kept in brown paper bags and stored in airtight containers at room temperature (25°C ± 2°C).

Fungal isolation was done using 'Blotter Method'[8] and 'Tissue Planting Method'[9]. Identification of the fungal isolates was determined based on morphological characteristics observed under a compound microscope following the standard literatures[10-14].

The evaluation of the isolated fungi's pathogenicity was conducted using Koch's postulates criteria and the seed inoculation method outlined by Reddy and Subbayya (1989)[15]. A comprehensive description of this test can be found in our previously published paper[16].

Five fungicides namely, Capvit 50 WP, Cynil 72 WP, Kochi 80 WDG, Sinozim 50 WP and Ridomil Gold MZ 68 WG were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. For each fungicide, a stock solution having the concentration of 10,000 ppm was prepared. The calculated amount of stock solution of a fungicide was supplemented with sterilized PDA medium to get the concentration of 100, 200, 300, 400 and 500 ppm, respectively. The concentrations of fungicides were expressed in terms of its active ingredients. In control set, required amount of sterilized water instead of fungicide solution was added to the PDA medium. Then 15 ml of medium was poured in each Petri plate and allowed them to solidify. *In vitro* fungitoxicity of these fungicides at 100, 200, 300, 400 and 500 ppm concentrations were evaluated against pathogenic fungi according to Chowdhury *et al.* (2015)[17].

Fresh leaves of five angiospermic plants namely, *Azadirachta indica* A. Juss., *Adhatoda vasica* Nees., *Psidium guajava* L., *Cassia alata* L. and *Citrus limon* were selected for evaluating their efficacy on the radial mycelial growth of pathogenic fungi. Leaves of the selected plants were collected from the Botanical Garden of Curzon Hall Campus, University of Dhaka and identified by a taxonomist and voucher specimens of collected leaves were preserved in Herbarium, Department of Botany, University of Dhaka.

Leaves of each plant were thoroughly washed in tap water, air dried and then used for fresh extract preparation. Leaf extracts were prepared by crushing known weight of fresh leaves with distilled water in ratio of 1:1 (w/v). In this method, the requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10, 15 and 20% concentrations. The medium thus prepared was poured into sterilized Petri plates and allowed to solidify. Each Petri plate was inoculated centrally with a 5 mm agar disc cut from the margin of actively growing culture of the test pathogens. In the control set, a Petri plate containing PDA medium with the requisite amount of distilled water instead of a plant extract was also inoculated with agar disc of the test pathogen in the same manner as described above. Three replications were maintained for both the experimental and control sets. The inoculated Petri plates were incubated at 25 ± 2˚C. The radial growth of the colonies of the test pathogen was measured after 5 days of incubation. The fungitoxicity of the fungicides and plant parts extracts in terms of percentage inhibition of mycelial growth were calculated by using the following formula: $I = \frac{C-T}{T} \times 100$ where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment. The data were collected as inhibition percentage of the radial growth of the pathogen in mm in each replication and evaluated by standard error of the mean (±SEM) with significant level at $p<0.001$, as determined by a Student's t-test.

Results and Discussion

During the tenure of July 2019-November 2019 a total of twelve seed borne fungi (having 6 genus) were isolated from selected BRRI rice varieties by using 'tissue planting' method. The isolated fungi were *Alternaria* sp., *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A. ochraceous* Wilhelm, *A. terreus* Thom, *Curvularia lunata*, *Curvularia* sp., *Drechslera oryzae*, *Penicillium* sp. 1, *Penicillium* sp. 2 and *Rhizopus* sp.

Moreover, six seed borne fungi such as *Aspergillus flavus* Link, *A. niger* van Tieghem, *C. lunata*, *D. oryzae*, *Penicillium* sp. and *R. stolonifer* were isolated from the same varieties by using 'blotter' method during the tenure of study. *A. niger* was the most prevalent fungi among all the varieties examined[16].

Through pathogenicity test, *Curularia lunata* and *Drechslera oryzae* were found to be pathogenic to selected BRRI rice varieties (Fig. 1). Fungicides and antifungal activity of angiospermic plant extracts were used in *vitro* control of pathogenic fungi *Curvularia lunata* and *Drechslera oryzae* isolated from selected BRRI rice varieties.

Chowdhury *et al.* (2015)[17] also found *C. lunata* and *D. oryzae* as pathogenic along with *Alternaria alternata*, *Fusarium moniliforme* and *Pestalotiopsis guepinii* on two rice varieties *viz.*, BRRI 29 (Boro) and Pajam (Aman).

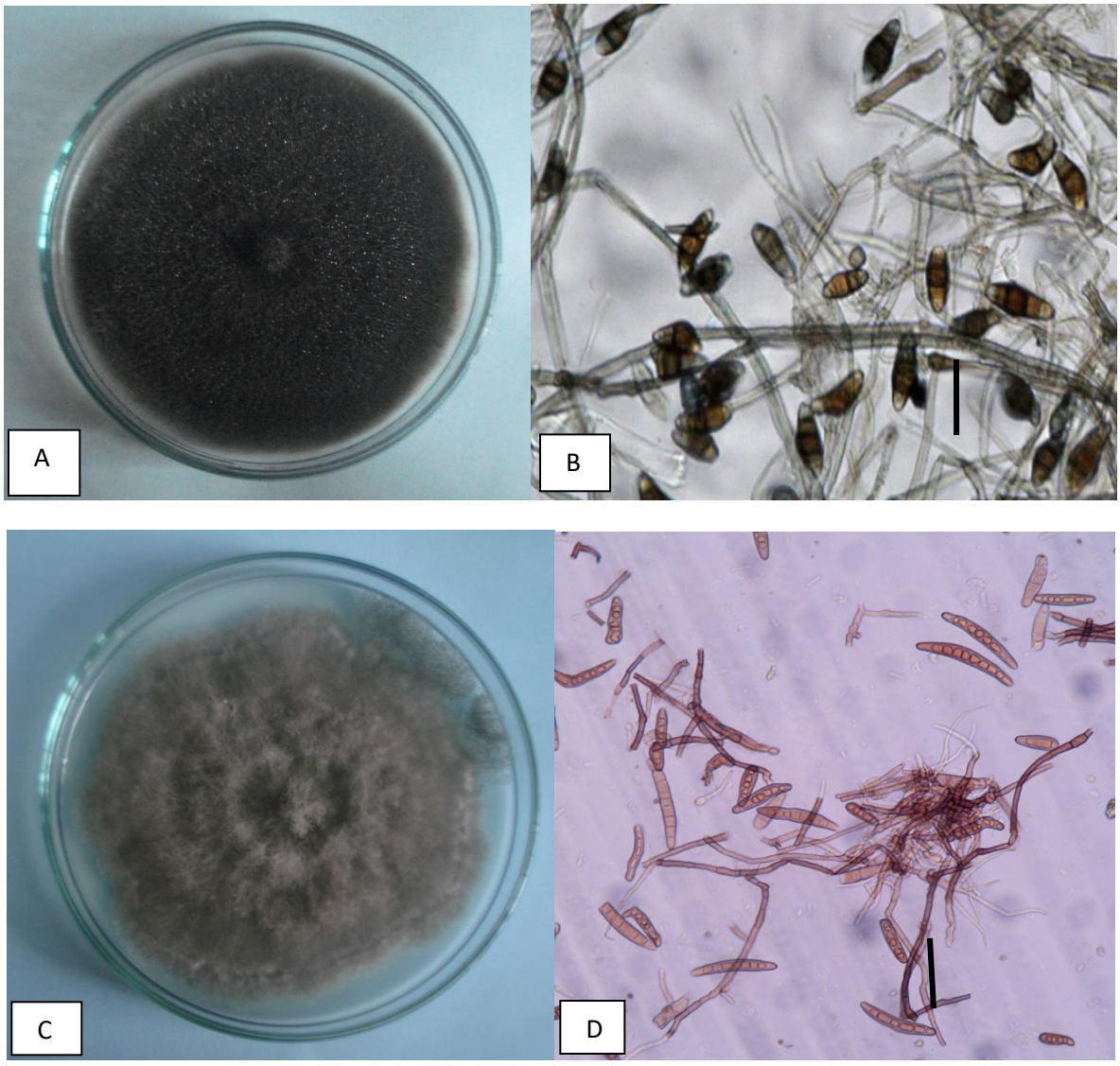

Figure 1. Colony, Mycelia, conidiophores and conidia of A-B. *C. lunata* and C-D. *D. oryzae*. (Bar = 50 µm).

Results of the effect of fungicides and plant extracts on the radial growth of *C. lunata* and *D.oryzae* are presented in Figs. 2 and 3. Amongst five selected fungicides, Capvit 50 WP showed complete inhibition of *C. lunata* at 300, 400 and 500 ppm concentrations and *D. oryzae* at 200, 300, 400 and 500 ppm concentrations followed by Cynil 72 WP and Ridomil Gold MZ 68 WG (Fig. 2).

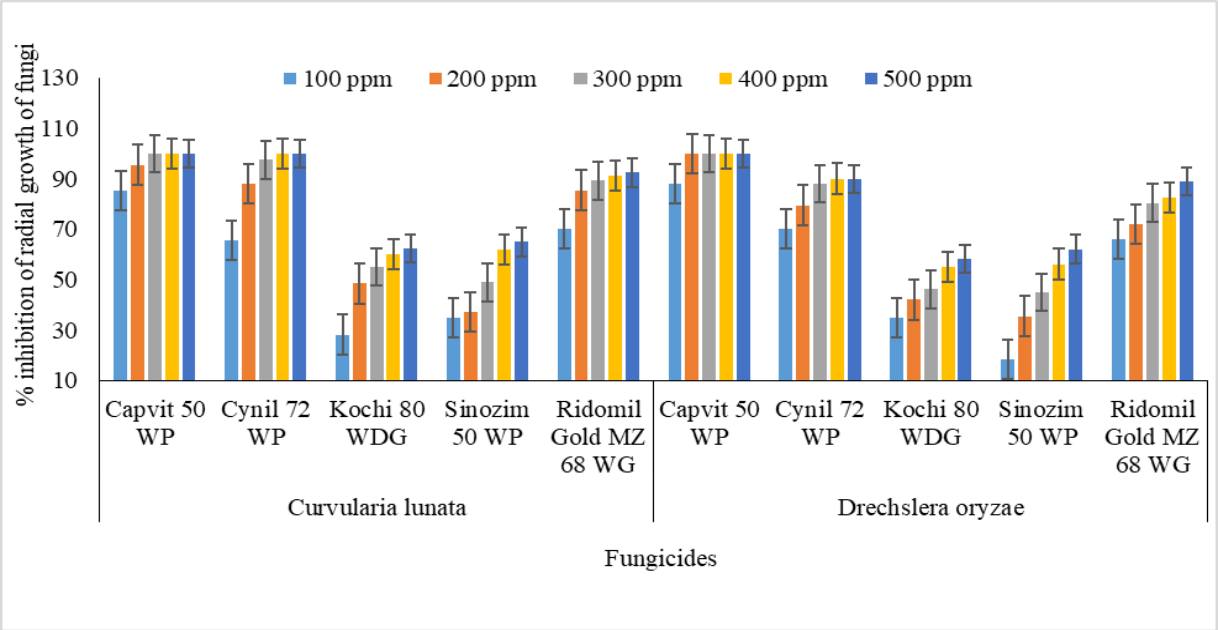

Figure 2. Per cent inhibition of radial growth of *C. lunata* and *D. oryzae* owing to fungicides at different concentrations.

Previously, Nahar and Shamsi (2020)[18] evaluated *in vitro* efficacy of five fungicides *viz.*, Acrobat MZ, Autostin 50 WDG, Capvit 50 WP, Nativo 75 WP and Thiovit 80 WG against *C. lunata* isolated from cotton seeds and found that Nativo completely inhibited radial growth of the tested fungus at all the concentrations used. In their study, Capvit 50 WP completely inhibited the radial growth of the fungus at 400 and 500 ppm concentration. It is evident from the results that the per cent growth inhibition of the test pathogens gradually increased with the increase in concentration of the fungicides. Leaf extracts of *A. indica*, *A. vasica*, *P. guajava*, *C. alata* and *C. limon* were evaluated against rice pathogens *C. lunata* and *D. oryzae*. Extracts of selected plant leaves showed varied degrees of growth inhibition of the test pathogens at different concentrations (Fig 3).

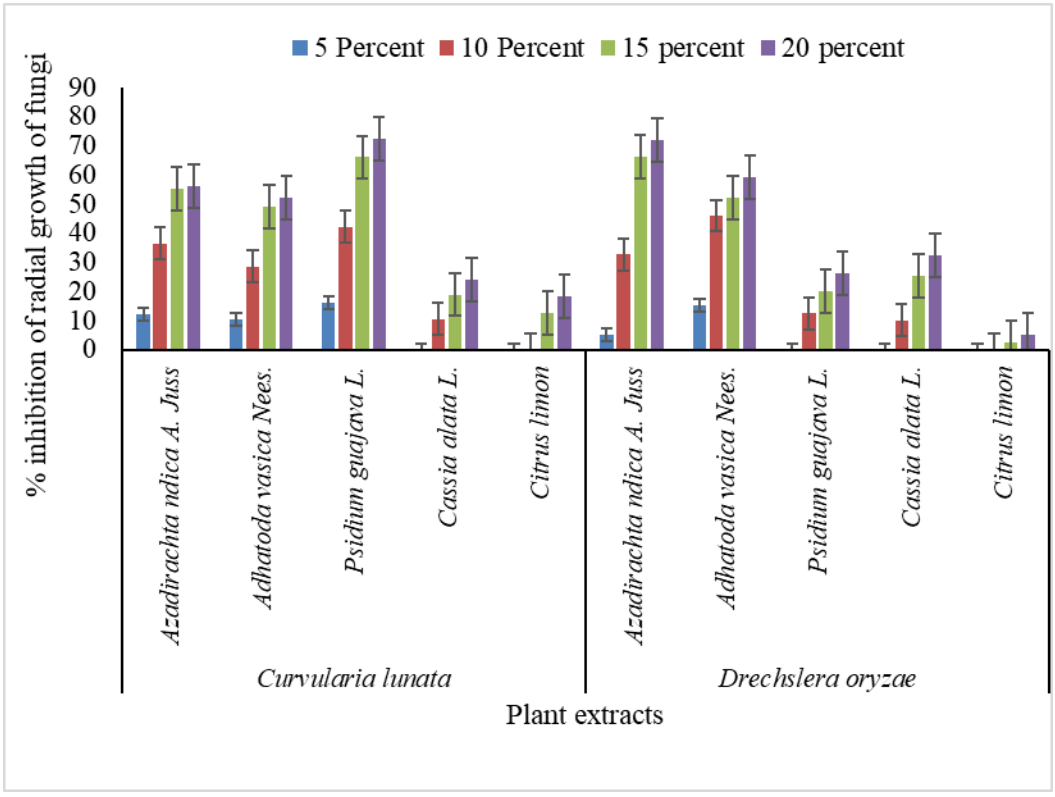

Figure 3. Per cent inhibition of radial growth of *C. lunata* and *D. oryzae* owing to plant extracts at different concentrations.

Out of the five plant extracts, *P. guajava* showed maximum radial growth inhibition (72.2) of *C. lunata* at 20% concentration followed by *A. indica*. (56%), *A. vasica*. (52.3%), *C. alata* (24%) and *C. limon* (18.1%). Similar type of result was reported by Nahar and Shamsi (2020)[18] who evaluated leaf extract of five angiospermic plants *viz., A. vasica*, *A. mermelos*, *A. indica*, *D. metel* and *P. guajava* and found *Psidium guajava* most active against *C. lunata* isolated from hill cotton. In the present study, *A. indica* showed maximum radial growth inhibition of *D. oryzae* (72%) at 20% concentration followed by *A. vasica* (59.25%), *C. alata*. (32.25%), *P. guajava*. (26%) and *C. limon* (5.25%). *Citrus limon* showed poor performance for both the tested pathogens. It is also clear from the results that the percent inhibition of the pathogens increases with the increase of the concentration of the plant extracts in culture medium. However, Chowdhury *et al*. (2015)[16] evaluated ten plant extracts against *C. lunata* and *D. oryzae i*solated from two rice varieties namely BRRI 29 and Pajam and found complete inhibition of the radial growth of *C. lunata* and *D. oryzae* at 20% concentration.

The present investigation suggests that out of five fungicides Capvit 50 WP is identified as the best inhibiting fungicides for pathogen C. *lunata* and *D. oryzae*. Cynil 72 WP completely inhibited *C. lunata*. *Psidium guajava* showed maximum inhibition of *C. lunata* and *A. indica* showed maximum inhibition of *D. oryzae.*

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