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Eco-Friendly Management of Sheath Blight Disease in Barnyard Millet (*Echinochloa crusgalli*) incited by *Rhizoctonia solani* Kuhn

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ABSTRACT

Sheath blight, caused by *Rhizoctonia solani*, threatens barnyard millet (*Echinochloa crusgalli*), a nutrient-rich cereal critical for food security in India. This study evaluates eco-friendly disease management strategies, including seed treatment, seed biopriming, and organic amendments colonized with biocontrol agents (*Trichoderma asperellum, Pseudomonas fluorescens, Bacillus subtilis*), under field conditions in Uttarakhand. The pathogen was identified via morphological and microscopic analyses. Field trials using a Randomized Block Design assessed disease incidence, severity, and control efficacy across 36 plots. Results showed that farm yard manure (FYM) pre-colonized with a consortium of bioagents achieved 100% disease control efficacy, outperforming individual seed treatments. The study highlights the synergistic potential of bioinoculants in reducing disease incidence and severity, offering a sustainable alternative to synthetic fungicides. These findings underscore the role of bioagents in promoting resilient, eco-friendly agriculture in India.

Key Words: biocontrol agents, bioinoculants, disease management, eco-friendly agriculture, organic amendments, sustainable practices.

INTRODUCTION

Millet is the oldest of the cultivated grain crops and a general term for a variety of small-grained cereal grass varieties (Kumar, 2016; Rawat *et al,* 2019). Vital nutrients are found in millets, and their protein content is regarded as being on par with or better than that of rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*). (Kumar et al, 2017). They are considered as Nutri Cereal crops and slowly being rediscovered by the agricultural research and development community (Kumar, 2016; Grovermann *et al*, 2018). Of the 30.73 million tons of millet produced worldwide, 11.42 million tons are produced in India, making up 37% of the total production. In India, millet is grown on about 24 million ha of land. (Sakamma *et al,* 2018).

Madhya Pradesh has the largest state area (84,000 ha,) among all the states that grow small millets, followed by Chhattisgarh (63,370 ha), Uttarakhand (53000 ha,), and Maharashtra (40980 ha,). With a productivity of 1711 kg/ha, Telangana leads the pack, followed by Tamil Nadu (1444 kg/ha) and Uttarakhand (1339 kg/ha). With an average productivity of 1034 kg/ha, India is the world's largest producer of barnyard millet, both in terms of area (0.146 m ha) and production (0.147 mt). One of the smallest and most resilient millets is barnyard millet (*Echinochloa crusgalli*), which is a member of the Poaceae family and the Panicoideae subfamily. It is known by a number of names, including sanwank, sawan, ooda, and oadalu. Indian barnyard millet, or *Echinochloa frumentacea (Roxb.) Link; syn. E. colona var. frumentacea* (allohexaploid, 2n = 6x = 54), is derived from wild *E. colona* (L.). Compared to other millets, barnyard millet has a relatively higher nutritional status. Nearly all of the essential components of a typical human diet are present in greater amounts in it, including protein

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(11.60g/100g), carbohydrates (74.30g/100g), minerals (4.70g/100g), calcium (14.00 mg/100g), crude fiber (14.70g/100g), and phosphorous (121.00 mg/100g). (Anbukkani *et al,* 2017). Similar to this, barnyard millet's alkaloids, steroids, carbohydrates, glycosides, tannins, phenols, and flavonoids have a variety of ethnomedical qualities, including the ability to heal wounds, reduce biliousness, and alleviate diseases linked to constipation. (Sharma *et al,* 2016; Sayani and Chatterjee, 2017).

Rhizoctonia solani-induced sheath blight has become a growing issue in Uttarakhand's hilly regions. (Kumar and Prasad, 2009; Kumar, 2016). The first reports of *Rhizoctonia solani*-caused sheath blight disease in barnyard millet date back to 2009 (Kumar and Prasad, 2009). Oval to irregular and light grey to dark brown lesions are the symptoms that were previously seen on the sheath but are now also seen on the leaves. Lesions initially developed on the leaves close to the soil's surface, but they quickly spread, merged to cover the upper leaves, and resulted in blighting of the foliage (Kumar, 2016). A series of copper and brown bands appeared across the leaf as the disease worsened, with the central parts of the lesions eventually turning white to straw with thin, reddish-brown borders (Kumar, 2016). Attempts to employ a group of bio-control agents to achieve long-term control of plant diseases have also been made in recent years (Chaube and Sharma, 2002; Rawat *et al,* 2011, 2012).

It is commonly known that *Trichoderma* works well as a biological control agent against a variety of plant diseases, including *R. solani*. (Hicks *et al,* 2014). According to several reports, *Trichoderma's* strong antagonistic and mycoparasitic activity effectively inhibits plant pathogens in the soil (Bhattacharjee and Dey, 2014; Rawat *et al,* 2016), in addition to direct impacts on plant roots, boosting the uptake of nutrients, enhancing seed germination, and boosting plant defenses against biotic and abiotic stressors (Hicks *et al,* 2014; Bhattacharjee and Dey, 2014). Among the most popular bacterial biological agents for combating a variety of soil phytopathogens, such as *Rhizoctonia solani*, are *Bacillus subtilis* and *Pseudomonas fluorescens* (Bhattacharjee and Dey, 2014; Kukreti *et al,* 2017). The synthesis of bioactive substances and/or extracellular hydrolytic enzymes may be the cause of *Bacillus subtilis* and *Pseudomonas fluorescens'* antagonistic activity (Saber *et al,* 2015; Kukreti *et al,* 2017). Using combinations of compatible biocontrol agents that have synergistic effects can result in biological control ability (Hicks *et al,* 2014; Ezzat *et al,* 2015). In order to assess the impact of seed treatment, seed biopriming, and the incorporation of organic amendments with bacterial or fungal antagonists used as soil treatment against the sheath blight disease in barnyard millet caused by *Rhizoctonia solani*, the current study was conducted.

MATERIALS AND METHODS

The current study was carried out at the College of Forestry's Plant Pathology Research Block and Laboratory in Ranichauri, Tehri Garhwal, Uttarakhand. A field trial was carried out in 2021 during the *Kharif* season. Randomized block design was used to lay out the field experiment.

Isolation and identification of the pathogen

In order to isolate the blight-inciting pathogen, the infected sheath sample was taken from the Vegetable Research Block and transported to the Plant Pathology Laboratory at the College of Forestry in Ranichauri. Following a thorough cleaning with sterile distilled water, these sheath samples were allowed to air dry. Both the healthy and diseased portions of the samples were then cut into tiny pieces, each measuring around 4 mm. Following surface sterilization with sodium hypochlorite and double-distilled water, these cut portions were allowed to air dry on sterile blotting paper. A laminar airflow chamber was used to create aseptic conditions for the inoculation of these cut portions onto the petri plates that contained the PDA medium. These plates were then incubated at 25±2 ºC in a BOD incubator for about 7-10 d for the fungus to grow. The fungus was then observed under a compound microscope for its identification.

Table 1. Details of treatments used under field conditions.

Sr. No.	Symbol	Treatment detail	Concentration
1.	T_1	Control/Untreated check	-
2.	T_2	Seed treatment with *Trichoderma asperellum*	@10g/kg seed
3.	T_3	Seed treatment with *Pseudomonas fluorescens*	@10g/kg seed
4.	T_4	Seed treatment with *Bacillus subtilis*	@10g/kg seed
5.	T_5	Seed bio-priming with *Trichoderma asperellum*	@10g/kg seed
6.	T_6	Seed bio-priming with *Pseudomonas fluorescens*	@10g/kg seed
7.	T_7	Seed bio-priming with *Bacillus subtilis*	@10g/kg seed
8.	T_8	Soil application of value added FYM (FYM pre-colonized with *Trichoderma asperellum*)	@5kg pre-colonized FYM/plot
9.	T_9	Soil application of value added FYM (FYM pre - colonized with *Pseudomonas fluorescens*)	@ 5kg pre-colonized FYM/plot
10.	T_{10}	Soil application of value added FYM (FYM pre - colonized with *Bacillus subtilis*)	@ 5 kg pre-colonized FYM/plot
11.	T_{11}	Soil application of value added FYM (FYM pre - colonized with *Trichoderma asperellum +Pseudomonas fluorescens +Bacillus subtilis*)	@ 5kg pre-colonized FYM/plot
12.	T_{12}	Seed treatment with Carbendazim	@ 2g/kg of seed

Table 2. Disease Rating scale for sheath blight disease severity (AICRP Small Millets Proceeding, 2020)

Grade	Description	Host reaction
1	<1% of plant area covered by lesion	Highly resistant (HR)
2	1-5% of plant area covered by lesion	Resistant (R)
3	6-10% of plant area covered by lesion	Resistant (R)
4	11-20% of plant area covered by lesion	Moderately resistant (MR)
5	21-30% of plant area covered by lesion	Moderately resistant (MR)
6	31-40% of plant area covered by lesion	Susceptible (S)
7	41-50% of plant area covered by lesion	Susceptible (S)
8	51-75% of plant area covered by lesion	Highly susceptible (HS)
9	>75% of plant area covered by lesion	Highly susceptible (HS)

Evaluation of fungal and bacterial bioagents and fungicide against sheath blight disease in barnyard millet, incited by *Rhizoctonia solani* Kuhn under field conditions.

The Plant Pathology Laboratory, College of Forestry, Ranichauri, Tehri Garhwal, V. C. S. G. Uttarakhand University of Horticulture and Forestry, Uttarakhand, provided the seed material for the current study. It included one variety of barnyard millet (*Echinochloa crusgalli L.*), known as PRJ-1, as well as pure cultures of three biocontrol agents: *Trichoderma asperellum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*. Prior testing revealed that the aforementioned

Table 3. Effect of different treatments on percent disease incidence, percent disease index, host reaction and per cent efficacy of disease control (PEDC).

Sr. No.	Treatment	Percent Disease Incidence	Percent disease index	Grade	Host Reaction	PEDC
1.	T1	43.36±0.15(41.172)	30.29 ±0.38 (33.38)	5	Moderately Resistant (MR)	0.00
2.	T2	22.58*±0.13(28.36)	12.44*±0.11(20.64)	4	Moderately Resistant (MR)	59.93
3.	T3	25.65*±0.06(30.42)	13.16*±0.02(21.26)	4	Moderately Resistant (MR)	56.55
4.	T4	28.40*±0.34(32.19)	16.44*±0.09(23.91)	4	Moderately Resistant (MR)	45.72
5.	T5	15.74*±0.07(23.37)	9.03*±0.14(17.48)	3	Resistant (R)	70.18
6.	T6	18.43*±0.04(25.41)	10.17*±0.21(18.58)	4	Moderately Resistant (MR)	66.42
7.	T7	19.81*±0.18(26.42)	11.10*±0.08(19.45)	4	Moderately Resistant (MR)	63.35
8.	T8	5.35*±0.02 (13.37)	1.21*±0.02(6.31)	2	Resistant (R)	96.00
9.	T9	7.51*±0.04 (15.89)	2.34*±0.01 (8.79)	2	Resistant (R)	92.26
10.	T10	10.85*±0.14(19.22)	6.70*±0.14 (15.00)	3	Resistant (R)	77.88
11.	T11	0.00*±0.00(0.00)	0.00*±0.00(0.00)	1	Highly Resistant (HR)	100
12.	T12	23.86*±0.19(29.22)	8.72*±0.21 (17.16)	3	Resistant (R)	71.21
	S.E (d)	0.17 (0.11)	0.23 (0.19)			-
	C.D. 0.05)	0.35 (0.24)	0.49 (0.41)			-

bioagents were compatible with one another. Before the seeds were planted, the field was prepared by two power tiller ploughings and the addition of the recommended fertilizer dosage (40:20:20) to the soil. Thirty-six plots measuring 2.0 m by 1.35 m were constructed with appropriate drainage channels, a path, and enough space between them to allow for the labeling and tagging of various replications. The details of these treatments are shown in Table 1.

Observations were made for per cent disease incidence, per cent disease severity, per cent disease control. The per cent disease incidence was recorded for the number of plants found infected in the field, and disease severity for the sheath blight disease was recorded by the scale (Table 2) and calculated by the formula given by Mayee and Datar (1986), and per cent efficacy of disease control (PEDC) was calculated by the formula given by Vincent (1927).

RESULTS AND DISCUSSION

Isolation and identification of the pathogen

Based on documented morphological and microscopic observations, the isolated fungus was determined to be *Rhizoctonia solani*. Under a microscope, the pathogen's mycelium appeared hyaline when it was young and turned brown as it matured. Branching was seen at a roughly right angle to the distal septum. The primary characteristic of the fungus *Rhizoctonia solani* is constriction and the formation of the septum in branches close to the point of origin. as shown in Fig. 1 B. The characters were found similar to those described in the literature of Chowdary and

Govindaiah (2007), Mishra *et al* (2011) along with Jain *et al* (2016).

Disease assessment and its control

Percent Disease Incidence

T1 (Control) had the highest disease incidence rate (43.36%), followed by T4 (*Bacillus subtilis* seed treatment) and T3 (*Pseudomonas fluorescens* seed treatment), with 28.40% and 25.65%, respectively. However, the treatment T11 (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) had the lowest disease incidence (0.00%), followed by T8 (FYM pre-colonized with *Trichoderma asperellum*) at 5.35 % and T9 (FYM pre-colonized with *Pseudomonas fluorescens*) at 7.51%.

Disease severity

Treatment T11 (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) had the lowest disease severity (0.00%) with 1G and highly resistant (HR) type of host reaction.T8 (FYM pre-colonized with *Trichoderma asperellum*) had the second-lowest disease severity (1.21%) with 2G scale and resistant (R) host reaction, and T9 (FYM pre-colonized with *Pseudomonas fluorescens*) had the second-highest disease severity (2.34%) with 2G scale and resistant (R) host reaction.The highest disease incidence (30.29%) was recorded in T1 (Control) with 5G and moderately resistant (MR) host reaction.

Per cent Efficacy of disease control (PEDC)

The treatment T11 (FYM pre-colonized with *Trichoderma asperellum* + Pseudomonas fluorescens + *Bacillus subtilis*) had the highest percentage of disease control efficacy (100.00%) of sheath blight, followed by T8 (FYM pre-colonized with *Trichoderma asperellum*) with 96.0 % and T9 (FYM pre-colonized with *Pseudomonas fluorescens*) with 92.26%. The lowest percentage of disease control efficacy (45.72%) was found in T4 (Seed treatment with *Bacillus subtilis*), followed by T3 (Seed treatment with *Pseudomonas fluorescens*) and T2 (Seed treatment with *Trichoderma asperellum*) with 56.55 % and 59.93 %, respectively. T11 (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) had the lowest percentage disease incidence and PDI, followed by T8 (FYM pre-colonized with *Trichoderma asperellum*) and T9 (FYM pre-colonized with *Pseudomonas fluorescens*). T1 (Control) had the highest percentage disease incidence and PDI. According to a number of studies in the literature, siderophores' production sequesters iron in the root environment, reducing its availability to competing harmful microflora and weakening pathogenic populations. This makes it simpler for rhizospheric biocontrol agents to lyse pathogens. (Bholay *et al,* 2012; Deshwal, 2012). Patro *et al,* (2014).

Morsy *et al* (2015 reported that, in field settings, *Pseudomonas fluorescens* significantly decreased the incidence of sheath blight disease caused by *R. solani*. In a similar vein, B. subtilis has also been shown to reduce the prevalence of disease by Morsy *et al* (2015) in rice crop. The present findings also corroborate with the earlier work of Rani *et al* (2013) in maize with lowest disease severity recorded in *Pseudomonas fluorescens* followed by *Trichoderma viride* and carbendazim against banded leaf and sheath blight disease. Patro *et al* (2020) found that the application of *Trichoderma viride* and *Pseudomonas fluorescens* significantly decreased the incidence of *Rhizoctonia solani*-caused sheath blight in little millet. The development of cereals and legumes, the biofortification of mineral nutrients in grains, and the suppression of phytopathogens under biotic and abiotic stressors have all been found to be successfully accomplished by bioinoculants. (Rawat *et al*, 2011, Rawat *et al,* 2013).

CONCLUSION

The current study makes it clear that every treatment was significantly more effective than the control, which was left untreated. The treatment of FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis* was the most successful among seed treatments, seed biopriming, and colonized compost in terms of disease parameters. This was followed by treatment of FYM pre-colonized with

Fig 1: The experimental field for the field evaluation of different treatments against the sheath blight disease of barnyard millet.

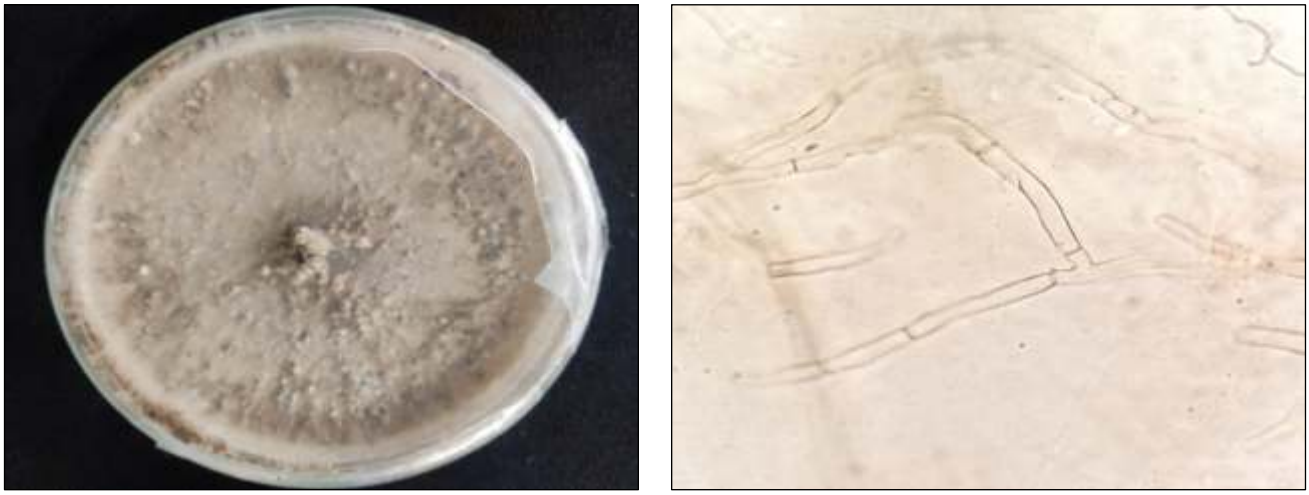

Plate 1 A. Isolated pathogen from infected sheath

Trichoderma asperellum and treatment of FYM pre-colonized with *Pseudomonas fluorescens*. The findings showed that when it came to lowering the incidence and severity of the sheath blight disease, the bioagents were not far behind. Therefore, it can be claimed that bioinoculants can be used in modern agriculture to reduce reliance on synthetic fungicides and can be used in conjunction with various integrated methods to control plant diseases.

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