IN VITRO CONTROL OF PATHOSENIC FUNGI ASSOCIATED WITH SELECTED SEEDS OF BRRI RICE VARIETIES

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ABSTRACT

The present study evaluated the efficacy of five chemical fungicides, viz., Capvit 50 WP, Knowin 50 WP, Ridomil Gold MZ 68, Thiovit 80 WG and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm concentrations against pathogenic fungi of selected rice varieties BRRI dhan 90 to BRRI dhan 99. Pathogenic fungi were *Aspergillus niger*, *Curvularia lunata*, and *Fusarium solani.* In this study, out of five fungicides Tilt 250 EC showed the complete growth inhibition of above mentioned three pathogenic fungi at all the concentrations used. Knowin 50 WP completely inhibited radial growth of *A. niger* at all the concentration used. Knowin 50 WP, also showed 94.00 % inhibition of the radial growth of *F. solani* at 100 ppm concentration. The fungicide showed complete inhibition of *F. solani* at 200, 300, 400 and 500 ppm concentrations used.

KEYWORDS: *In vitro* control, Pathogenic fungi, Selected BRRI rice seeds, Bangladesh

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Introduction

In Bangladesh, several researches have been done separately on fungal association with BRRI rice varieties and their management up to BRRI dhan 89, except BRRI dhan 79 and BRRI dhan 85) in different time span [1-3]. A total of 19 fungal species were isolated from the seeds of selected rice varieties (BRRI dhan 90 to BRRI dhan 99) following 'Tissue planting method' and 'Blotter method'.The isolated fungi were *Aspergillus niger*, *A. ochraceus*, *A. oryzae*, *A. tamarii*, *A. terreus*, *Chaetomium globosum*, *Cladosporium oxysporum*, *Colletotrichum gloeosporioides*, *Corynespora cassiicola*, *Curvularia lunata*, *Curvularia soli*, *Daldinia eschscholtzii*, *Fusarium solani*, *Penicillium oxalicum*, *Penicillium sclerotiorum*, *Pestalotiopsis guepinii*, *Pyricularia oryzae*, *Rhizopus stolonifer* and *Trichoderma veredi* (Nishi *et al*. 2024)[4]. Out of 19 species of fungi *Aspergillus niger, Curvularia lunata*, and *Fusarium solani were* found to be pathogenic to the selected rice varieties (Nishi and Shamsi (2026)[5].

The study targets BRRI dhan 90 to BRRI dhan 99, which represent modern, recently released rice varieties in Bangladesh. Identifying effective crop protection measures for these specific seeds offers direct value to local farmers and agricultural expansion officers.

Material and Methods

Ten varieties of BRRI rice seeds i.e. BRRI dhan 90 to BRRI dhan 99 were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. Samples were collected during August 2021. Fungi associated with selected BRRI rice varieties were isolated with following "Tissue planting method" on PDA medium and "Blotter Method[6-7]. Identification of the isolates was determined following standard literatures[8-14]. The specimens were preserved in the Herbarium, Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh.

***In vitro* fungitoxicity assessment of fungicides against the test pathogens**

Five fungicides with different active ingredients viz., Knowin 50 WP, Thiovit 80 WG, Ridomil Gold MZ 68, Capvit 50 WP and Tilt 250 EC were collected from the Siddique Bazar Gulistan, Dhaka (Table 1). *In vitro* fungitoxicity of these fungicides at 100, 200, 300, 400 and 500 ppm concentration were evaluated against pathogenic fungi isolated from selected rice varieties *Aspergillus niger, Curvularia lunata*, and *Fusarium solani.*

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. Therefore, at the center of the plate 5mm agar disk of test pathogen was inoculated. Three replications were maintained in each case. The plates were incubated at 25±2°C in an incubator. The radial growth of control and treatment plates were measured at 5 days of incubation [15].

The growth inhibition of each test fungi was calculated by using the following formula:

$$I = (C-T) / C \times 100$$

Where, I = Percent growth inhibition

C = Growth in control

T = Growth in treatment.

Table 1. Particulars of the fungicides used in the study

SL No.	Fungicides	Active ingredients (s)	Manufacturer
1.	Knowin 50 WP	50% Carbendazim	Sundat (S) Pte. Ltd.
2.	Thiovit 80WG	Sulphur	Syngenta (BD) Ltd.
3.	Ridomil Gold MZ 68	4% Metalaxyl and 64% Mancozeb	Syngenta Production, France.
4.	Capvit 50 WP	Copper oxychloride	Jiangsu Hong Ze Chemical and Industry Company Limited, China.
5.	Tilt 250 EC	25% Propiconazole	Syngenta Crop Production, Switzerland

Analysis of data

Data on different parameters were analyzed following computer package MSTAT-C and means were compared using Duncan's Multiple Range Test (DMRT).

The experiment was performed twice. The data were collected as inhibition percentage of the radial growth of the pathogen in mm in each replication and evaluated by analysis of variance (ANOVA) by using STAR statistical program.

Results and Discussion

The efficacy of five chemical fungicides, viz., Capvit 50 WP, Knowin 50 WP, Ridomil Gold MZ 68, Thiovit 80 WG, and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm concentrations were evaluated against there pathogenic fungi of selected rice varieties BRRI dhan 90 to BRRI dhan 99. Pathogenic fungi were *Aspergillus niger*, *Curvularia lunata*, and *Fusarium solani.*

A total of 19 fungal species were isolated from the seeds of selected rice varieties (BRRI dhan 90 to BRRI dhan 99) following 'Tissue planting method' and' Blotter method'.The isolated fungi were *Aspergillus niger*, *A. ochraceus*, *A. oryzae*, *A. tamarii*, *A. terreus*, *Chaetomium globosum*, *Cladosporium oxysporum*, *Colletotrichum gloeosporioides*, *Corynespora cassiicola, Curvularia lunata, Curvularia soli, Daldinia eschscholtzii, Fusarium solani, Penicillium oxalicum*, *Penicillium sclerotiorum*, *Pestalotiopsis guepinii*, *Pyricularia oryzae*, *Rhizopus stolonifera* and *Trichoderma viride*. Out of 19 species of fungi *Aspergillus niger, Curvularia lunata*, and *Fusarium solani were* found to be pathogenic to the selected rice varieties Fig. 1**.** A,B and C..

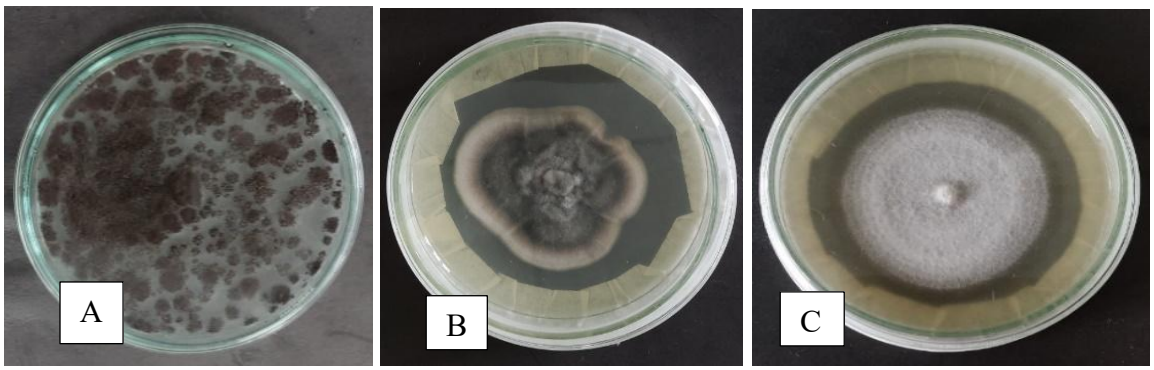

Figure 1. Colony on PDA medium A. Aspergillus niger B. Curvularia lunata and C. *Fusarium solani*

Effect of selected fungicides on three pathogenic fungi are presented in Table 2-4 and Figure 2-5.

Fungitoxicity of fungicides against *Aspergillus niger*

Table 2. Per cent inhibition of radial growth of *Aspergillus niger* at different concentrations

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Capvit 50 WP	45.35^{c}	62.57^{c}	64.08^{c}	69.85^{c}	75.44^{c}
Known 50 WP	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Ridomil Gold MZ 68	59.78^{b}	67.56^{b}	69.87^{b}	73.47^{b}	67.56^{b}
Thiovit 80 WG	0^{d}	0^{d}	5.3^{d}	10.64^{d}	18.08^{d}
Tilt 250 EC	100a	100a	100a	100a	100a
CV (%)	1.35	1.53	0.65	0.82	1.2

Mean followed by same letter within a column did not differ significantly at 5% level By DMRT

Remarks of efficiency gradient of fungicides against *Aspergillus niger* at 100 ppm concentration: Tilt 250 EC / Knowin 50 WP > Ridomil Gold MZ68 > Capvit 50 WP >Thiovit 80 WG.

Out of five fungicides complete inhibition of *Aspergillus niger* was observed with Tilt 250EC and Knowin 50WP at all the treated concentrations. At 500 ppm concentration Ridomil Gold MG 68 and Capvit 50WP showed 67.56 and 75.44 % radial growth inhibition of the fungus. At 100 ppm concentration Capvit 50 WP showed lowest growth inhibition of the fungus and that was 45.35%, Thiovit 80 WG did not showed any growth inhibition of *A. niger* colony at 100 ppm and 200 ppm. (Table 2 and Fig. 2-3). The toxicity of the fungicides against *A. niger* at 100 ppm concentration was in descending order was Tilt 250 EC / Knowin 50 WP > Ridomil Gold MZ 68 > Capvit 50 WP >Thiovit 80 WG.

Fungitoxicity of fungicides against *Curvularia lunata*

Table 3. Per cent inhibition of radial growth of *Curvularia lunata* at different concentrations

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Capvit 50 WP	32.57^{b}	59.43^{b}	75.01^{b}	97.00^{a}	100^{a}
Known 50 WP	38.92^{c}	56.63^{c}	60.78^{c}	67.85^{b}	74.14^{b}
Ridomil Gold MZ 68	35.67^{c}	50.79^{d}	58.56^{d}	66.45^{b}	96.00^{a}
Thiovit 80 WG	28.82^{bc}	34.23^{e}	40.55^{e}	51.12^{c}	63.20^{c}
Tilt 250 EC	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV (%)	6.49	3.57	1.81	2.05	3.25

Mean followed by same letter within a column did not differ significantly at 5% level By DMRT

Remarks of efficiency gradient of fungicides against *Curvularia lunata* at 100 ppm concentration: Tilt 250 EC > Knowin 50 WP > Ridomil Gold MZ 68 > Capvit 50 WP > Thiovit 80 WG.

The radial growth of *C. lunata* was completely inhibited by Tilt 250 EC at all the treated concentration. Ridomil Gold MZ 68, Knowin 50 WP and Thiovit 80 WG showed 96.00, 74.14 and 63.20% inhibition of the fungus at 500 ppm concentration. The lowest activity (28.82%) was shown by Thiovit 80WG at 100 ppm concentration. The toxicity of these fungicides against *C. lunata* at 100 ppm concentration in descending order was Tilt 250 EC > Knowin 50 WP > Ridomil Gold MZ 68 > Capvit 50 WP > Thiovit 80 WG. (Table 3and Fig. 4-5).

Fungitoxicity of fungicides against *Fusarium solani*

Table 4. Per cent inhibition of radial growth of *Fusarium solsni* at different concentrations

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)				
	100	200	300	400	500
Capvit 50 WP	15.27^{d}	28.62^{d}	39.43^{d}	48.56^{c}	67.33^{d}
Known 50 WP	94.00^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Ridomil Gold MZ 68	35.63^{b}	45.89^{b}	6.970^{b}	67.54^{b}	72.93^{b}
Thiovit 80 WG	23.65^{c}	34.85c	47.73^{c}	50.55^{c}	57.34^{c}
Tilt 250 EC	100a	100a	100a	100a	100a
CV (%)	5.16	3.66	3.11	3.43	2.51

Mean followed by same letter within a column did not differ significantly at 5% level By DMRT

Remarks of efficiency gradient of fungicides against *Fusarium solani* at 100 ppm concentration: Tilt 250 EC > Knowin 50 WP > Ridomil Gold MZ 68 > Capvit 50 WP> Thiovit 80 WG.

Tilt 250 EC showed complete inhibition of radial growth of *Fusarium solani* at the treated concentrations. Knowin 50 WP, showed 94.00 % inhibition of the radial growth of the fungus at 100 ppm concentration. The fungicide showed complete inhibition of *F. solani* at 200, 300, 400 and 500 ppm concentrations used. At 500 Ridomil Gold MZ 68, Capvit 50 WP and Thiovit 80 WG. showed 72.93, 67.33 and 57.34% radial growth inhibitions of the fungus. At 100 ppm concentration Capvit 50 WP showed the lowest radial growth inhibition of the fungus 15.27%. (Table 4 and Fig.5) The fungitoxicity of the selected fungicides against *F. solai* at 100 ppm concentration in descending order was Tilt 250 EC, Knowin 50 WP, Ridomil Gold MZ 68, Capvit 50 WP and. Thiovit 80 WG.

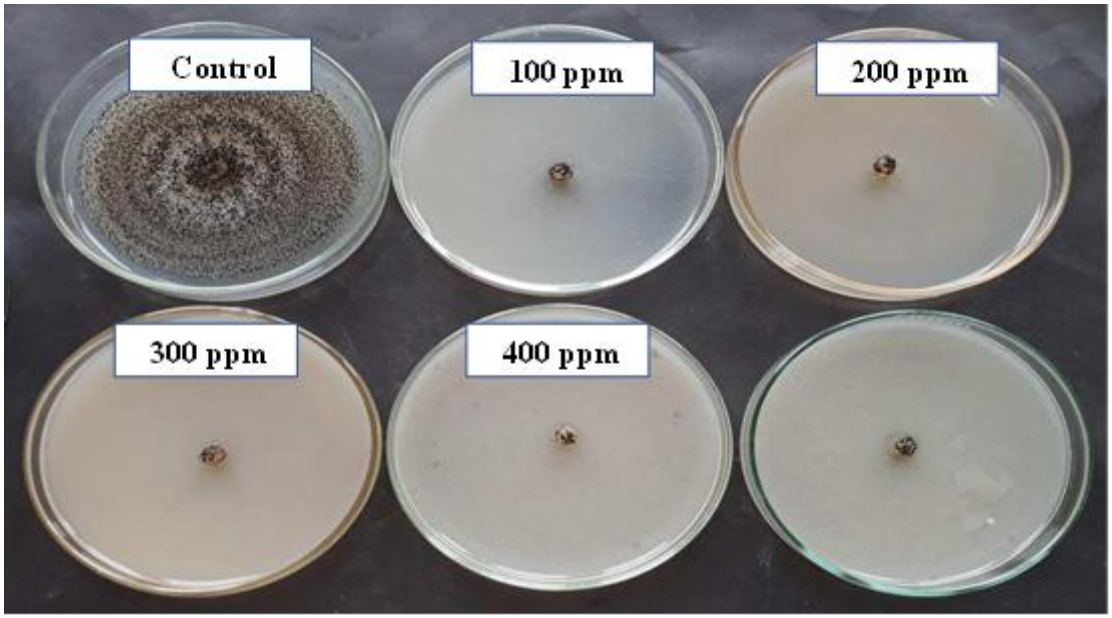

Figure 2. Effect of Knowin 50 WP on the radial growth of *Aspergillus niger* at different concentrations.

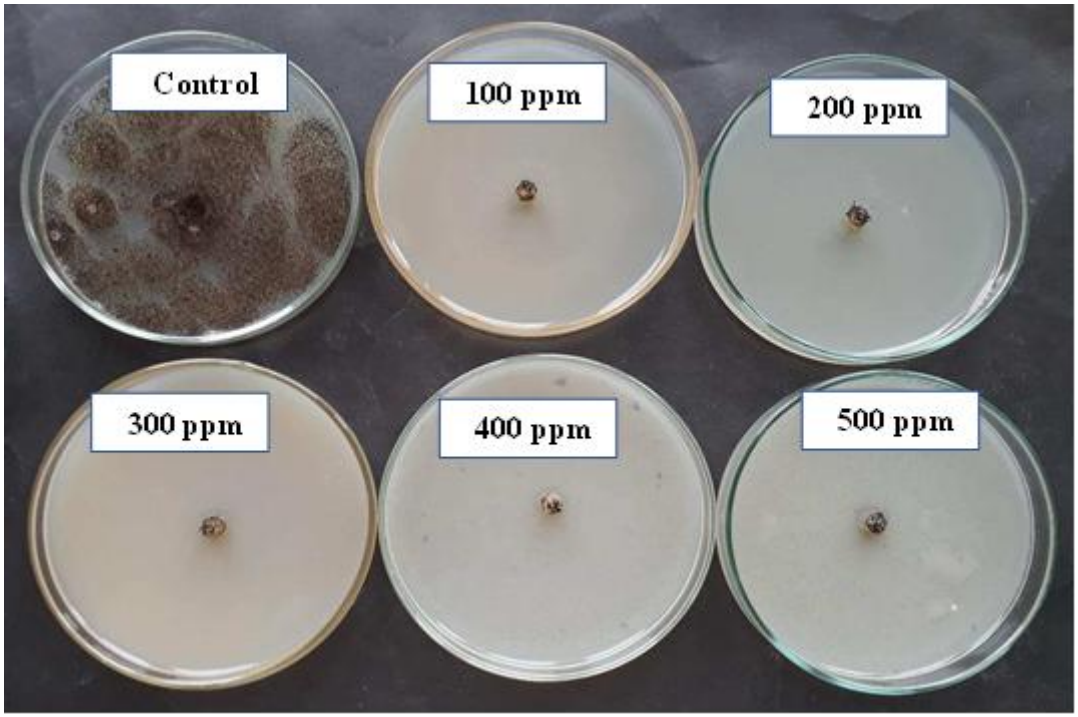

Figure 3. Effect of Tilt 250 EC on the radial growth of *Aspergillus niger* at different concentrations.

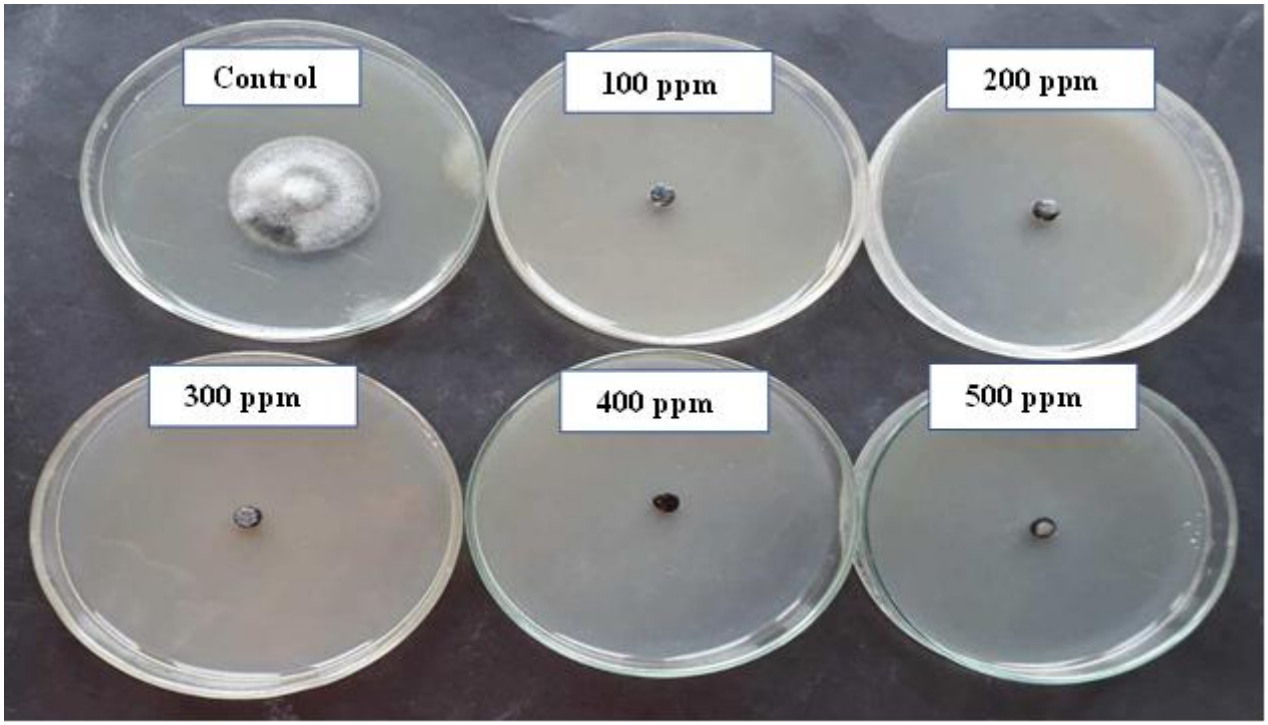

Figure 4. Effect of Tilt 250 EC on the radial growth of *Curvularia lunata* at different concentrations**.**

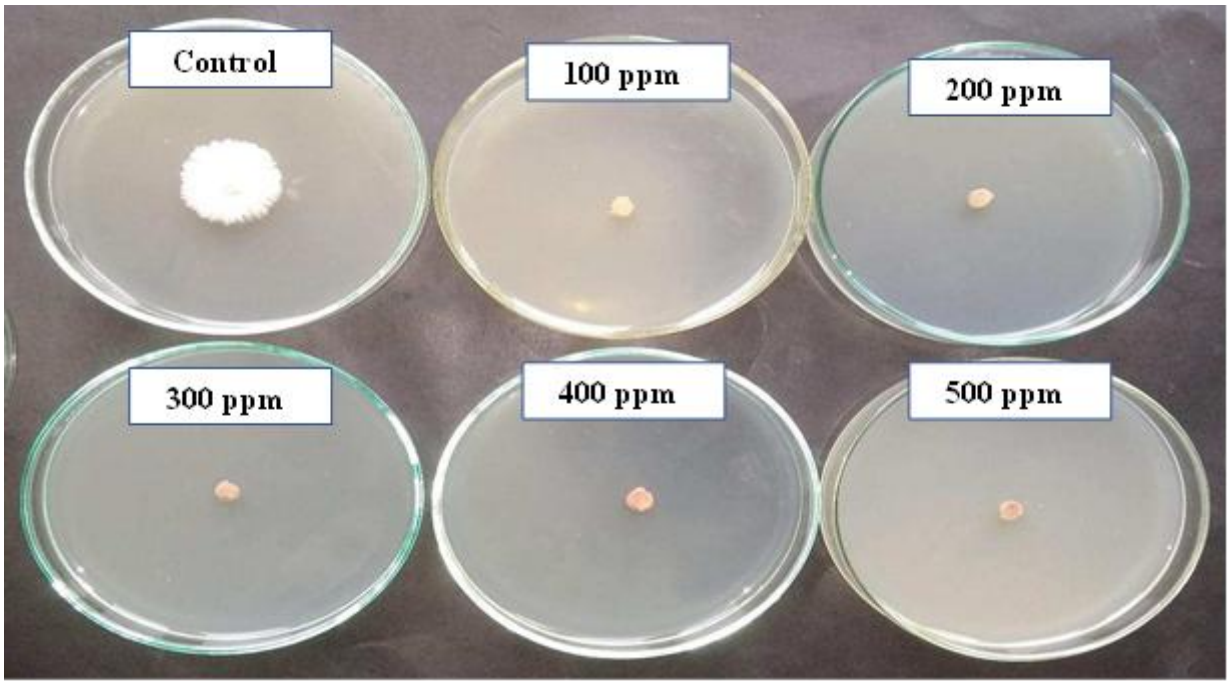

Figure 5. Effect of Tilt 250 EC on the radial growth of Fusarium solani at different concentrations.

Chowdhury *et al*. (2021)[16] reported *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *Curvularia lunata* (Wakker) Boedijn, *Drechsler aoryzae* Breda de Haan (Subramanian and Jain), *Fusarium moniliforme* Sheldon, *F. solani* (Mart.) Sacc., *Microdochium oryzae* (Hashloka and Yokogi) Sam. and Hal., *Pestalotiopsis guepinii* (Desm.) Stay. and *Sarocladium oryzae* (Sawada) W. Gams and D. Hawks were found to be pathogenic to rice seeds.

Chawdhury (2019)[17] reported that Tilt 250 EC completely inhibited radial growth of C. *lulata* and *Fusarium solani* isolated from seeds of selected rice varieties at 100,200,300 400 and 500 ppm concentration. She also reported that Bavistin 50WP completely inhibited radial growth of C. *lulata* and *F. solani* isolated from seeds of selected rice varieties at 100, 200,300 400 and 500 ppm concentration.

Sultana *et* al (2020)[18] reported 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 fisher* iand *Nigrospora oryzae* showed pathogenic potentiality following seed inoculation technique.

Sultana (2021)[19] reported Knowni 50 W P and Tilt 250 EC completely inhibited radial growth of *C. lulata* isolated from seeds of selected at 100, 200, 300, 400 and 500 ppm concentrations.

Hosen *et al* (2024)[20] 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 against the radial mycelial growth of two pathogenic fungi, *Curvularia lunata* and *Drechslera oryzae* associated with newly released BRRI rice varieties at varying concentrations. In this study 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

Present investigation indicates that Tilt 250 EC showed the complete growth inhibition of above mentioned three pathogenic fungi at all the concentrations used. Knowin 50 WP exclusively inhibited complete growth inhibition of *A. niger* at all the concentrations used.

Conclusion

The study evaluated the in vitro effectiveness of five chemical fungicides at varying concentrations (100, 200, 300, 400, and 500 ppm) against three specific pathogenic fungi isolated from ten selected Bangladeshi rice varieties (BRRI dhan 90 to BRRI dhan 99). Tilt 250 EC is universally effective at low doses, while Knowin 50 WP serves as a powerful alternative specifically

for *Aspergillus niger* and *Fusarium solani***.**

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