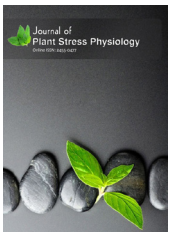
Journal of Plant Stress Physiology 2023, 9: 10-17
doi: 10.25081/jpsp.2023.v9.8459
https://updatepublishing.com/journal/index.php/jpsp

Research Article



ISSN: 2455-0477

Comparative study between biological and chemical agents for control sheath blight disease of rice

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ABSTRACT

Biological control measures are indispensable to sustain global food security, due to it being economically profitable and environmentally sound. A comparative study was conducted to know the effectiveness of biological control measures compared with contact fungicide. *Trichoderma* spp. based bio fungicides Bioquick and Biospark were applied as preventive measures and contact fungicide as a curative measure for controlling sheath blight disease in rice varieties BR 71 and IR 24. Biospark and Bioquick were applied before disease development while, contact fungicide was used after the initiation of sheath blight disease. At the early stage of disease development, the effect of Bioquick, Biospark, and fungicide in terms of reducing percent relative lesion height and percent tiller infection are comparable. At 14 DAI and 18 DAI, contact fungicide performed best among the three control measures based on the two parameters. The genotypes of the rice accessions used in the study also appeared to be a factor in disease development, as evidenced by higher horizontal and vertical disease severity in BR71 than in IR24. Between comparison of Bioquick and Biospark in terms of reducing percent relative lesion height, percent tiller infection, and percent disease control, appeared to be higher in Biospark in both varieties. From this study, we can conclude that farmers can use Biospark as a biofungicide to get maximum benefit considering rice yield and ecology. However, its efficacy is slightly lower than chemical fungicides for controlling sheath blight disease of rice.

KEYWORDS: Biological Control, Disease, Rice, Sheath Blight, *Trichoderma*

Received: June 27, 2023
Revised: August 23, 2023
Accepted: August 26, 2023
Published: September 05, 2023

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INTRODUCTION

Sheath blight is an important and worldwide distributed threatening disease for rice cultivation. Sheath blight is a soil-borne fungal disease caused by *Rhizoctonia solani* AG1-1A (Kühn) with teleomorph stage *Thanatephorus cucumeris* (A. B. Frank) Donk (Lee & Rush, 1983; Agrios, 2005; Uppala & Zhou, 2018). Sheath blight disease of rice causes an average of 10% to 30% yield losses which could reach up to 50% under favorable environmental conditions (Uppala & Zhou, 2018). The introduction of modern semi-dwarf nitrogen-responsive rice cultivars caused the increased incidence of rice sheath blight in many rice-growing areas in the world (Teng, 1990).

Under the microscope, the mycelium of *R. solani* exhibits branching at a right angle with septa close to the branching point (Agrios, 2005). In the field, distinct signs of pathogen infection include the appearance of white web-like hyphae and brownish sclerotial bodies measuring 1 mm to 3 mm (Uppala & Zhou, 2018). Sheath blight develops during the late tillering to early internode elongation stage of rice. The disease is characterized initially as a water-soaked to greenish-gray oval to ellipsoidal lesion measuring 1 cm to 3 cm along the leaf sheath situated 0.5 cm to 3 cm below the leaf collar, which enlarges forming bleached centers and irregular purplish borders (Lee & Rush, 1983; Uppala & Zhou, 2018; IRRI Rice Knowledge Bank, 2019). When the environmental conditions are more favorable, these lesions could spread to the upper part of the leaf sheath, to the leaves, and adjacent tillers. In leaves, symptoms are seen as irregularly shaped lesions with grayish-white centers and dark green, yellow, orange to brown margins. The disease causes the senescence of infected leaves and the softening of infected tillers, resulting in stem lodging (IRRI Rice Knowledge Bank, 2019). Superficial white sclerotia are produced on or near the infected tissues after 6 days which then turn brown and are easily dislodged by running water (Lee & Rush, 1983).

Sheath blight pathogen penetrates the rice tissue through natural openings or appressoria. Some of the factors that

favor *R. solani* infection include the use of susceptible semi-dwarf rice varieties, too much nitrogen application, and environmental conditions such as temperature ranging from 15 °C to 35 °C, ≥ 95% relative humidity, and dense heavily developed canopy (Lee & Rush, 1983; Agrios, 2005; Uppala & Zhou, 2018). In addition, high planting density and overuse of nitrogen fertilizer could induce excessive vegetative growth and dense canopies resulting in low relative humidity within the microclimate of the plants.

Various control measures are being applied to prevent or mitigate the negative effects of the disease. Of all the control strategies being applied to manage rice sheath blight, fungicides are still the most effective reducing disease levels to 4% to 27% depending on the variety planted (Groth & Bond, 2007). However, fungicide application increases the cost of rice cultivation. Furthermore, continued use of these chemicals is accompanied by the risk of the emergence of fungicide-resistant pathogens, making the disease harder to control in the future. The use of fungicides could also threaten different environmental components such as soil, water, and soil-living beneficial microbes.

Some plants inhibiting microbes can suppress plant disease through competition, predation, or antagonism against plant pathogens, or through induction of a plant defense system (Compant *et al.*, 2005; Niranjan *et al.*, 2006). Antagonistic bacteria isolated from plant surfaces, soil and rhizosphere have been extensively used to control major crop diseases caused by various fungal and bacterial diseases (Kanjanamaneesathian *et al.*, 1998; Kazempour, 2004). These microorganisms can be used alone or combined with other chemical or biological control agents for various crop diseases (Paulitz *et al.*, 1992; Datnoff *et al.*, 1995; Duffy & Weller, 1995; Pinson *et al.*, 2010). Important bacterial and fungal biological agents proven to control sheath blight in rice like *Bacillus*, *Pseudomonas*, *Serratia*, and *Erwinia*, *Trichoderma*, *Penicillium Myrothecium verrucaria*, *Chaetomium globosum*, and *Laerisaria arvalis* (IRRI Rice Knowledge Bank, 2019). *Trichoderma* spp. is a mycoparasite that produces low pH antibiotics antagonistic to *R. solani*. Other species such as *T. hamatum* and *T. harzianum* produce enzymes such as chitinase and glucanase, which cause lysis of both the hyphae and sclerotia of *R. solani*. In the Philippines, *Aspergillus* is found to have mycoparasitic interaction with *R. solani* through coiling on the hyphae of the pathogen (Bestil, 2012).

With the increasing interest in environmentally safe and sustainable management strategies to control plant diseases such as rice sheath blight, this study aims to test the efficacy of fungal biological agents against *R. solani* causing sheath blight in rice. Specifically, this experiment aims to:

1. To test the efficacy of *Trichoderma* based commercial biological control products against *R. solani* in screen house conditions; and
2. Compare the efficacy of the commercial *Trichoderma*- based biological agent with a new contact fungicide.

MATERIALS AND METHODS

This experiment was conducted at the Institute of Weed Science, Entomology and Plant Pathology (IWEP) screen house in the University of the Philippines Los Baños (UPLB) from January to May 2019.

Sowing and Transplanting of Rice Plants

Two rice varieties, BR71 and IR24, were used in this experiment. Seeds were sown in seed trays measuring (12 x 4 x 2) inches. The seed trays were placed inside the greenhouse and transferred under sunlight when three shoots emerged for hardening. After 21 days, the rice seedlings were transplanted in clay pots (10 in diameter) containing sterilized lowland soil according to treatments. The optimum dose of urea, Triple superphosphate (TSP), and Muriate of potash (MoP) fertilizer (14-14-14) were applied in three splits: one as a basal dose and others in tillering and panicle initiation stage. During the duration of the experiment, plants were adequately watered and monitored for signs of insect infestation.

Experimental Design and Preparation of Treatments

The experiment was laid out in split plots of two varieties arranged in a randomized complete block design (RCBD) with five replications. Five treatments were used in this experiment. These treatments are as follows

i) Bioquick
ii) Biospark
iii) Contact fungicide (Fungutek© 500SC)
iv) Positive control/Disease control
v) Negative control/Healthy control

For each replicate, two (2) hills with one (1) rice seedling were planted at the appropriate distance. Finally, all plants were placed in the screen house and were labeled appropriately.

Biospark Treatment

Biospark© containing *Trichoderma* sp. as an organic fertilizer was used according to the manufacturer's recommendation. For each variety, 1.5 g of Biospark© was placed into separate plastic cups containing 100 mL distilled water. After the mixture was thoroughly mixed, roots of 21 day old BR71 and IR24 rice seedlings were soaked and left for 21 hours to allow the fungus to enter the natural openings in the root hairs before transplanting in pots (Figure 1).

Bioquick Treatment

Bioquick©, a compost 'ripener' containing *Trichoderma* sp., was also used according to the recommendation label. For each variety, 10 g of Bioquick© was mixed with 10 kg of sterile dry soil. After mixing, for each variety, 1 kg of soil was placed into 5 clay pots and watered to facilitate the transplanting of the rice seedlings.

Contact Fungicide

Following the recommended application rate, the new commercial contact fungicide Fungutek© 500SC containing

chlorothalonil (500 g/L) was used. For the experiment, the fungicide solution was prepared 2 DAI. One liter solution was prepared by mixing 6.5 mL of the fungicide in sterile distilled water. The solution was thoroughly mixed and transferred into a 1 L plastic spray bottle to facilitate application. Fungicides were applied four days after inoculation and observed for confirmation of disease initiation.

Disease Control

Plants were artificially inoculated like other biological and chemical treatments, but no treatments were applied here to control disease pressure. Instead, this treatment was used as a positive or disease control to compare the highest disease intensity and severity with other disease control agents.

Negative Control/Healthy Control

Plants under this treatment were not inoculated with *Rhizoctonia solani* and were cultivated without any control. Therefore, this treatment is considered as negative control/healthy control.

Preparation of Inoculum

A pure culture of *R. solani* was obtained from the Plant Pathology Preparation Room of IWEP. In addition, new pure cultures were revived from the stock culture of *R. solani* to ensure the virulence and viability of the inoculum.

Preparation of Media

Potato dextrose agar (PDA) was used in making new pure cultures of *R. solani*. One liter of PDA was prepared using a standard protocol in PDA making. Potatoes weighing 200 g were thoroughly washed with clean water, sliced, and boiled in distilled water until cooked. When the potatoes were already soft, the broth was filtered through three layers of clean cheesecloth into a microwaveable container containing 20 g dextrose and 20 g PTC Agar. The mixture was homogenized and cooked inside the microwave for 7 minutes until homogenized. The mixture was transferred into separate reagent bottles and was sterilized at 15 psi for 15 minutes in the autoclave. Before plating, five drops of freshly prepared lactic acid (80%) were dropped into the PDA to prevent bacterial contamination. Approximately 20 mL of melted PDA was transferred into 20 individual sterilized Petri plates and allowed to cool down inside the laminar.

Preparation of Pure Culture

Using a flame sterilized 5 cm^2 cork borer, mycelial plugs of uniform sizes were bored from the original pure culture of *R. solani*. Then, using a flamed sterilized wire needle, individual mycelial plugs were transferred and placed at the center of each Petri plate containing PDA. The plates were sealed completely using Parafilm to prevent contamination and entry of insects and were incubated at room temperature for five days.

Inoculation of *R. Solani*

Five-day-old pure culture of *R. solani* was used for inoculation (Figure 2). First, the PDA medium containing culture was cut into eight (8) sections of uniform sizes using a clean popsicle stick on each Petri plate. Then, one (1) mycelial plug (pie) of *R. solani* culture was placed between the rice stem and sheath in two tillers per hill 1 inch above the soil surface of the pot. That indicates the water level of these pot plants. Inoculations were done at the maximum tillering stage of the rice plants. To induce the development of the disease, all inoculated plants were covered with translucent plastic sprayed with sterile distilled water. This practice will also provide optimum moisture and avoid desiccation of the mycelial plug (Figure 3). After 24 hours, the plastics were removed. Untreated rice plants were placed as border plants to increase the plant density and provide a more favorable microclimate for disease development.

Data Collection and Analysis

Data on plant height, % relative lesion height, % disease control, the total number of tillers, number of infected tillers, and % tiller

Figure 1: Before Transplanting in main pots soaking rice seedlings in Biospark for 24 hours

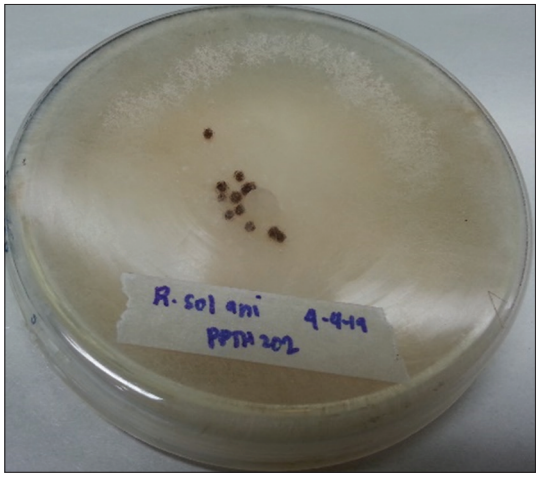

Figure 2: Mycelial culture with sclerotia of *R. solani* on Potato Dextrose Agar (PDA) media

infection were measured 6, 10, 14, and 18 days after inoculation (DAI). Using these data, the relative lesion length, the average number of infected tillers, and the amount of disease or area under the disease progress curve were computed as follows:

$$\%\text{Relative Lesion Length}(\text{RLH}) = \frac{Length\,of\,lesion}{Plant\,height} \times 100$$

$$\%\ \text{Tiller infection} = \frac{Number\,of\ \text{infected}\,tillers}{Total\,number\,of\,tillers} \times 100$$

$$\%\ \text{Disease control} = \frac{highest\,RLH - calculated\,RLH}{Highest\,RLH} \times 100$$

Analysis

The data were analyzed using R software (version 3.5.3). Analysis of Variance (ANOVA) and level of significance was tested by Duncan's Multiple Range Taste (DMRT). For the disease progress, the AUDPC was calculated using the formula:

$$AUDPC = \sum_{i=1}^{n=1} \frac{y_i + y_{i+1}}{2} \times t_{i+1} - t_i$$

Where: y is the proportion of the disease at the i^{th} observation, it is time in days after inoculation (DAI) at the i^{th} observation, and n is the total number of observations done.

RESULTS

In this study two, *Trichoderma* based biological controlling tools Bioquick and Biospark were used to compare their effectiveness in controlling sheath blight with one commercial contact chemical fungicide, Fungutek© 500SC containing chlorothalonil (500 g/L). Pure culture of the sheath (Figure 2) blight inoculum with PDA media was placed between the stem and leaf sheath of rice plants.

After one day of inoculation, growing mycelia were observed on the epidermal surface of the stem (Figure 3) in all treatments. At the primary stage of disease development, the observed mycelial growth is similar in all treatments for rice varieties BR71 and IR24. The negative control was free from the disease (Figure 4).

At 4 DAI, water-soaked lesions already develop in all rice varieties' treatments and start blight symptoms. Furthermore, blighted symptoms were observed to move in an upward direction of the plants by producing typical sheath blight symptoms. Moreover, disease symptoms start to develop vertically and horizontally. Therefore, after initiating blight symptoms, fungicide was sprayed at 2 DAI in rice varieties BR71 and IR24.

Effect at 6 Days after Inoculation (DAI)

During the first data collection at six days after inoculation (DAI), the effects of Bioquick, Biospark, fungicide, and positive control were similar in terms of parameters like plant height, percent (%) relative lesion height (RLH), and percent (%)

Figure 3: Mycelial growth of *R. solani* on stem surface inoculated with PDA culture media one day after inoculation (DAI). Red color rectangular shows Initial whitish mycelia on plant stem

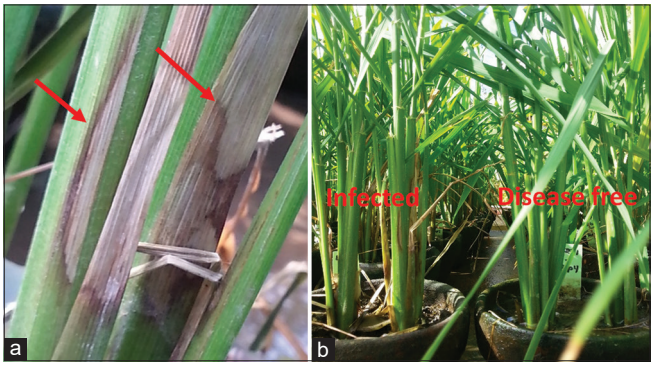

Figure 4: Sheath blight symptom at the base of plants a) Ideal Sheath blight symptom on rice by *R. solani* and b) Infected and disease-free plants in pot. Red arrow indicate sheath blight disease symptom

disease control in BR71 and IR24. Percent (%) tiller infection in plants applied with fungicide was observed to be lower 33.69% and 17.17%, respectively, in BR71 and IR24 (Table 1).

Percent tiller infections were reduced significantly in both rice varieties. However, the percent relative lesion height and percent disease control have no significant difference compared to the positive control treatment.

Effect at 10 Days after Inoculation (DAI)

At 10 DAI, the efficacy of chemical fungicide was observed to be better than the biological agents Bioquick and Biospark in terms of relative lesion height, percent tiller infection, and disease control. Percent relative lesion height and percent tiller infection in fungicide-applied plants are lower, respectively, 14.90 and 34.36 in rice variety BR71 (Table 2). In reducing the percent relative lesion height and percent tiller infection, biological measures bioquick and biospark showed moderate results. Fungicides showed good results in the case of disease control like 37.66% and 29.39% in the variety BR71 and IR24, respectively (Table 2). For controlling the disease, it was found that the performance of Bioquick and

Biospark were similar and were comparatively lower than that of chemical fungicides. Moreover, fungicide showed highly significant results in the case of all parameters on both rice varieties BR71 and IR24.

Effect at 14 Days after Inoculation (DAI)

At 14 DAI, contact fungicide showed better results than Bioquick and Biospark. The percent relative lesion height was 15.40 while the percent tiller infection was 49.49 (Table 3). Similarly, the percent disease control performance was also higher than both biological control measures. Comparison between the two biological control measures showed that Biospark is better than Bioquick in controlling relative lesion height (17.88 and 18.26) and percent tiller infection (68.53 and 46.96) in rice varieties BR71 and IR24, respectively (Table 3). However, vertical disease spreading (RLH) was found to be similar among the treatments and rice varieties, but horizontal disease progress like percent tiller infection was higher in BR71.

Effect Disease Severity 18 Days after Inoculation (DAI)

At the maximum time (18 DAI) of sheath blight disease development, contact fungicide performed best in controlling relative lesion height than the other two biological treatments. In controlling vertical disease progress of like percent relative, lesion height was lower in Biospark than Bioquick in rice variety BR71 and IR24 (Figure 5).

Effect of Treatments on AUDPC

In the case of Area under the disease progress curve of disease severity, the values were found to be increasing smoothly over the whole 18-day assessment period (Figure 6). Disease severity was lowest in contact fungicide in every disease severity measurement time, followed by Biospark and Bioquick in rice variety BR71. On the contrary, the increasing disease severity of rice variety IR24 is not smooth over the whole 18 day disease assessment period. Instead, this variety IR 24, increasing trend of disease severity followed a wavy curve pattern (Figure 7).

Table 1: Effect of biological and chemical agents for controlling rice Sheath blight disease 6 Days after Inoculation (DAI)

Treatment	BR71			IR24		
	%RLH	% Disease control	% Tiller infection	%RLH	% Disease control	% Tiller infection
Bioquick	15.04 a	27.61 b	68.78 a	14.23 a	36.36 b	43.63 b
Biospark	14.24 a	31.42 b	59.09 a	15.68 a	29.92 b	45.15 b
Fungicide	15.13 a	27.17 b	33.69 b	13.62 a	39.14 b	17.17 c
Positive control	13.60 a	34.53	65.36 a	14.77 a	33.95 b	71.94 a
Negative control	0.00 b	100.00 a	0.00 c	0.00 b	100.00 a	0.00 d
LSD value	3.06	14.72	20.79	3.80	16.98	13.88
CV	19.65	24.86	34.16	24.30	26.46	29.09
F value	NS	NS	0.001**	NS	NS	0.001**

RLH = Relative humidity

Table 2: Effect of biological and chemical agents for controlling rice Sheath blight disease 10 Days After Inoculation (DAI)

Teatment	BR71			IR24		
	%RLH	% Disease control	% Tiller infection	%RLH	% Disease control	% Tiller infection
Bioquick	17.40 a	15.30 c	67.51 a	16.23 ab	18.86 bc	53.01 b
Biospark	16.21 ab	24.13 bc	61.52 a	17.40 a	13.00 c	49.50 b
Fungicide	14.90 b	37.87 b	34.36 b	14.12 b	29.39 b	17.17 c
Positive control	17.61 a	14.22 c	71.67 a	15.66 ab	21.70 bc	71.94 a
Negative control	0.00 c	100.00 a	0.00 c	0.00 c	100.00 a	0.00 d
LSD value	2.12	16.15	15.40	2.29	11.46	15.49
CV	11.98	31.46	24.43	13.48	23.36	30.14
F value	0.001***	0.001***	0.001***	0.001***	0.001***	0.001***

RLH = Relative humidity

Table 3: Effect of biological and chemical agents for controlling rice Sheath blight disease 14 Day After Inoculation (DAI)

Treatment	BR71			IR24		
	%RLH	% Disease control	% Tiller infection	%RLH	% Disease control	% Tiller infection
Bioquick	19.05 a	4.74 c	74.25 a	19.99 a	22.54 c	58.92 ab
Biospark	17.88 a	10.59 c	68.53 a	18.26 ab	29.24 bc	46.96 b
Fungicide	15.40 b	23.02 b	49.49 b	16.11 b	37.60 b	29.61 c
Positive control	19.59 a	2.06 c	77.52 a	20.58 a	20.27 c	68.02 a
Negative control	0.00 c	100.00 a	0.00 c	0.00 c	100.00 a	0.00 d
LSD value	2.28	11.40	14.29	3.24	12.53	16.85
CV	11.82	30.27	19.76	16.10	22.30	30.88
F value	0.001***	0.001***	0.001***	0.001***	0.001***	0.001***

RLH = Relative humidity

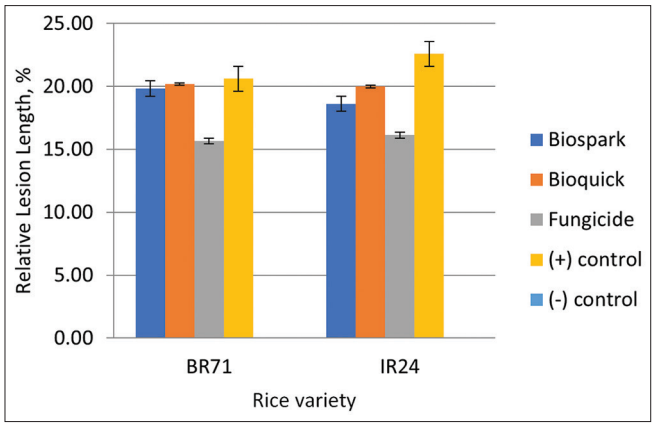

Figure 5: Disease severity of rice variety BR71 and IR24 at 18 Days After Inoculation (DAI)

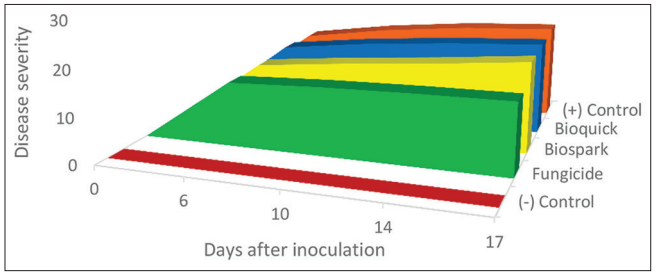

Figure 6: Area Under Disease Progress Curve (AUDPC) for rice variety BR71

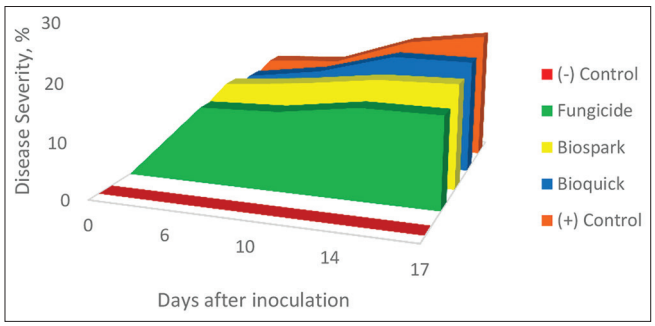

Figure 7: Area Under Disease Progress Curve (AUDPC) for rice variety IR24

DISCUSSION

Rice sheath blight disease control in ambient condition or field level by chemical fungicide showed promising results in many cases, but biological control measure at a satisfactory level is complicated. However, disease control by microbial agents like fungi, bacteria are environmentally sound and economically beneficial. On the other hand, the use of chemical control agents for disease control is not eco-friendly and could create a major problem for flora and fauna in the long run. Therefore, biological control of rice sheath blight is now considered a significant promising alternative approach to chemical fungicide (Wiwattanapatapee *et al.*, 2007; Karthiba *et al.*, 2010; Cuong *et al.*, 2011). Moreover, controlling disease pressure in rice plants is costly and risks to farmers for rice cultivation. This study used contact fungicide that showed better performance than biological control measures Bioquick and Biospark. Both biological agents are *Trichoderma* sp-based biofungicide.

Generally, to control sheath blight disease in farmer's fields, chemical fungicide is mainly used; for that reason, long-term continuous use of chemical fungicides *R. solani* becomes resistant to chemicals. As a result, to control disease, farmers apply more amounts and higher doses of chemicals, which become hazardous to the environment and increase production costs. Moreover, in some cases, the residual effect of chemical fungicides causes a negative impact on rice beneficial microbiomes and soil microbes. Therefore, they are considering different detrimental issues of chemical fungicides experts advocate to increase the use of bio-fungicides. However, the efficacy of bio-agents is comparatively lower than chemical fungicides for controlling diseases.

Bioquick and Biospark, both biofungicide, can reduce the Percent relative lesion height and percent tiller infection. This study found that Biospark showed significant disease reduction ability at 6 DAI, 10 DAI, 14 DAI, and 18 DAI in rice varieties BR71 and IR24. At 14 Days after inoculation (DAI), Biospark and chemical fungicide showed percent disease control 10.59, 23.02, and 29.24, 37.60 for rice varieties BR71 and IR24 respectively (Table 3). Effect of percent disease control, tiller infection, and relative lesion height Biospark showed comparatively better results than Bioquick. The fungus *Trichoderma* spp. was the most commonly used antidotes for various plant diseases. Biological control has become a more realistic option for rice disease management due to research conducted over the previous two decades (*Tsahouridou et al.*, 2002). Furthermore, fungal species of the genus *Trichoderma* have been found in many countries rice fields like the Philippines, China, and Bangladesh (Shovan *et al.*, 2008). *Trichoderma* isolates could be used to develop a biological control agent for F. fujikuroi and a replacement for bakanae management (Ng *et al.*, 2015). *Trichoderma* spp. effectively controls brown spot disease and promotes rice plant growth (Harish *et al.*, 2007). *T. Harzianum* spore suspension sprayed on rice plants resulted in a significant reduction in disease severity under greenhouse conditions (Abdel-Fataah *et al.*, 2007). *Trichoderma* species can colonize the root surface and rhizosphere from treated seeds, protecting them from fungal diseases and stimulating plant growth and productivity (Bakkar, 1988).

Due to their ability to grow much faster than pathogenic fungi, *Trichoderma* isolates inhibit the growth of the target organisms, allowing them to compete for space and nutrients more effectively. According to Barbosa *et al.* (2001) *Trichoderma* spp. inhibits pathogen growth by the production of amylase, partly responsible for antagonists' rapid growth in potato dextrose agar medium. In addition, *Trichoderma* species have been found to produce extracellular cellulose and pectinase enzymes capable of hydrolyzing the cell walls of other fungi, in addition to amylase (Marco *et al.*, 2003). Bacillus strains provide several mechanisms for biocontrol, including induced

systemic resistance, production of antimicrobial compounds, competition for nutrients, and colonization sites with pathogens (Kloepper *et al.*, 2004).

To suppress the microbial community structure caused by the pathogen *R. solani*, Biospark is employing a commercial bioagent, but no significant changes in the root microbiome occur. Indeed, if this bio-fungicide was applied to rice plants prior to disease inoculation, Biospark demonstrated significant antimicrobial activity in suppressing the development of sheath blight disease in rice.

This study found that several naturally occurring fungal isolates can inhibit *R. solani* Growth. Therefore, future research on Bioquick and Biospark should concentrate on their antagonistic ability against various pathogens that cause important plant diseases and the characterization of the molecular and genetic structures responsible for these two bio-agent's antimicrobial activities in order to uncover their underlying mechanism. Furthermore, this study suggests some intriguing future research directions. These include determining whether the antagonist has the same level of efficacy in natural field conditions, determining whether mixtures of biocontrol agents are more effective than a single strain at controlling disease severity and determining the best formulation of promising *Trichoderma* isolates.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENT

This study was supported by the Institute of weed science, Entomology, and plant pathology (IWEP) under the University of the Philippines Los Banos, Laguna, Philippines. We want to give heartfelt gratitude to Prof. Dr. Cubes for helping to get Bio-fungicide Bioquick and Bio spark. We also want to give special thanks to the plant pathology laboratory and field staff for their support during the experiment. Finally, we wish to thank Prof. Ireneo B. Pangga for planning and supervising this research work and Prof. Christian Joseph R. Cumagun for his valuable guideline to prepare the manuscript.

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