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Management of sheath blight of rice using *Bacillus amyloliquefaciens* based biofungicide

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ABSTRACT

Rice (*Oryza sativa* L.) is an important cereal crop belonging to *Gramineae* family. The production and productivity of the rice crop are affected by many fungal, bacterial, and viral diseases. Out of these diseases sheath blight (*Rhizoctonia solani*) is one of the most devastating diseases. Among different management strategies, biocontrol seems to be promising, as the use of chemical fungicides in disease management has many limitations including environmental hazards and development of resistance to fungicides. Considering the importance of the disease, the present study was conducted with the potential biocontrol agent against sheath blight of rice. In this study, the biopesticide *Bacillus amyloliquefaciens* was tested against sheath blight of rice under *in-vitro* and *in-vivo* conditions. Results showed that biopesticide *Bacillus amyloliquefaciens* formulation when applied as seedling plus foliar spray treatment, achieved disease control of 47.20% and 30.04% during both *Kharif* seasons 2022 and 2023, respectively. Meanwhile, the application of the fungitoxicant Amistar top 325 SC, a part of farmers' practices, exhibited significant disease control of 48.80% and 36.50% at a dosage of 200ml in 200 liters of water per acre over the control treatment during the same period (2022 & 2023). The biopesticide *Bacillus amyloliquefaciens* did not show any phytotoxicity on rice crop in both the years respectively. Therefore, based on observations of two years, biopesticide *Bacillus amyloliquefaciens* formulation as seedling plus foliar spray treatment was found effective in managing the sheath blight of rice caused by *Rhizoctonia solani* as compared to other treatments and comparable with framer's practices with plant protectants like fungitoxicant Amistar top 325 SC.

Key words: *Bacillus amyloliquefaciens, Rhizoctonia solani,* rice (*Oryza sativa* L.), sheath blight

Rice (*Oryza sativa* L.) is the most important and extensively grown food crop in the world. It is the staple food of more than 60 per cent of the world population. Predominantly grown and consumed in Asia, India holds the title for the largest area dedicated to paddy cultivation globally and secures the second position in production. In India, area under its cultivation is 46.38 Mha with production of 130.29 Mt and productivity of 2809 kg/ha (Anonymous, 2022). Rice, a important cereal crop, faces challenges from a range of fungal, bacterial, and viral diseases. Out of the various fungal diseases sheath blight is one of the most devastating diseases. The sheath blight pathogen, known as *Rhizoctonia solani* Kühn, has two stages: the anamorph stage and the teleomorph stage, identified as *Thanatephorus cucumeris* (Frank) Donk. It is part of the Basidiomycota division. *R. solani* is a necrotrophic fungus that creates sclerotia, which are tough and consistent in texture, varying in size but capable of staying dormant for extended periods (Mukherjee, 1978). Sheath blight is a fungal disease transmitted through the soil. It typically infects rice plants during the seedling, tillering, and booting stages. In paddy fields, the infection usually initiates near the water line of the rice plants. As it progresses, lesions form and spread upward from the lower leaf sheaths to the upper ones and leaf blades. These lesions feature a grayish-white center with a brown margin. Over time, multiple spots merge, resulting in characteristic blight symptoms. It leads to yield losses ranging from 59 to 69 per cent (Singh *et al*., 2016). Sheath blight disease interrupts grain filling and decreases rice production by 39%, but the loss can increase to 50% in terms of kg/ha of milled whole grain rice because of weakened grains that tend to break during milling. If sheath blight infections climb to 90% of the plant height, the expected loss in

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milled rice yields is about 46% (Suman *et al*., 2017)

Nowadays farmers mostly use single chemical which may lead to undesirable effects such as residual toxicity and environmental pollution, and also increase the risk of resistance development (Brent and Hollomon,1998). Recent advancements in crop disease management involve the utilization of environmentally friendly bio-products and the creation of biological control agents, serving as safe alternatives to chemical treatments. *Bacillus* spp. are notably intriguing in this aspect. They produce secondary metabolites possessing antifungal and antibacterial properties, along with siderophores, enhancing the efficacy of these strains (Khan *et al*., 2022). *Bacillus* spp. have undergone testing as biocontrol agents against diverse phytopathogens. For instance, *B. amyloliquefaciens* demonstrated effectiveness as an antagonistic bacterial strain capable of managing bacterial leaf blight disease and fungal plant diseases (Ji *et al*., 2013). Therefore, the present study was undertaken to evaluate the bioefficacy of *Bacillus amyloliquefaciens* for control of sheath blight of rice.

MATERIALS AND METHODS

The bioefficacy of *Bacillus amyloliquefaciens*, (NBRI Lucknow) was checked under both *in-vitro* and *in-vivo* conditions along with farmers practice with plant protectants like fungi-toxicant Amistar top 325 SC. The field trials were conducted in the experimental area of Department of Plant Pathology, Punjab Agricultural University, Ludhiana during two consecutive cropping seasons (*Kharif* 2022 and 2023). *Bacillus amyloliquefaciens* was procured from CSIR – National Botanical Research Institute (NBRI), Lucknow. The strain was routinely transferred onto Nutrient agar (NA) slants for maintenance, as described by Pathak *et al*. (2019), and stored at 4°C for future experimentation.

The *in-vitro* evaluation of antagonistic potential of *Bacillus amyloliquefaciens* against *Rhizoctonia solani* was carried out by dual culture technique on Nutrient agar medium. Five mm disk of the pathogen along with antagonist (by streaking method) were placed on opposite sides in the Petri plates. Three repetitions of each treatment were kept and the Petri plates with only pathogen served as control. All the plates were incubated at temperature of 28 ± 2ºC and the radial growth of the test organism and pathogen was measured after 7 days.

The formula provided by Vincent (1947) was used to calculate the per cent growth inhibition (PGI).

$$I = \frac{C-T}{C} \times 100$$

Where

I = Per cent growth inhibition

C = Average diameter of mycelial colony of control set

T = Average diameter of mycelial colony of treated set

Based on *in vitro* studies the biopesticide *Bacillus amyloliquefaciens* strain from NBRI, Lucknow was also tested under field conditions at Department of Plant Pathology, Ludhiana during *Kharif* (2022 & 2023). The crop was raised and artificial epiphytotics were created by the standard protocols as given by NBRI Lucknow. For this purpose, PR121 variety was sown at experimental area of Department of Plant Pathology, Punjab Agricultural University Ludhiana along with four different treatments *viz*., (i) (T_1) Untreated control (ii) (T_2) Famers practices with plant protectants like fungi toxicant Amistar top 325 SC @ 200 ml in 200 litre of water per acre spray (iii) (T_3) Seedling dip treatment in formulation only (iv) (T_4) Seeding dip treatment in formulation + foliar spray of water suspended formulation. The seedling treatment was done by forming the suspension of the formulation in the water at the rate 20 g / L and dipping the root seedlings in the suspension for approximately 30 min before transplanting. This treatment was done in shade or during early morning or late afternoon to avoid direct sunlight. The foliar application of water suspended formulation @ 20 g/ liter was sprayed on plants after one month of tillering stage.

The observations were taken regarding number of tillers per hill, disease severity (PDI), plant height, yield (q/ha) and plant biomass(kgm^{-2}) at the time of harvesting. Percent disease index (PDI) was calculated by using 0-5 scale, where; 0 = Healthy plant; 1 = Plants showing initial wilting on one leaf; 2- Plants showing continued wilting in more than one leaf; 3 = Plants with all the leaves wilted; 4 = Plants with all the leaves wilted and stem collapsing; 5 = Dead Plants .

Plant disease index was calculated by using formula given by (Wheeler *et al*.,1969).

$$\text{Plant disease index} = \frac{\text{Sum of numerical disease ratings}}{\text{Total no. of leaf examined} \times \text{Maximum of disease rating scale}} \times 100$$

The experiment followed a Randomized Block Design (RBD) with four treatments, each replicated three times. Each plot measured 2.5m × 2.5m. All data were collected in triplicate and analyzed using oneway ANOVA through the statistical software CPCS1 (Completely Randomized Design-Arcsine Transformation) and were determined by the significant magnitude of *f* value (P≤0.05).

RESULTS AND DISCUSSION

Bacillus amyloliquefaciens (NBRI Lucknow) exhibited strong antagonism against *Rhizoctonia solani* under *in vitro* conditions. There was 52.85 per cent inhibition of growth of *Rhizoctonia solani.* Biopesticide *Bacillus amyloliquefaciens*-NBRI formed a clear inhibition zone of 0.61 cm in size. It was later tested in field conditions at Department of Plant Pathology, Ludhiana against sheath blight of rice. The data obtained in field conditions indicated that PR121 variety of rice when treated with liquid bioformulation of *Bacillus amyloliquefaciens*, was found effective in managing sheath blight of rice over untreated control. The results obtained during the *Kharif* (2022 & 2023) on the adequacy of biopesticide *Bacillus amyloliquefaciens* formulation and fungitoxicant like Farmers practices with plant protectants Amistar top 325 SC (Azoxystrobin 18.2% + Difenconazole 11.4%) @ 200 ml in 200 litre of water per acre spray against sheath blight of rice caused by *Rhizoctonia solani* are presented in Table 1 and Table 2. Findings revealed that during *Kharif* 2022, the biopesticide *Bacillus amyloliquefaciens* formulation as seedling plus foliar application reduced the disease incidence by 47.20% as compared to untreated control. On the other hand, the farmer practices with plant protectants like fungitoxicant Amistar top 325 SC exhibited 48.80% disease control over untreated treatment. The plant growth parameters like plant height and number of tillers (93.59 cm, 12.87) were statistically significant than untreated control. The grain yield and plant biomass were also numerically significant as compared to untreated control.

In the *Kharif* 2023 season also, *Bacillus amyloliquefaciens*-based biopesticide, applied as a seedling dip treatment followed by a foliar spray, proved effective in managing sheath blight in rice. This approach achieved 30.04% disease control compared to the untreated control. The farmers' practice involving fungitoxicant treatment achieved

Table 1. Evaluation of bio-efficacy of biopesticide *Bacillus amyloliquefaciens*-NBRI formulation against sheath blight of rice caused by *Rhizoctonia solani* during *Kharif* 2022

Treatment	Treatment details	Plant height (cm)	No. of tillers per hill	Percent disease index (PDI)	Percent disease control (PDC)	Yield (q/ha)	Plant biomass at harvesting (kg/m^2)
T1	Control	86.36	11.82	83.33 (66.19)	0.00	47.66	1.44
T2	Farmer's Practice with plant protection (Amistar top 325 SC)	95.31	13.88	42.67 (40.72)	48.80	51.88	2.00
T3	Seeding dip treatment in formulation only (*Bacillus amyloliquefaciens)*	92.93	12.77	55.33 (48.28)	33.60	49.45	1.60
T4	Seeding dip treatment in formulation + foliar spray treatment (*Bacillus amyloliquefaciens)*	93.59	12.87	44.00 (41.53)	47.20	50.76	1.84
CD (p≤0.05)		2.23	1.317	12.83		NS	NS
CV (%)		1.27	5.369	13.64		6.55	16.56

Figures in parentheses indicate arc sine transformation for Percent Disease Index

Table 2. Evaluation of bio-efficacy of biopesticide *Bacillus amyloliquefaciens*-NBRI formulation against sheath blight of rice caused by *Rhizoctonia solani* during *Kharif* 2023

Treatment	Treatment details	Plant height (cm)	No. of tillers per hill	Percent disease index (PDI)	Percent disease control (PDC)	Yield (q/ ha)	Plant biomass at harvesting (kg/m^2)
T1	Control	86.01	11.87	87.67 (70.45)	0.00	49.33	1.51
T2	Farmer's Practice with Plant Protectants (Amistar top 325 SC)	95.50	14.06	55.67 (48.24)	36.50	57.67	2.11
T3	Seeding dip treatment in formulation only (*Bacillus amyloliquefaciens)*	92.04	13.02	66.33 (54.40)	24.33	52.33	1.76
T4	Seeding dip treatment in formulation + foliar spray treatment (*Bacillus amyloliquefaciens)*	93.76	13.26	61.33 (51.53)	30.04	53.67	1.94
CD (p≤0.05)		3.87	NS	7.04		3.69	NS
CV (%)		2.99	3.99	6.23		3.31	17.65

Figures in parentheses indicate arc sine transformation for Percent Disease Index

a 36.50% disease control. Although all treatments were numerically similar, they showed statistically significant improvements in controlling sheath blight in rice compared to the untreated control. Regarding plant growth, all treatments exhibited statistically significant improvements in plant height compared to the untreated control. In terms of tiller numbers, treatments showed numerical advantages over the untreated control. The grain yield at harvest was statistically significantly higher in the treatments using the biopesticide *Bacillus amyloliquefaciens* formulation as seedling plus foliar spray and the farmers' fungitoxicant treatment, compared to the untreated control. The plant biomass was also numerically better than untreated control. In Koch's (1999) research, only a handful of commercial bioformulations demonstrated consistent and lasting disease control. However, in our study, the performance of treatments was stable across the experiments conducted over a two-year field period. Data from two consecutive years revealed that the bioformulation of *Bacillus amyloliquefaciens* (NBRI) used in T2 and T4 treatments across both the *Kharif* seasons (2022-2023) showed a significant reduction in disease.

Our study also substantiated the study of Heflish *et al*. (2017). They reported that out of seventeen isolates of *Pseudomonas flourescens*- pf14 isolate showed highest inhibition zone (12.78,11.56) against *Rhizoctonia solani* and *Rhizoctonia oryzae* Also, application of Pf14 isolate of *Pseudomonas flourescens* under greenhouse conditions reduced the disease severity upto 81.03 per cent by seedling root dip + foliar spray treatment. Reshu *et al.* (2017) observed that seed+ soil application of *Trichoderma* and *Pseudomonas* showed lowest sheath blight severity (9.50%) whereas, 10.6 per cent sheath blight severity was recorded in plot receiving seed and foliar application of carbendazim. Similarly, Mishra *et al*. (2019) studied the different strains of *Trichoderma* against *Rhizoctonia solani*. The isolates BHU-11, T4, BHU-8 when applied as seed+ root dip treatment showed reduction of disease incidence and also increased the number of tillers/ hill, plant height and yield of the crop. Murugavel and Kannan (2020) concluded that seed+ foliar application of *Pseudomonas flourescens* showed maximum per cent disease over control (73.74%) against sheath blight of rice. These findings are also in conformity with the observation of Thakur *et al.* (2022) who has reported the bioefficacy of *Bacillus subtilis* against sheath blight of rice. They examined the different isolates of *Bacillus*. Among the eleven isolates tested, BS4 emerged as the most effective in controlling disease, with a minimal per cent disease index of 33.29% compared to other isolates. Mawaddah *et al*. (2023) verified that *Pseudomonas flourescens* when used

either alone or in combination with seed, root, soil, and foliar treatments was effective to control sheath blight of rice and thereby prompted plant growth and increased the rice yield.

Based on two years of consecutive field trial data, it was concluded that the biopesticide *Bacillus amyloliquefaciens* formulation, when used as a seedling and foliar spray treatment, is effective in managing sheath blight in rice caused by *Rhizoctonia solani.* It proved to be more effective than other treatments and was comparable to standard farmer practices using plant protectants like the fungicide Amistar Top 325 SC at a dosage of 200 ml in 200 liters of water sprayed per acre**.**

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