Bangladesh Rice J. 24 (1) : 47-58, 2020, doi.org/10.3329/brj.v24i1.53239

Biological Control of Rice Sheath Blight Disease (*Rhizoctonia solani*) Using Bio-pesticides and Bio-control Agents

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ABSTRACT

A series of experiments were conducted in Plant Pathology Laboratory at Bangladesh Rice Research Institute (BRRI), Gazipur to know the efficacy of four bio-pesticides Recharge, Microtech1, Agroplus and Chitin and two bio-control agents (*Trichoderma harzianum* and *Bacillus subtilis*) on the major rice disease sheath blight (ShB) caused by *Rhizoctonia solani* in *in-vitro* and field condition. One fungicide Nativo (Tebuconazole 50% + Trifloxystrobin 25%) and one disease control (without any treatments) were also included as treatment for comparison. Radial mycelia growth of *R. solani* was mostly inhibited by a Bangladesh *Trichoderma harzianum* (BT1), Recharge and chemical fungicide Nativo 75 G at 48 hours of dual contact of *R. solani* and tested bio-pesticides or bio-control agents in *in-vitro*. The other bio-pesticides along with *B. subtilis* didn't show any inhibition effect on the mycelia growth of *R. solani* which was similar to control (only water) treatment. Fungal inhibition 87.5% and 86.3% of *Rhizoctonia solani* was obtained by Recharge and *Trichoderma harzianum* (BT1), respectively which was nearly similar to the chemical treatment Nativo (96.3%) in *in-vitro* dual culture method compared to control treatment. In net house condition, 74% sheath blight disease was inhibited by soil application of Recharge (0.3 g/m^2 with 50 ml water) for two times at the time of transplanting and 30 days after transplanting (DAT), whereas similar soil application of *B. subtilis* (OD_{600}=0.3) was not found effective to control the ShB disease (< 30% disease reduction). The highest ShB disease was reduced by the chemical control Nativo 75 G (94.2%) followed by BT1 (89%), bio-pesticide Recharge (70.8%), Microtech1 (37.4%) and Chitin (61.3%) compared with the disease control when the tested materials were sprayed for two times (3 days before and 4 days after inoculation).

Key words: Efficacy, bio-pesticide, fungal inhibition, *Rhizoctonia solani*.

INTRODUCTION

Bio-pesticides offer powerful tools to create a new generation of sustainable agriculture products. They are the most likely alternatives to some of the most problematic chemical pesticides currently in use. Bio-pesticides offer solutions to concerns such as pest resistance, traditional chemical pesticides and public concern about side effects of pesticides on the surrounding environment and ultimately on human health. Bio-pesticides are the rich combination of beneficial microorganisms which are vital to soil health as well as pathogen inhibition (Mishra *et al.*, 2015; Sindhu *et al.*, 2016; Islam *et al.*, 2019; Naeimi *et al.*, 2019; Raj *et al.*, 2019).

There are several constraints of rice production and its low yield in Bangladesh. A total of 79 diseases of rice was recorded world-wide (Ou, 1985) and 20 rice diseases was found in Bangladesh and sheath blight was found one of the major diseases of rice (Mian et al. 1983). Among the major rice diseases sheath blight (ShB) caused by the fungal pathogen *Rhizoctonia solani* (*R. solani*) is one of the most economically important diseases in Bangladesh and the world. It is a global rice production constraint incurring economic loss to an extent of 4% annually (Zhong *et al.*, 2007). Sheath blight is becoming as an increasing problem for rice cultivation in Bangladesh for all the three seasons Aus, Aman and Boro (Miah *et al.*, 1985; BRRI, 2018). Sheath blight of rice takes place in all rice growing areas of the world (Ou, 1985; Savary *et al.*, 2006) and may cause up to a 50% decrease in the rice yield under favourable conditions around the world

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(Zheng *et al.*, 2013). Sheath blight caused 14-17% yield loss in different varieties during Aus, Aman and Boro seasons in Bangladesh (Shahjahan *et al.*, 1986). High temperature and relative humidity during crop growth favor development of sheath blight disease, high tillering, short stature and high nitrogen responsive varieties are comparatively more susceptible as the micro climates inside the rice canopy is more favorable than those of the traditional ones of tall plant type with low tillering ability (Miah *et al.*, 1985). Application of fungicides is the major measure for controlling sheath blight of rice for over three decades in Asian countries (Zheng *et al.*, 2013).

Fungal bio-pesticides can be used to control insects, bacteria, nematodes, fungi and weeds (Manoharachary *et al.*, 2014). Mechanism of bio-control varies and depends on both the pesticidal fungus and the target pest. *Trichoderma* secretes enzymes such as chitinolytic enzymes, glucanases, cellulases, and proteases that help in the biological control of plant diseases (Mishra *et al.*, 2015). However, extensive and continuous use of a single chemical may lead to undesirable effects such as residual toxicity and environmental pollution, and also increases the risk of resistance development (Brent and Hollomon, 1998). Therefore, it is necessary to develop environmentally friendly, low residual and effective alternative methods for the management of sheath blight (ShB) disease.

Cultural practices, chemical and biological control, disease forecasting, host genetic resistance, typically major gene resistance are commonly used for controlling ShB. But cultural practices are not found effective in all locations and its efficacy mainly depends on disease incidence records. Agrochemicals and their behaviour of natural degradation harm the environment, causing major ecological and health problems. An eco-friendly and sustainable crop production in agriculture is possible by using bio-pesticides, a formulated product using bio-control agents. The use of biological agents has not widely popular for controlling ShB even though biological control is an environmentally friendly and cost-effective substitute to chemical. Now-a-days, the use of antagonistic bacteria as biological control is considered as the best alternative way to reduce the application of chemicals in field (Yang *et al.*, 2007; Misk and Franco, 2011; Raj *et al.*, 2019).

The extensive use of synthetic organic chemicals in the past decades has led to a number of long-term environmental problems (Arora *et al.*, 2012). Chemical fertilizers and pesticides are continuously accumulating in the environment, harming the ecosystem, causing pollution, and inflicting diseases at alarming levels (Gerhardson, 2002; Arora *et al.*, 2010). The heavy use of pesticides has already caused grave damage to health, ecosystems, and groundwater. Therefore, this study was undertaken to know the efficacy of environment friendly bio-pesticides along with bio-control agents against one of the major rice disease sheath blight.

MATERIALS AND METHODS

The experiments were conducted at Plant Pathology Division, Bangladesh Rice Research Institute (BRRI) during 2016-17. Three bio-pesticides namely Recharge (Russel IPM, UK), Microtech (*B. subtilis*) and Chitin; two bio-control agents *Trichoderma harzianum* (BT1) and *Bacillus subtilis*; one chemical Nativo were tested in the experiments.

Recharge is a rich combination of beneficial soil microorganisms (*Glomus* spp, *Bacillus* spp. *Trichoderma* spp.) which are vital to soil health. The application of Recharge vitalizes the soil and restores its ability to function properly by providing vital background protection to the crop from invasive pests and diseases. Recharge only puts back what the soil has already lost due to excessive pesticide applications. Recharge is a new product came from Russel IPM, UK for field evaluation in Bangladesh. The other three bio-pesticides Microtech1 (*Bacillus subtilis*), Agroplus and Chitin were supplied by different pesticide companies of Bangladesh.

Two bio-control agents *Trichoderma harzianum* (BT1) isolated from rhizosphere of rice plant and *Bacillus subtilis* was derived from *Datura metel* seed extract (Wang *et al.*, 2018). A commonly used effective fungicide Nativo 75 G (Tebuconazole +Trifloxystrobin) of Bayer Crop Science was used as standard chemical control treatment to compare the efficacy of bio-pesticides or the bio-control agents following the method of Groth (2005).

Isolation of *R. solani*. The test pathoges *R. solani* was isolated from the infected sheath. Infected sheath samples were cut off with a pair of sterilized scissors, kept in a sterilized polyethylene bag and brought into the laboratory for microscopic study and isolation work. The fungus was isolated from infected parts of the rice plants following tissue planting method (Bashar *et al.*, 2010). Sheath blight infected sheath with culm was cut into small pieces (5-6 mm) with the help of sterilized scissors. The cut pieces were washed with sterile water for two minutes and then surface sterilized by 5% Clorox for 2-3 minutes. Finally, these pieces of sheath were washed with sterile water for 2-3 minutes and were dried on sterile tissue paper. The dried samples were placed on potato dextrose agar (PDA) plate and incubated for seven days at room temperature (25±2 ^{o}C). The isolate was purified by the hyphal tip method which consists of cutting the hyphal tip of a growing mycelium. Fungus was identified based on mycelial growth, colony character, sclerotia formation and sclerotial size. The fungal strain was stored on PDA medium at 4°C.

The experiments were conducted both in *in vitro* and also in net house conditions to confirm the efficacy of the treatments.

***In-vitro* experiment.** *In-vitro* experiment was conducted in the Plant Pathology Laboratory, BRRI, Gazipur. The treatments were T1: *Trichoderma harzianum* (BT1) @ OD600=1, T2: Microtech1 containing *Bacillus. subtilis* @ 20 µl/ml, T3: *B. subtilis* @ OD600=1, T4: Agroplus @ 20 µl/ml, T5: Recharge (*Glomus* spp, *Bacillus* spp. and *Trichoderma* spp.) @ 20 mg/ml, T6: Chitin @ 20 µl/ml, T7: Nativo 75 G @ 20 mg/ml as a chemical control and T8: Control (only water). The *in vitro* antagonistic assay was performed according to the dual culture method (Wang *et al.*, 2018) on PDA medium. Bio-pesticides and chemical @ 0.3% (w/v or v/v) were over lapped (50 µl) on the PDA plates. Then six mm mycelial block of *R. solani* isolated from pure culture was disposed at the center of Petri dishes and incubated at 25±2 ^{o}C for 2-3 days. The antagonistic activity of the bio-pesticides was estimated by the inhibition of the fungal growth in comparison to a solely cultivated fungal agar disk. The fungal growth was monitored by measuring the diameter in centimeter of the colony. Each treatment was tested in three different plates and the experiment was carried out twice. Effect of bio-pesticides, bio-control agents and chemical on the growth inhibition of *R. solani* over control treatment was calculated as percent inhibition using the following formula reported by Satish *et al.*, 2007 and Dubey *et al.*, 2009.

Fungal growth inhibition% = (C-T/C)×100, Where C = *R. solani* growth in the control treatment, T = *R. solani* growth in the bio-pesticide or bio-control agent or chemical treatments.

Net house experiments. These experiments were conducted to know the efficacy of some bio-pesticides and bio-control agents on sheath blight (ShB) disease of rice in net house condition using soil application, root dipping and spray methods. Two seedlings of test variety BR11 were transplanted maintaining three hills per pot. Three methods namely soil application, root dipping and spray were used for treatments application. In soil application and root dipping method, the treatments were T_1 = Recharge (0.3 g/m^2 with 50 ml water) by soil application at two times; 0 days after transplanting (DAT), 30 DAT, T_2 = Root dipping with Recharge (0.3 g/m^2 with 50 ml water) of seedlings for 30 min, $T_3 = T_1 + T_2$, T_4 = *Bacillus subtilis* (*Bs*) (OD_{600}=0.3) by soil application at two times (0 DAT, 30 DAT), T_5 = Root dipping of seedlings for 30 min with *Bs* (OD_{600}=0.3), $T_6 = T_4 + T_5$, T_7 = Control (without Recharge or *Bs*. Artificial inoculation of ShB was done at 60 days after transplanting (DAT) in plants. *Rhizoctonia solani* was cultured on PDA medium and incubated at 26-28 ^{o}C. After 7-10 days of incubation upto sclerotia formation, the inoculums were placed on the beneath of the rice plant into the sheath.

In spray method, bio-pesticides and others were applied by spraying at maximum tillering stage. The bio-pesticides along with chemical were sprayed at three days before inoculation and 4 days after inoculation @ 3% w/v or v/v. Spray was done both as preventive and curative. Percent disease reduction was calculated over diseased control treatment. The bio-pesticides, bio-control agents along with chemical fungicide were sprayed at three days before inoculation and four days after inoculation @ 3% (w/v or v/v) during maximum tillering stage of rice plant. Treatments were Recharge, Microtech1, Agroplus, Chitin, Chemical control (Nativo) and disease control (ShB inoculation but no spray).

Disease assessment. For assessing the percent relative lesion height (RLH) of sheath blight disease, data were taken from nine hills from each treatment following the Standard Evaluation System (IRRI, 2013). Plant height and lesion height were measured at 21 days after inoculation. The percent relative lesion height (RLH) was calculated following the formula:

Relative Lesion Height, RLH (%) = (Lesion height/Total plant height) × 100.

Experimental design and data analysis. The experiments were conducted by completely randomized design (CRD) with three replications. Whenever necessary, the data were distorted before statistical analysis following appropriate methods.

RESULTS

Efficacy of bio-pesticides and bio-control agents in *in-vitro* against *R. solani*

After 24 hours of dual contact, mycelial growth of *R. solani* was very low in *Trichoderma harzianum* (BT1), Recharge and chemical fungicide Nativo 75 WP. Similar and higher growth was observed in other bio-pesticides (Microtech 1, Agroplus, Chitin), *Bacillus* strain and control treatment (Figs. 1 and 3). In the dual contact, the radial growth of *R. solani* was obtained fully at 48 hours in control treatment. The radial growth of the fungus was significantly stopped on the PDA medium-containing BT1, Nativo 75 WP and Recharge Bio-pesticide at 48 hours of incubation (Figs. 1 and 4).

Inhibition of mycelial growth over control. Inhibition of mycelial growth of *R. solani* over control at 48 hours after culturing was calculated. In case of fungal inhibition, highest percent inhibition of mycelial growth of *R. solani* was obtained in the PDA medium containing chemical fungicide Nativo (96%) followed by Bio-pesticide Recharge (87%) and *Trichoderma* strain BT1 (86%) (Fig. 2). Other bio-pesticides (Microtech1, Agroplus and Chitin), *Bacillus subtilis* were not effective for inhibiting mycelia growth of *R. solani* as similar growth of ShB in this treatment was observed with control (no treatment) at 48 hours after dual culture (Figs. 2 and 4). This result suggests that Recharge and BT1 has the effective antifungal activity to *R. solani*, and they showed similar results as like chemical fungicide Nativo 75 WP.

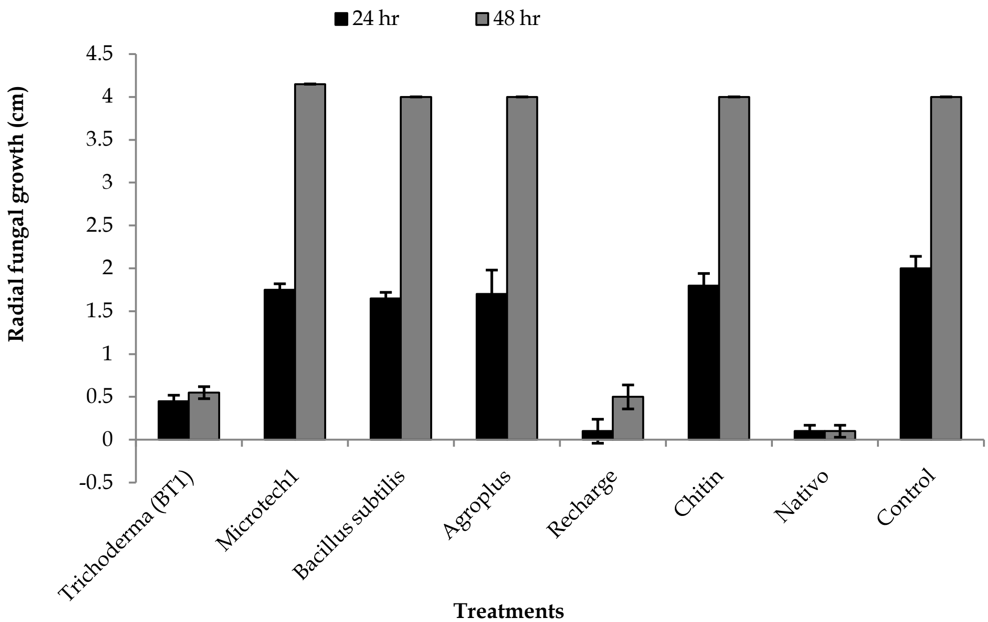

Fig. 1. Mycelial growth of *Rhizoctonia solani* on PDA medium in different bio-pesticides, bio-control agents and chemical fungicide at 24 and 48 hours of direct contact.

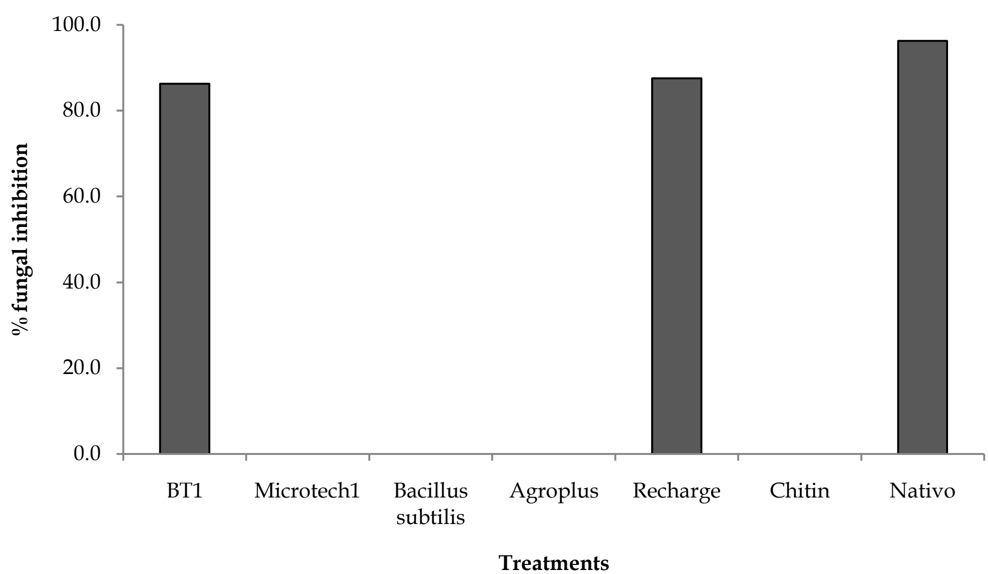

Fig. 2. Inhibitory actions of bio-pesticides, bio-control agents and chemical fungicide over control treatment at 48 hours in *in-vitro*.

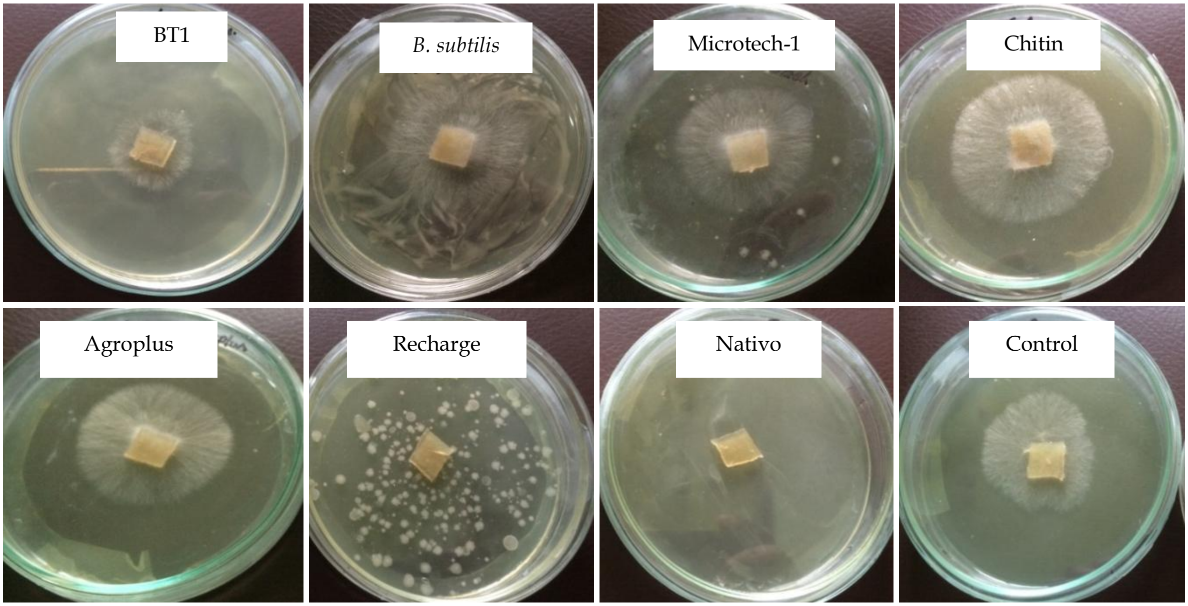

Fig. 3. Dual culture assay of *Rhizoctonia solani* with bio-pesticides, bio-control agents compared to chemical fungicide (Nativo) and control treatment on PDA medium at 24 hours after direct contact.

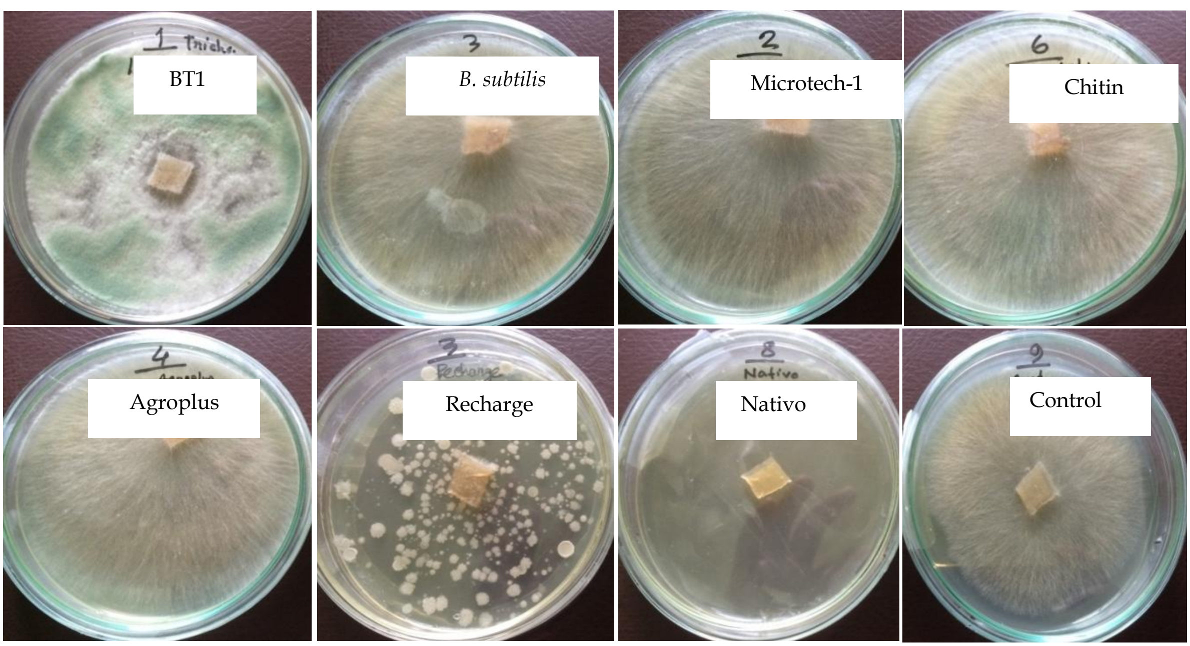

Fig. 4. Dual culture assay of *Rhizoctonia solani* with bio-pesticides, bio-control agents compared to chemical fungicide (Nativo) and control treatment on PDA medium at 48 hours after direct contact.

Efficacy of bio-pesticides in net house condition against ShB disease of rice

Soil application and root dipping method. In this study, one bio-pesticide Recharge was found most effective in *in-vitro* experiment and that's why the effectiveness and appropriate application method of the bio-pesticide were investigated. Sheath blight disease was significantly decreased by the application of Recharge @ 0.3g/m2 in soil for two times during transplanting and 30 days after transplanting (DAT) compared to the other treatments (Fig. 5). Root dipping of the seedling with Recharge was also effective to some extent but not at acceptable level. *Bacillus subtilis* was not found effective on the ShB disease reduction. About 76% disease was reduced over control treatment by soil application of Recharge followed by root dipping (52%) of the seedlings before transplanting (Fig. 6). This result indicates that efficacy of Recharge is more effective by soil application to control sheath blight disease of rice.

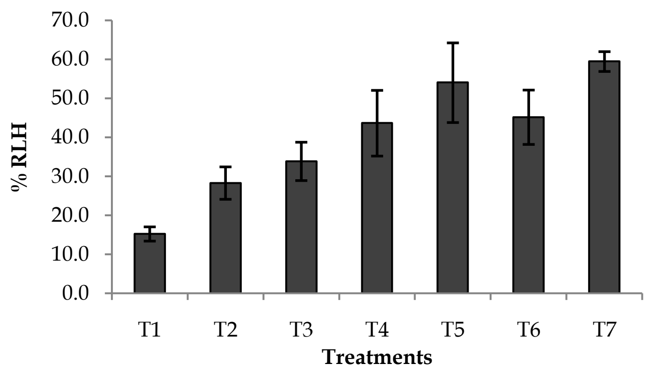

Fig. 5. Efficacy of bio-pesticides and bio-control agents against ShB disease of rice in net house condition by soil application method. % Relative Lesion Height (RLH) of ShB disease showed. T_1 = Use of Recharge (0.3 g/m^2 with 50 ml water) by soil application two times (0 DAT, 30 DAT), T_2 = Root dipping with Recharge (0.3 g/m^2 with 50 ml water) of seedlings for 30 min, $T_3 = T_1 + T_2$, T_4 = *Bacillus subtilis* (*Bs*) (OD_{600}=0.3) by soil application two times (0 DAT, 30 DAT), T_5 = Root dipping of seedlings for 30 min with *B. subtilis*, $T_6 = T_4 + T_5$, T_7 = Control (without bio-pesticides).

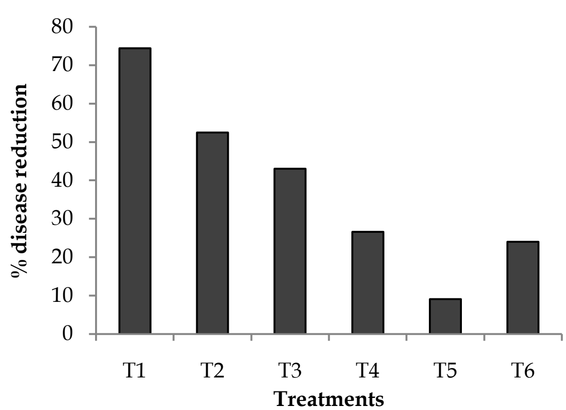

Fig. 6. Percent ShB disease reduction over control treatment in net house condition by application in soil. T_1 = Use of Recharge (0.3 g/m^2 with 50 ml water) by soil application two times (0 DAT, 30 DAT), T_2 = Root dipping with Recharge (0.3 g/m^2 with 50 ml water) of seedlings for 30 min, $T_3 = T_1 + T_2$, T_4 = Use *Bacillus subtilis* (*Bs*) (OD_{600}=0.3) by soil application two times (0 DAT, 30 DAT), T_5 = Root dipping of seedlings for 30 min with *Bs*, $T_6 = T_4 + T_5$, T_7 = Control (without Recharge or *Bs*).

Spray method. After 21 days of inoculation, sheath blight disease was significantly reduced by spraying of *Trichoderma harzianum* (BT1) and recharge (Fig. 7). Disease was mostly controlled by spraying the chemical fungicide Nativo 75 WP. Disease reduction of 89 % was obtained by spraying *Trichoderma harzianum* (BT1) and 94 % by chemical fungicide Nativo 75 WP and 71% disease was inhibited by Recharge over diseased control treatment (Fig. 8). Though the chemical fungicide performed the best but besides the chemicals, Recharge and *Trichoderma harzianum* (BT1) can be the environmentally friendly alternative option of chemicals for controlling ShB disease of rice. Further field experiments with Recharge and formulations of *Trichoderma harzianum* (BT1) are needed in different locations to clarify the present study.

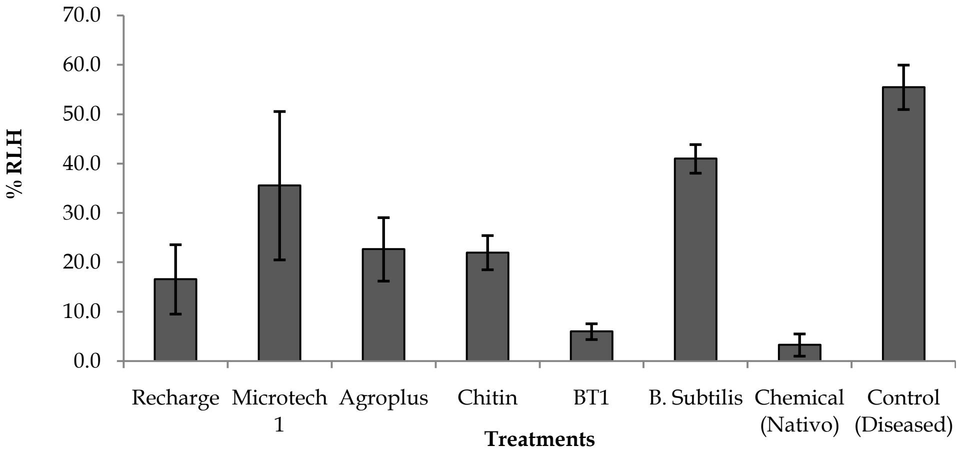

Fig. 7. Efficacy of bio-pesticides and bio-control agents against ShB disease of rice in net house condition by spray method. % Relative Lesion Height (RLH) of ShB disease data were collected during 21 days after inoculation. The bio-pesticides, bio-control agents along with chemical were sprayed at three days before inoculation and four days after inoculation @ 3% w/v or v/v.

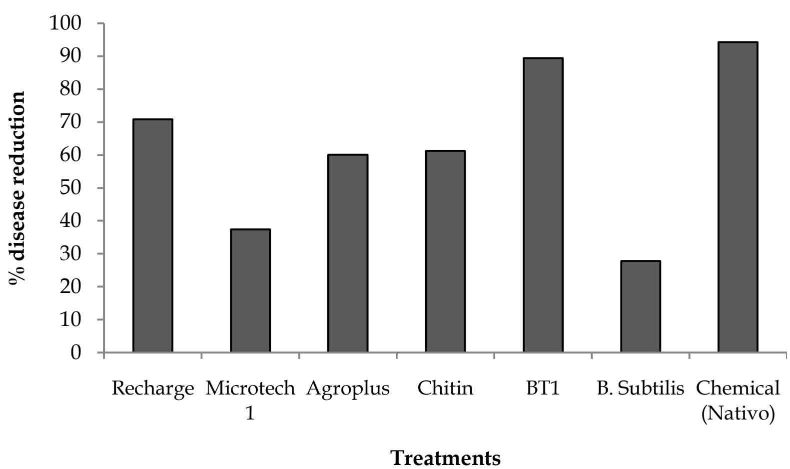

Fig. 8. Percent ShB disease reduction over diseased control treatment in net house condition by spray method. Data of % Relative Lesion Height (RLH) of ShB disease were used for calculating disease reduction over diseased control.

DISCUSSION

Bio-pesticides are potential alternatives to chemical pesticides. Microbial bio-pesticides are living natural enemy organisms and/or their products including plant and microbial products and/or their byproducts and they could reduce pathogen populations. In the present decade, bio-pesticides are widely acceptable and demanded for sustainable agriculture and for production of safe foods. It was significantly considered that, bio-pesticides are eco-friendly, target-specific, easily biodegradable and safer alternatives. Economically, chemical pesticides are very expensive in comparison to bio-pesticides. Many scientists in the world developed and experimentally validated different bio-pesticides and bio-control agents for controlling different diseases including rice diseases (Kazempour, 2004; Chowdhury *et al.*, 2013; Sindhu *et al.*, 2016; Naeimi *et al.*, 2019; Raj *et al.*, 2019).

The biological control of plant diseases is a promising alternative approach to maintaining plant health and promoting crop yield. Several bacterial isolates including *B. subtilis* from soil are effective against the fungal pathogen *R. solani* (Kang *et al.*, 2015; Raj *et al.*, 2019). One UK's Bio-pesticide (Recharge), three Bangladeshi company's bio-pesticides (Microtech 1, Agroplus, Chitin), two bio-control agents (*Trichoderma harzianum* and *Bacillus subtilis*) were tested against one of the major rice diseases sheath blight caused by *Rhizoctonia solani* in *in vitro* and net house conditions and the results were compared with one chemical control and another is control (no treatments, only sterilized water). Bio-pesticide Recharge was found effective antagonist to *R. solani* in *in vitro* and in net house condition. The findings of the present study also supporting by the findings of Hossain *et al.* (2001) and Bashar *et al.* (2010), who showed bacterial isolates exhibited comparatively higher growth inhibition of *R. solani*. Bashar *et al.* (2010) reported that ShB disease development reduced tremendously when antagonistic bacteria treated sclerotia were inoculated. The tested isolates of antagonistic bacteria reduced sheath blight disease development upto 35% over to control.

Elkahoui *et al.* (2012) reported that two bacterial strains, *Bacillus subtilis* and *Bacillus cereus,* showed a clear antagonism against *R. solani* on potato dextrose agar (PDA) medium. Elkahoui *et al.* (2011) also found some bacterial isolates collected from Marine Bio-Films for antifungal activity against *R. solani*. The tested strain of *Bacillus subtilis* didn't show any inhibitory reaction on *R. solani* in the same condition. It might be the differences of the species of *Bacillus* strains. Many researchers reported different bio-control agents effective to control sheath blight causing pathogen *R. solani* (Harman *et al.*, 2004; Jacobsen *et al.*, 2004; Kloepper *et al.*, 2004; Kumar *et al.*, 2009a & 2009b; Peng *et al.*, 2014; Mishra *et al.*, 2015; Shrestha *et al.*, 2016).

Our results also showed that one strain of *Trichoderma harzianum* (BT1) can reduce more than 70% sheath blight disease and similar results was found with the report of Naeimi *et al.* (2019). Sharma *et al.* (2012) also reported that *Trichoderma*-based enzymes (chitin and glucans) are known to show pest resistant activity. Studies concerning commercialization and field applications of integrated stable bio-formulations of *Trichoderma harzianum* (BT1) as an effective bio-control strategy would be needed in future. Recharge bio-pesticide was found comparatively better than the other bio-pesticides tested. The recommended application methods of Recharge Bio-pesticide are soil application and spray method. In order to fully exploit Recharge potential, further studies on field experiments are required to establish it as an effective Bio-pesticide for controlling sheath blight disease along with other major rice diseases.

CONCLUSION

From this study, *Trichoderma harzianum* (BT1) could be suggested as Bio-pesticide with suitable formulation followed by field trial. Bio-pesticide Recharge could also be used as Bio-pesticide for controlling sheath blight disease of rice after some field trial by using soil application as well as spray method. Bio-pesticides can be a satisfactory substitute to the chemical pesticide when used as part of an overall integrated disease management plan. Advances in Bio-pesticide technology like use of beneficial microbes, nanopesticide, encapsulation, Recombinant DNA technology make Bio-pesticide more effective, selective or specific and cause less environmental pollution and less toxic to mammals as compared to conventional pesticides.

AUTHORS' CONTRIBUTION

MMR, MAIK and MAL generated idea; MAH, NH, MMR and MAL coordinated the experiment/research/project; MMR, MAIK and MAL developed methodology; MAH and NH provided scientific insights; MMR, MRB and HAD gathered data and carried out analysis and synthesis; MMR did the writings for all versions of the manuscript; MAL and MAIK performed critical review and editing; All authors read and approved the final manuscript.

ACKNOWLEDGMENT

Authors are very much grateful to Russel IPM, UK and authority of Bangladesh Rice Research Institute (BRRI) Gazipur for providing financial and research support, respectively.

DECLARATION OF INTERESTS

Every author accepts and consents to the publishing of the manuscript. The authors declare having no conflicting interests.

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