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Research Article

In-vitro Tests to Determine the Efficacy of Plant Extracts, BAU-Biofungicide and Fungicides on the Inhibitory Effects on Some Important Rice Pathogen

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Abstract: Extract of garlic (*Allium sativum*), Neem (*Azadirachta indica*), BAU-Biofungicide (*Trichoderma* based preparation), Bavistin DF (Carbandazim) and Potent 250 EC (Propiconazole) were studied in *in-vitro* test to evaluate the efficacy of plant extracts, BAU-Biofungicide and fungicides on the inhibitory effects on some selected rice pathogen. BAU-Biofungicide (2 & 3%) showed significant effect in reducing mycelial growth of *Bipolaris oryzae, Cercospora oryzae, Rhizoctonia solani* and *Ustilaginoidea virens.* Potent (0.1%) showed excellent effect in controlling mycelial growth of *Ustilaginoidea virens*, *Sarocladium oryzae*, *Rhizoctonia solani*, *Bipolaris oryzae* and *Cercospora oryzae*. Bavistin (0.1%) was found to have better result in inhibiting the mycelial growth of *Cercospora oryzae, Sarocladium oryzae* and *Rhizoctonia solani*. Highest increase (16.67%) in seed germination was recorded over control when seeds were treated with BAU-Biofungicide (3%). BAU-Biofungicide (3%) also resulted in higher increase vigour index compared to control.

Keywords: BAU-Biofungicide, *Bipolaris oryzae*, *Cercospora oryzae*, *Rhizoctonia solani*, *Ustilaginoidea virens*, Vigour index

INTRODUCTION

Rice is the staple food crop in Bangladesh. As a rice producer the country is occupying 4th position in the world (USDA, 2016). It provides nearly 48% of rural employment, about two-third of total calorie supply and about one-half of the total protein intakes of an average person in the country. The cultivation of rice is increasing day by day, while the yield is comparatively lower. The national average yield of husked rice is 3.05 t/ha (BBS, 2016). There are many factors responsible for low yield of rice in Bangladesh. Among these, diseases are considered as major and the productivity yield must be greatly enhanced by providing additional nutrient input and through effective control of phytopathogens (Sivasakthi *et al*., 2013).

Rice disease management strategies mainly aim at preventing epidemics through the use of host plant resistance and chemical pesticides. The use of chemicals, no doubt, has been found very effective in controlling fungal diseases of plant, but some major problems threaten to limit the continuous use of fungicides. Many researchers tried to find out safe and economical control of plant diseases by using extracts of different plant parts (Akhter *et al*., 2006; Bdliya and Alkali, 2008). Control of plant disease by biological means instead of using chemicals has drawn special attention all over the world. Out of different biotic stresses which influence the performance of rice crop, brown spot of rice caused by (*Bipolaris oryzae*) is disease that impairs grain quality and results in about vary widely from 4 to 52% yield losses (Barnwal *et al*., 2013). Narrow brown leaf spot (*Cercospora oryzae*) causes a great loss both in the storage and field with reduced the seed viability (Arunyanrat *et al*., 1981). Rice Sheath blight disease causes 30% yield loss in very susceptible rice fields with artificial inoculation (Groth, 2008) and may reach up to 50% during prevalent years (Meng *et al*., 2001). Naeimi *et al*. (2003) reported that sheath rot of rice occurred in most rice growing areas of the world. It usually causes 20-85% yield losses. The rice false smut has already changed from a minor disease to a major disease in all rice-growing countries in Asia since 1970. The highest disease incidence (61.20%) and yield loss (14.18%) were reported (Singh *et al*., 2012) and yield losses caused by RFS (rice false smut) disease is attributed to both smut balls as well as chaffiness, reduction in grain weight and infertility of the spikelet near the smut balls (Rani, 2014). The rice false smut disease not only reduces yield but also affects grain quality and imposes

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health hazards significantly in all rice producing areas. Research is still concentrated on the identification, evaluation and formulation of potential biocontrol agents for deployment. Some species of *Trichoderma* produce antibiotics. *Trichoderma hamatum* and *Trichoderma harzianum* produce hydrolytic enzymes (chitinases and glucanases) that attack the hyphae and sclerotia of the pathogen (Gajera *et al*., 2013). Plant extracts and biofungicide are available to treat seed (Koike *et al*., 2011). BAU-Biofungicide has its strong potentiality as a seed treating bioagent (Hossain, 2011). It also increases the plant growth and plant stand of rice (Mahmud and Hossain, 2016). The present study has been designed to control major fungal diseases of rice by using plant extracts and biocontrol agent as an alternative option avoiding environmental pollution.

MATERIALS AND METHODS

Isolation: *Bipolaris oryzae* and *Cercospora oryzae* were isolated from leaf and seed collected from the rice field. *Rhizoctonia solani* and *Sarocladium oryzae* from infected sheath of rice plants and *U. virens* were isolated from the collected seed following the method of Ming-Xia *et al*. (2009). Isolation of fungi from seed was done following ISTA rules (1996). The single conidium of each fungi from the infected leaf piece was transferred to PDA plate for incubation at 25 ± 1 °C for 10-15 days. Pure culture of the pathogen was preserved in PDA with the help of hyphal tip culture method aseptically and stored in a refrigerator at 4 °C for further study.

Preparation of plant extracts: Healthy leaves of neem and garlic cloves were collected and washed thoroughly under running tap water followed by Sterile Distilled Water (SDW). The extracts were prepared by homogenizing plant parts using a blender and prepared at 1 and 2% concentration by dilution with water and kept in conical flasks separately before use.

Use of BAU-Biofungicide and Fungicide: BAU-Biofungicide was used at 2 and 3%. BAU-Biofungicide is a *Trichoderma* based preparation (Hossain, 2011). Bavistin DF (Carbendazim) and Potent 250 EC (Propiconazole) were also used at 0.1 and 0.05% concentration.

Bioassay of plant extracts, BAU-Biofungicide, bavistin and potent on collected fungi: Potato dextrose agar medium was prepared and poured into 9 cm Petri plates at 20 mL/plate. After solidification, three 5 mm discs of the medium were scooped from three places maintaining equal distance of 4 cm from the centre using a sterilized disc cutter. One milliliter of each of plant extracts, suspension of BAU-Biofungicide, Bavistin DF and Potent 250 EC were put into each hole and the plates were stored over night. Next day, the plates were inoculated at the center with 6 mm blocks of 15 days old culture of fungi and incubated at 24 ± 1 °C. Each treatment was replicated thrice and only water was used for control treatment.

Collection of data and statistical analysis: Observation was made regularly to record the mycelial growth. After 4, 6, 8, 10 and 12 days of inoculation, Linear mycelial growth of fungi was measured (Nene and Thaplial, 1993) and percent inhibition of growth was calculated using the following formula as suggested by Sundar *et al*. (1995):

$$\text{Inhibition (\%)} = \frac{X - Y}{X}$$

where,

X = Mean mycelial growth (radial) of pathogen in control plate

Y = Mean mycelial growth (radial) of pathogen in treatment

Seed treatment and vigour index determination: Three hundred harvested seeds of rice susceptible variety BRRI dhan40 of control plot were treated with each plant extracts, separately over time by weight basis at 1 and 2%, and also treated with BAU-Biofungicide at 2 and 3% and both with Bavistin and Potent at 0.1, 0.05% of seed weight. The experiment was conducted in the nethouse of the Seed Pathology Centre, BAU, Mymensingh. Sand was collected from Brahmaputra river, Mymensingh. Collected sand was sterilized with formalin (40%) at the rate of 5 mL formalin diluted with 20 mL of water for 4 kg sand (Dasgupta, 1988). Formalin treated soil was covered with polythene sheet for 48 h and then exposed for 48 h for aeration before setting experiment. The plastic trays (12″×8″) were filled with sand. The experiment was carried out in CRD with three replications. Three hundred seeds of each treatment were sown in plastic trays (100 seeds/tray) maintaining equal distances among the seeds. Plants were watered for maintaining proper moisture. Randomly selected 10 seedlings were uprooted carefully from each tray and washed thoroughly with running tap water. Data was recorded for each treatment at 14 days after sowing on different parameters. Vigour index (VI) was computed using formula of Abdul-Baki and Anderson (1973):

$$\text{Vigor index} = (\text{mean shoot length} + \text{mean root length}) \times \text{\% germination}$$

Statistical analyses: All the recorded data on different parameters were analysed statistically using MSTAT-C computer program to find out the significance of variation resulting from experimental treatments. The difference between the treatment means were evaluated for significance using Duncan's Multiple Range Test

(DMRT) following the procedure as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Maximum (56.80%) reduction of average radial mycelial growth of *B. oryzae* was observed in BAU-Biofungicide (2%) followed by Potent (0.1%) over control and highest mycelial growth was recorded in control. Garlic (2%) and neem (2%) were found to have good effect in controlling mycelial growth (Table 1). Khalili *et al*. (2012) reported that mycelial growth of *B. oryzae* was reduced by up to 100% by producing volatile and nonvolatile metabolites of antagonistic fungi, such as *T. harzianum*. These findings were in accordance with the observation of Manimegalai *et al*. (2011) and Abdel-Fattah *et al*. (2007). Ahmed *et al*. (2002) reported that four fungicides *viz*., Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 were evaluated against *B. oryzae*. Tilt 250 EC inhibited highest (95.58%) mycelial growth inhibition at concentration of 500 ppm.

BAU-Biofungicide (3%) resulted in the highest average radial mycelial growth inhibition of *C. oryzae* by 59.03% followed by BAU-Biofungicide (2%) and Bavistin (0.1%) over control. Garlic (2%) and neem (2%) exhibited good effect in controlling mycelial growth (Table 2). Manurung *et al*. (2014) tested antagonism ability of endophytic fungi to control *C. oryzae in-vitro*. Interaction between the pathogen *C. oryzae* and the endophytic fungi *Trichoderma* spp. affected significantly to the inhibiting zone where *C. oryzae* was showed on (*C. oryzae* + *Trichoderma* spp.) with 67.56% in inhibiting zone.

Maximum 60.65%, 55.16%, 57.14%, 52.22% and 50.19% reduction of mycelial growth of *Rhizoctonia solani* were found with Potent (0.1%) at 4, 6, 8, 10 and 12 DAI, respectively over control followed by BAU-Biofungicide (3%) as shown in Table 3. Mayo *et al*. (2015) evaluated *Trichoderma* isolates for their potential to antagonize *R. solani* by dual culture and *Trichoderma* spp. to over grew the pathogen and found growth inhibition of *R. solani* between 86 and 58%. The findings were also supported by Naeimi *et al*. (2010). Rahman (2007) tested that *T. harzianum* showed linear over growth against *R. solani* and significantly reduced the pathogen.

Highest (50.08%) reduction of average radial mycelial growth of *S. oryzae* was noted with Potent (0.1%) over control, while BAU-Biofungicide (3%) showed 42.08% reduction. Garlic (2%) and neem (2%) were noticed with good effect in controlling mycelial growth (Table 4). Pérez Torres *et al*. (2013) tested antagonic activity of *T. harzianum* against *S. oryzae a*t 240 h and observed mycelial growth inhibition 64.4%. Similar finding was also reported by Kalaiselvi and Panneerselvam (2015). Venkateswarlu and Chauhan (2005) tested the efficacy of 15 systemic, non-systemic and antibiotic fungicides against *S. oryzae*. Jagannathan and Sivaprakasam (1996) tested the

Table 1: *In-vitro* evaluation of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Potent on radial mycelial growth of *Bipolaris oryzae*

	Radial mycelial growth (mm)		
Treatment (dose)	4 DAI	6 DAI	8 DAI
Garlic (1%)	10.33b (18.47)	15.00bcd (18.17)	19.00bcd (26.92)
Garlic (2%)	8.00c (36.86)	13.00d (29.08)	16.67cde (35.88)
Neem (1%)	10.33b (18.47)	16.33b (10.91)	20.33bc (21.81)
Neem (2%)	9.33bc (26.36)	14.00cd (23.62)	17.00cde (34.62)
BAU-Biofungicide (2%)	5.67e (55.25)	8.00e (56.36)	11.33f (56.42)
BAU-Biofungicide (3%)	6.00de (52.64)	9.33e (49.10)	13.00ef (50.00)
Bavistin DF (0.1%)	10.67b (15.79)	15.00bcd (18.17)	18.00bcd (30.77)
Bavistin DF (0.05%)	11.33ab (13.18)	15.67bc (14.51)	22.00b (15.38)
Potent 250 EC (0.1%)	5.00e (60.54)	8.00e (56.36)	12.33f (52.58)
Potent 250 EC (0.05%)	7.67cd (39.46)	10.00e (45.44)	15.00def (42.31)
Control (water)	12.67a	18.33a	26.00a

	Radial mycelial growth (mm)		
Treatment (dose)	10 DAI	12 DAI	Average mycelial growth (mm)
Garlic (1%)	25.00bc (19.35)	29.33bc (22.82)	19.73 (21.15)
Garlic (2%)	23.33c (24.74)	27.67bc (27.18)	17.73 (30.75)
Neem (1%)	26.67b (13.97)	31.00b (18.42)	20.93 (16.72)
Neem (2%)	20.00d (35.48)	26.00cd (31.58)	17.27 (30.33)
BAU-Biofungicide (2%)	13.00e (58.06)	16.00g (57.89)	10.80 (56.80)
BAU-Biofungicide (3%)	15.00e(51.61)	18.00fg (52.63)	12.27 (51.20)
Bavistin DF (0.1%)	20.00d (35.48)	23.00de (39.47)	17.33 (27.94)
Bavistin DF (0.05%)	25.00bc (19.35)	28.00bc (26.32)	20.40 (17.75)
Potent 250 EC (0.1%)	15.00e (51.61)	18.00fg (52.63)	11.67 (54.74)
Potent 250 EC (0.05%)	18.00d (41.94)	21.00ef (44.74)	14.33 (42.78)
Control (water)	31.00a	38.00a	25.20

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; DAI = Days After Inoculation; Data represent the means of three replications; Data in parentheses indicate % growth inhibition over control

Table 2: *In-vitro* evaluation of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Potent on radial mycelial growth of *Cercospora oryzae*

Treatment (dose)	Radial mycelial growth (mm) 5 DAI	7 DAI	9 DAI
Garlic (1%)	8.33b (30.58)	11.67bc (35.17)	16.33b (31.01)
Garlic (2%)	7.00bc (41.67)	10.00cde (44.44)	14.00cd (40.85)
Neem (1%)	8.00bc (33.33)	12.33b (31.50)	14.67c (38.02)
Neem (2%)	8.33b (30.58)	11.00bcd (38.88)	13.00de (45.08)
BAU-Biofungicide (2%)	6.67bc (44.42)	8.00ef (55.56)	10.00h (57.75)
BAU-Biofungicide (3%)	6.33c(47.25)	7.67f (57.39)	8.33i (64.81)
Bavistin DF (0.1%)	7.33bc (38.92)	9.00def (50.00)	11.00gh (53.53)
Bavistin DF (0.05%)	8.00bc (33.33)	10.00cde (44.44)	12.33ef (50.70)
Potent 250 EC (0.1%)	7.17bc (40.25)	9.33def (48.17)	11.33fg (47.91)
Potent 250 EC (0.05%)	8.33b (30.58)	10.33bcd (42.61)	13.00de(45.08)
Control (water)	12.00a	18.00a	23.67a

Treatment (dose)	Radial mycelial growth (mm) 11 DAI	13 DAI	Average mycelial growth (mm)
Garlic (1%)	18.00b(32.51)	19.33b (33.34)	14.73 (32.52)
Garlic (2%)	16.33bc (38.77)	17.00c (41.38)	12.87 (41.42)
Neem (1%)	16.67bc (37.50)	17.33c (40.24)	13.80 (36.12)
Neem (2%)	15.00cd (43.76)	16.00c (44.83)	12.67 (40.63)
BAU-Biofungicide (2%)	10.67fg (59.99)	11.00ef (62.07)	9.27 (55.96)
BAU-Biofungicide (3%)	10.00g (62.50)	10.67f (63.21)	8.60 (59.03)
Bavistin DF (0.1%)	11.33fg (57.52)	12.00ef (58.62)	10.13 (51.72)
Bavistin DF (0.05%)	13.33de (50.02)	14.00d (51.72)	11.53(46.04)
Potent 250 EC (0.1%)	12.33ef (53.77)	12.67de (56.31)	10.57(49.28)
Potent 250 EC (0.05%)	13.33de (50.02)	14.00d (51.72)	11.80 (44.00)
Control (water)	26.67a	29.00a	21.87

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; DAI = Days after inoculation; Data represent the means of three replications; Data in parentheses indicate % growth inhibition over control

Table 3: *In-vitro* evaluation of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Potent on radial mycelial growth of *Rhizoctonia solani*

Treatment (dose)	Radial mycelial growth (mm) 4 DAI	6 DAI	8 DAI
Garlic (1%)	14.17de (33.07)	23.33b (15.16)	31.00b (11.43)
Garlic (2%)	15.00cd (29.15)	21.33bc (22.44)	28.00cd (20.00)
Neem (1%)	17.33b (18.14)	22.50bc (18.18)	30.00bc (14.29)
Neem (2%)	15.83c (25.22)	20.83c (24.25)	27.33d (21.91)
BAU-Biofungicide (2%)	13.17ef (37.79)	16.67d (39.38)	18.33f (47.63)
BAU-Biofungicide (3%)	12.33f (41.76)	17.00d (38.18)	18.00f (48.57)
Bavistin DF (0.1%)	14.00de (33.87)	17.00d (38.18)	20.00f (42.86)
Bavistin DF (0.05%)	14.17de (33.07)	17.50d (36.36)	22.50e (35.71)
Potent 250 EC (0.1%)	8.33g (60.65)	12.33e (55.16)	15.00g (57.14)
Potent 250 EC (0.05%)	9.17g (56.68)	13.17e (52.11)	18.67f (46.67)
Control (water)	21.17a	27.50a	35.00a

Treatment (dose)	Radial mycelial growth (mm) 10 DAI	12 DAI	Average mycelial growth (mm)
Garlic (1%)	37.50b (9.64)	39.67b (9.49)	29.13 (15.76)
Garlic (2%)	36.00b (13.25)	40.17b (8.35)	28.10 (18.64)
Neem (1%)	30.83c (25.71)	38.83b (11.41)	27.90 (17.55)
Neem (2%)	28.33c (31.73)	34.67c (20.90)	25.40 (24.80)
BAU-Biofungicide (2%)	20.00e (51.81)	22.33e (49.05)	18.10 (45.13)
BAU-Biofungicide (3%)	20.33e (51.01)	21.67e (50.56)	17.86 (46.02)
Bavistin DF (0.1%)	21.67de (47.78)	22.67e (48.28)	19.07 (42.19)
Bavistin DF (0.05%)	24.17d (41.76)	25.83d (41.07)	20.83 (37.59)
Potent 250 EC (0.1%)	19.83e (52.22)	21.83e (50.19)	15.46 (55.07)
Potent 250 EC (0.05%)	22.67de(45.37)	24.00de (45.24)	17.54 (49.21)
Control (water)	41.50a	43.83d	33.80

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; DAI = Days after inoculation; Data represent the means of three replications; Data in parentheses indicate % growth inhibition over control

Table 4: *In-vitro* evaluation of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Potent on radial mycelial growth of *Sarocladium oryzae*

	Radial mycelial growth (mm)		
Treatment (dose)	4 DAI	6 DAI	8 DAI
Garlic (1%)	14.00bc (24.32)	23.00b (12.65)	30.83b (12.74)
Garlic (2%)	13.83bc (25.24)	20.50bc (22.14)	28.83bc (18.40)
Neem (1%)	16.00b (13.51)	23.00b (12.65)	30.67b (13.19)
Neem (2%)	14.17bc (23.41)	21.50bc (18.34)	27.67c (21.68)
BAU-Biofungicide (2%)	12.33cd (33.35)	17.00d (35.43)	20.00d (43.39)
BAU-Biofungicide (3%)	12.00cd (35.14)	16.67d (36.69)	20.33d (42.46)
Bavistin DF (0.1%)	11.00d (40.54)	14.00e (46.83)	17.33e (50.95)
Bavistin DF (0.05%)	12.00cd (35.14)	15.67d (40.49)	18.67de (47.16)
Potent 250 EC (0.1%)	11.00d (40.54)	13.67e (48.08)	17.00e (51.88)
Potent 250 EC (0.05%)	12.33cd (33.35)	16.00d (39.23)	18.33de(48.12)
Control (water)	18.50a	26.33	35.33a
	Radial mycelial growth (mm)		Average mycelial growth (mm)
Treatment (dose)	10 DAI	12 DAI	
Garlic (1%)	33.50bc (19.61)	39.50b (13.51)	28.17 (16.57)
Garlic (2%)	31.33c (24.81)	36.00c (21.17)	26.10 (22.35)
Neem (1%)	34.50b (17.21)	39.00b (14.60)	28.63 (14.23)
Neem (2%)	32.00c (23.21)	34.00c (25.55)	25.87 (22.44)
BAU-Biofungicide (2%)	22..33d (46.41)	23.00d (49.64)	18.93 (41.64)
BAU-Biofungicide (3%)	22.00d (47.20)	23.33d (48.92)	18.87 (42.08)
Bavistin DF (0.1%)	18.83e (54.81)	20.00e (56.21)	16.23 (49.87)
Bavistin DF (0.05%)	20.00de (52.00)	22.00de (55.83)	17.67 (46.12)
Potent 250 EC (0.1%)	19.00e(54.40)	20.33e (55.49)	16.20 (50.08)
Potent 250 EC (0.05%)	20.00de (52.00)	21.33de (53.30)	17.60 (45.20)
Control (water)	41.67a	45.67a	33.50

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; DAI = Days after inoculation; Data represent the means of three replications; Data in parentheses indicate % growth inhibition over control

Table 5: *In-vitro* evaluation of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Potent on radial mycelial growth of *Ustilaginoidea virens*

	Radial mycelial growth (mm)		
Treatment (dose)	4 DAI	6 DAI	8 DAI
Garlic (1%)	9.00c (26.05)	10.67c (25.54)	12.33c (26.03)
Garlic (2%)	7.67de (36.98)	9.67cd (32.52)	11.50c (31.01)
Neem (1%)	11.33ab (6.90)	13.67a (4.61)	15.00b (10.02)
Neem (2%)	11.00b (9.61)	12.00b (16.26)	13.00c (22.02)
BAU-Biofungicide (2%)	8.33cd (31.55)	9.33d (34.89)	10.00d (40.01)
BAU-Biofungicide (3%)	8.33cd (31.55)	9.00d (37.19)	9.30d (44.21)
Bavistin DF (0.1%)	6.33f (47.99)	8.33de (41.87)	9.00de (46.01)
Bavistin DF (0.05%)	6.67ef (45.19)	9.00d (37.19)	9.67d (41.99)
Potent 250 EC (0.1%)	4.67g (61.63)	6.67f (53.45)	6.67f (59.99)
Potent 250 EC (0.05%)	5.17g (57.52)	7.33ef (48.85)	7.67ef (53.99)
Control (water)	12.17a	14.33a	16.67a
	Radial mycelial growth (mm)		Average mycelial growth (mm)
Treatment (dose)	10 DAI	12 DAI	
Garlic (1%)	14.17c (25.42)	17.50c (26.07)	12.73 (25.82)
Garlic (2%)	12.67cd (33.32)	15.00d (36.63)	11.30 (34.09)
Neem (1%)	16.33b (14.05)	19.67b (16.90)	15.20 (10.50)
Neem (2%)	14.17c (25.42)	17.33c (26.78)	13.50 (20.02)
BAU-Biofungicide (2%)	10.20ef (46.32)	10.33fg (56.36)	9.64 (41.83)
BAU-Biofungicide (3%)	9.60efg (49.47)	10.00fg (57.75)	9.25 (44.03)
Bavistin DF (0.1%)	10.67ef (43.84)	12.33e (47.91)	9.33 (45.52)
Bavistin DF (0.05%)	11.17de (41.21)	14.00d (40.85)	10.10 (41.29)
Potent 250 EC (0.1%)	8.00g (57.89)	9.00g (61.98)	7.00 (58.99)
Potent 250 EC (0.05%)	9.33fg (50.89)	10.50f (55.64)	8.00 (53.38)
Control (water)	19.00a	23.67a	17.17

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; DAI = Days after inoculation; Data represent the means of three replications; Data in parentheses indicate % growth inhibition over control

ability of neem (*A. indica*) derivatives (neem oil and neem seed kernel extract) and carbendazim to control sheath rot of rice compared with control.

Highest reduction by 61.63%, 53.45%, 59.99%, 57.89% and 61.98% of radial mycelial growth of *U. virens* over control were found with Potent (0.1%) at 4, 6, 8, 10 and 12 DAI, respectively followed by Potent (0.05%), Bavistin (0.1%) and BAU-Biofungicide (3%) (Table 5). Kumar *et al*. (2014) evaluated five isolates of *Trichoderma* spp. by dual culture along with *U. virens*

Table 6: Effect of seed treatments with extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Potent on germination (%) and vigour index at 14 days after sowing of seeds of cv BRRI dhan40 following Tray method

Treatment (dose)	Germination (%)	Normal seedling (%)	Diseased seedling (%)	Germin. failure (%)	Hard seed (%)
Garlic (1%)	93.00a (+10.71)	81.00b (+24.62)	8.00b (-22.56)	3.00cd (-70.00)	4.00b (-33.33)
Garlic (2%)	94.00a (+11.91)	83.00ab (+27.69)	8.00b (-22.56)	3.00cd (-70.00)	3.00c (-50.00)
Neem (1%)	94.00a (+11.91)	80.00b (+23.08)	8.00b (-22.56)	4.00bc (-60.00)	2.00cd (-66.67)
Neem (2%)	95.00a (+13.10)	85.00ab (+30.77)	6.00cd (-41.92)	3.00cd (-70.00)	2.00cd (-66.67)
BAU-Biofungicide (2%)	97.00a (+15.48)	90.00a (+38.46)	4.00e (-61.28)	2.00de (-80.00)	1.00d (-83.33)
BAU-Biofungicide (3%)	98.00a (+16.67)	90.00a (+38.46)	5.00de (-51.60)	1.00e (-90.00)	1.00d (-83.33)
Bavistin DF (0.1%)	93.00a (+10.71)	81.00b (+24.62)	7.00bc (-32.24)	4.00bc (-60.00)	3.00c (-50.00)
Bavistin DF (0.05%)	90.67ab (+7.94)	78.00bc (+20.00)	7.67b (-25.75)	5.00b (-50.00)	4.33b (-27.83)
Potent 250 EC (0.1%)	96.00a (+14.29)	67.00d (+3.08)	5.00de (-51.60)	2.00de (-80.00)	2.00cd (-66.67)
Potent 250 EC (0.05%)	97.00a (+15.48)	71.00cd (+9.23)	5.00de (-51.60)	1.00e (-90.00)	2.00cd (-66.67)
Control (water)	84.00b	65.00d	10.33a	10.00a	6.00a
Treatment (dose)	Shoot length (cm)	Root length (cm)	Shoot weight (mg)	Root weight (mg)	Vigour Index
Garlic (1%)	12.23ab (+11.18)	13.00abc (+17.12)	36.67ab (+14.59)	38.00cd (+7.56)	2347.47c (+26.65)
Garlic (2%)	12.84ab (+16.72)	13.21abc (+19.01)	38.67a (+20.84)	40.33abc (+14.15)	2445.24c (+31.92)
Neem (1%)	11.70b (+6.36)	12.00bc (+8.11)	36.67ab (+14.59)	40.33abc (+14.15)	2233.33c (+20.49)
Neem (2%)	12.18ab (+10.73)	12.67abc (+14.14)	37.33ab (+16.66)	41.67abc (+17.95)	2354.32c (+27.02)
BAU-Biofungicide (2%)	13.74a (+24.91)	14.60a (+31.53)	39.00a (+21.88)	44.00ab (+24.54)	2750.04ab (+48.37)
BAU-Biofungicide (3%)	13.78a (+25.27)	14.60a (+31.53)	40.00a (+25.00)	44.33a (+25.47)	2781.69a (+50.07)
Bavistin DF (0.1%)	12.92ab (+17.45)	13.72ab (+23.60)	38.00ab (+18.75)	42.33ab (+19.81)	2479.32bc (+33.76)
Bavistin DF (0.05%)	11.83ab (+7.55)	12.78abc (+15.14)	37.00ab (+15.63)	40.00bc (+13.22)	2226.91c (+20.14)
Potent 250 EC (0.1%)	7.05c (-35.91)	11.80bc (+6.31)	34.00bc (+6.25)	33.00e (+6.59)	1806.43d (-2.54)
Potent 250 EC (0.05%)	7.44c (-32.36)	12.23bc (+10.18)	32.00c (+0.00)	32.00e (+9.43)	1910.52d (+3.07)
Control (water)	11.00b	11.10c	32.00c	35.33de	1853.55d

In a column, figures having same letter (s) do not differ significantly at 5% level of significance by DMRT; Data represent the means of three replications; Data in parentheses indicate % increased (+) and % decreased (-) over control; DAS = Days after sowing

and observed maximum antagonistic potential against *U. virens* after 96 h and 120 h of incubation. Similar finding was also supported by Kannahi *et al*. (2016). Chen *et al*. (2013) tested fungicides, viz., prochloraz, difenoconazole, propiconazole and tebuconazole against *U. virens* isolates for their sensitivity during the stage of mycelial growth and found good result.

In this study no good effect of the extract of *Azadirachta indica* was observed, while Rajappan *et al*. (1999) reported that formation of neem oil had significant inhibitory effect against *S. oryzae* even at the lowest concentration (0.2%) although extracts of neem (2%) and garlic (2%) were found to have good effect in reducing the mycelial growth of *C. oryzae*.

Highest 38.46% increase of normal seedlings obtained from treated seeds was marked with BAU-Biofungicide (2 & 3%). Maximum 61.28% reduction of diseased seedling was noted with BAU-Biofungicide (2%) followed by BAU-Biofungicide (3%) and Potent (0.1 & 0.05%) each having 51.60% over control. Higher 90.00% reduction of germination failure was achieved both with BAU-Biofungicide (3%) and Potent (0.05%). Maximum 25.27% increase of shoot length was appeared with BAU-Biofungicide (3%). Higher increase 31.53% of root length was found over control by BAU-Biofungicide (2 & 3%) followed by Bavistin (0.1%). Maximum 16.67% increase of seed germination was increased when seeds were treated with BAU-Biofungicide (3%) over control, while BAU-Biofungicide (3%) resulted 50.07% higher increase in vigour index over control followed by BAU-Biofungicide (2%) and Bavistin (0.1%). BAU-Biofungicide (3%) also resulted the highest shoot weight 40.00 mg and root weight 44.33 mg indicating 25.00% and 25.47% higher increase in shoot and root weight, respectively over control followed by BAU-Biofungicide (2%) and Bavistn (0.1%) as shown in Table 6. The results are supported by Ora *et al*. (2011) and Mahmud and Hossain (2016). Ora *et al*. (2011) showed better performance in terms of lowest pathogenic incidence, rotten seed, dead seed, seed germination and seedling vigour index. Mahmud and Hossain (2016) reported that 45.7% and 32.7% vigour index were increased over control when rice seeds of BR11 variety were treated with BAU-Biofungicide (3%) and Bavistin (0.1%), respectively at 14 days after sowing. These findings were in accordance with the observation of Hossain *et al*. (2015). Mahmud and Hossain (2016) also reported that highest shoot weight (40.0) mg, maximum root weight (36.0) mg and highest reduction of diseased seedling were observed when seeds were treated with BAU-Biofungicide (3%) and Bavistin (0.1), respectively. In case of lowest diseased seedling, and growth of shoot and root development which can be correlated with the work of Barbosa *et al*. (2001), Hossain *et al*. (2015) and Mahmud and Hossain (2016).

CONCLUSION

From this study, BAU-Biofungicide (*Trichoderma harzianum*) may be recommended for treating seeds as biological agent to improve seed health, plant stand as well as controlling mycelial growth of seed borne fungi of rice *in-vitro* test by avoiding chemicals as an alternative option in controlling diseases of rice.

Besides this, by using of agricultural chemicals in our water, soil and food affect the health, safety and environment. As a result, the use of biological control needs to be emphasized strongly.

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