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Effective management strategies for sheath blight disease of barnyard millet (*Echinochloa crusgalli* L.) incited by *Rhizoctonia solani* in hills of Uttarakhand

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ABSTRACT: Among small millets, barnyard millet has emerged as very important feed as well as fodder crop. The crop is most likely affected by the sheath blight disease incited by *Rhizoctonia solani* in hilly areas of Uttarakhand. Keeping this in view, a field experiment was conducted to evaluate the effect of seed treatment, seed priming and soil application of value added FYM with bioinoculants on growth parameters, yield, yield contributing traits, sheath blight disease suppression and economics of barnyard millet. With respect to plant growth parameters, T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) was found best for the plant height, number of tillers per plant, number of productive tillers per plant and ear length with 148.45 cm, 5.20, 4.06 and 18.62 cm, respectively. The treatment T₁₁ also performed best for yield (22.67 q/ha) and disease suppression (100.00 per cent efficacy of disease control) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with yield of 21.88 q/ha and disease suppression of 96.00 per cent over control. The highest cost-benefit ratio was recorded in treatment T₁₁ with 1: 2.47 followed by T₈ with 1: 2.38. In the present study, the soil application of FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis* (@ 5 kg pre-colonized FYM/plot) was found to be most effective and promising for enhancing morpho-physiological growth, yield, economics and at the same time provided resistance against sheath blight disease in barnyard millet incited by *Rhizoctonia solani*. The present research, thus, offers a novel approach and merits further attention and may also pave the way for the use of bioagents application (in combination) through value added FYM for improving growth, yield and enhanced disease tolerance in barnyard millet.

Key words: Barnyard millet, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Rhizoctonia solani*, sheath blight, *Trichoderma asperellum*

Small millets are grown in many parts of the world because of their unique adaptability in the marginal fertile soil, and moisture stressed condition (Gowda *et al.*, 2006). Among small millets, barnyard millet (*Echinochloa crusgalli* L.) is one of the hardiest millets and is a fair source of protein, which is highly digestible and is an excellent source of dietary fibre (Hadimani and Malleshi, 1993; Veena *et al.*, 2005). In India, barnyard millet is the second important small millet after finger millet having production and productivity of 87 thousand tonnes and 857 kg/ha, respectively (Padulosi *et al.*, 2009).

The *Echinochloa* species generally has potential resistance against various biotic and abiotic stresses. However, cultivated species are threatened by various pests and diseases (i.e., shoot fly, stem borer, leaf spot, leaf blight, grain smut, head smut and sheath blight) at different growth stages of the crop (Jain *et al.*, 1997; Jagadish *et al.*, 2008; Kumar and Prasad, 2009). Among all the diseases, sheath blight caused by *Rhizoctonia solani* Kuhn is one of the most serious diseases worldwide and has been an increasing problem in mid and high hilly areas of Uttarakhand (Kumar and Prasad, 2009; Kumar, 2016). The disease manifests itself as oval to irregular and light grey

to dark brown lesions on the leaf sheath. The central portions of the lesions later turned white to straw colored with narrow, reddish-brown borders, which appears as series of copper and brown colour bands across the leaf sheaths giving a very characteristic banded appearance compared to healthy leaf sheaths. Lesions first appear on the sheaths of leaves near soil level and rapidly extend, coalesce with one another to cover the upper sheaths and cause blighting of the foliage (Kumar, 2016).

It's a challenge to the farmers of hilly areas of Uttarakhand to protect the crop from pests and diseases particularly. Use of expensive fungicides and alternative chemicals to protect the crop is too expensive to the small and marginal farmers in mid and high hills of Uttarakhand, furthermore, these are harmful for the nutritional aspects of the crop. The use of chemicals to control diseases in crop like barnyard millet seems to be uneconomical mainly in hilly areas where the crop is generally grown by small and marginal farmers under rainfed conditions. The use of chemicals also degrades the standard of millets and aren't useful as compared to alternative cereal crops. However, these problems can be overcome by using fungal and

bacterial biocontrol agents like *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., etc. because bio-agents having bio-control and plant growth promoting (PGP) activities may be a viable alternative to minimize the use of synthetic chemicals and their hazardous effects, and to provide protection to the plants against resident pathogen populations (Lugtenberg and Bloemberg, 2001).

Bio-agents can be applied as seed treatment, seed coating, seed priming and soil application, of which, most effective technique is seed bio-priming and soil application using value added FYM/compost as it improves germination, vigour, seedling establishment, yield in crops and reduces various biotic and abiotic stresses (Talebian *et al.*, 2008; Rawat *et al.*, 2011; Rawat *et al.*, 2012). Bio-priming is a seed treatment system that integrates the biological and physiological aspects of disease control, involves coating the seed with fungal or bacterial biocontrol agents. This technique helps seeds to evenly germinate even under adverse soil conditions. Bio-priming of seeds is gaining importance in the management of many plant pathogens as another alternative to chemical fungicides in recent times (Callan *et al.*, 1990; Conway *et al.*, 2001; Rawat *et al.*, 2016). Furthermore, soil application with FYM pre-colonized by bioagents has significantly reduced the incidence of various seed borne diseases in various crops (Wilson and Jackson, 2013).

Considering the above facts, the present study was made to evaluate the effect of seed treatment, seed priming and soil application of value added FYM with bioinoculants on growth parameters, yield, yield attributing traits, management of sheath blight disease and economics of barnyard millet crop.

MATERIALS AND METHODS

The present study was conducted during two *Kharif* seasons of 2020 and 2021 at Plant Pathology Research Block, College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand.

Seed material and biocontrol agents

The seed material for the present investigation was comprised of one variety viz., PRJ-1 of barnyard millet (*Echinochloa crusgalli* L.) which was obtained from Plant Pathology Research Block, College of Forestry, Ranichauri. The pure culture of three biocontrol agents viz., *Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis* and of the pathogen *Rhizoctonia solani* causing sheath blight disease in barnyard millet were obtained from Plant Pathology Laboratory, College of

Forestry, Ranichauri, Tehri Garhwal, V. C. S. G. Uttarakhand University of Horticulture and Forestry, Uttarakhand. The above bioagents were previously tested and were found compatible with each other.

Laboratory experiments (*in vitro*)

In vitro evaluation of bioagents and fungicide against *Rhizoctonia solani* causing sheath blight disease of barnyard millet

Before conducting the field experiments, the bioinoculants and fungicide were evaluated against the pathogen *Rhizoctonia solani* under *in vitro* conditions. The bio-efficacy treatments included three bioagents alone (*Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis*), one combination of bioagents (*Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*), one fungicide (Carbendazim) and a control against the pathogen.

In vitro evaluation of antagonistic activity of bioagents against the pathogen

An attempt was made to study the antagonistic nature of fungal and bacterial bioagents against the pathogen by following the “Dual culture technique” (Johnson and Curl, 1972). For this purpose, 15 ml of PDA was aseptically poured in sterilized petri dishes and allowed to solidify. The 5 mm mycelial disc was cut from the periphery of 5 days old culture of the pathogen and *Trichoderma asperellum*, with the help of sterilized cork borer and needle, placed on opposite sides in petri dishes under aseptic conditions. The same procedure was used to determine the efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis*. A mycelial disc (5 mm) of the isolated pathogen was placed at one end of the plate (10 mm away from the periphery) and the bacterial antagonist was streaked at another end (10 mm away from periphery) of the petri dish just opposite to the mycelial disc of the pathogen. The pathogen inoculated without bio-control agent at one side in petri dish served as check. Those plates were then incubated at 27 ± 2 °C in BOD incubator. For each treatment, three replications were maintained, and mycelium growth of the pathogen was recorded when the control plate was fully covered by the pathogen.

In vitro evaluation of antagonistic activity of the combination of *Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against the pathogen

An attempt was made to study the antagonistic efficacy of the combination of *Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against the pathogen. 15 ml of molten PDA was aseptically poured in test and control petriplates. After the solidification of

media, mycelia disc (5 mm) of five days old culture of the pathogen was placed at the center of the plate whereas 1.6 mm mycelia disc of five days old culture of *Trichoderma* was kept at one corner and 1.6 mm mycelia disc each of the *Pseudomonas fluorescens* and *Bacillus subtilis* were streaked at remaining two corners of the isolated pathogen which were approximately 10 mm away from the periphery of the test plate. PDA medium inoculated only with the pathogen was used as control. Triplicates were maintained of each test and control plates. The plates were then incubated at $27 \pm 2^\circ\text{C}$ and observations were taken when the control plate was fully covered by the pathogen.

***In vitro* evaluation of chemical fungicide against the pathogen**

The efficacy of chemical fungicide against the pathogen was carried out by poisoned food technique (Nene and Thapliyal, 1993). The required amount of carbendazim was weighed and prepared the double strength stock solution in sterile distilled water. The double strength PDA media was prepared and distributed into 500 ml capacity conical flask, which was then autoclaved for 15 minutes at 15 lbs psi (121.6°C). After autoclaving of media, the prepared fungicide stock solution suspension was incorporated into the flask containing sterilized melted PDA. The media toxicated with fungicide at desired dose was poured in to 90 mm sterilized petri plates. Non toxicated media was poured into petri plate to serve as a control plate. After the solidification of media, all the plates were inoculated with an actively growing 5 mm mycelial disc of the pathogen taken from the five days old culture and incubated at a $27 \pm 2^\circ\text{C}$ temperature. The mycelial growth of the pathogen was measured in test plates when the control plate was fully covered by the pathogen.

Observations recorded for *in vitro* study

Mycelial growth of the pathogen (mm)

A measuring scale was used to observe the size of the fungal colony on the petriplates from two opposite ends. Their mean was calculated and was considered as a measure for the mycelial growth of the pathogen.

Per cent mycelium inhibition of the pathogen

Per cent inhibition of the mycelial growth of the test pathogen *Rhizoctonia solani* by the bioagents and fungicide was calculated in relation to the control as described by Vincent (1947).

Preparation of bio-formulations

Formulation of *Trichoderma asperellum*

Talc powder was used as the carrier material to produce

the bio-formulation of *Trichoderma asperellum*. *Trichoderma* was multiplied on pre-soaked and sterilized seeds of barnyard millet and incubated for 12 days at $25 \pm 2^\circ\text{C}$. The colonized seeds were then air-dried, grinded and passed through the 50 and 80 mm mesh size sieve. This spore powder was mixed with pre-sterilized talc powder at 1: 2 (1.0×10^8 cfu was maintained) and stored at 25°C to 27°C in clean and sterilized polythene bags (Zaidi and Singh, 2004) for further use.

Formulation of *Pseudomonas fluorescens* and *Bacillus subtilis*

A loopful of effective bacterial isolate was inoculated into the sterile King's B broth for *P. fluorescens*, Nutrient broth for *B. subtilis* and incubated in a rotatory shaker at 150 rpm for 48-72 hours, at room temperature of $28 \pm 2^\circ\text{C}$. After the incubation period, the bacterial broth containing 9×10^8 cfu ml^{-1} was thoroughly mixed in pre-sterilized raw talc containing 10g carboxymethyl cellulose (CMC). This mixture was then dried in shade and stored in pre-sterilized polythene bags (Nandikumar *et al.*, 2001; Udhayakumar *et al.*, 2019).

Preparation of value added FYM (Colonization of FYM by bio-control agent)

FYM before use was supplemented with bioagents (@ 250g bioagent formulation /quintal of FYM). Bioagents viz., *Trichoderma asperellum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were mixed separately with FYM. The mixture was spread as a 6-10 inch layer under the shade and covered with leaves or rice straw. The supplemented FYM was left for 2 to 3 weeks, with water poured at frequent intervals to keep the FYM heap moist. The FYM heaps were colonized by bioagents after 2 to 3 weeks and were ready for usage since each FYM heap had a large population of inoculated bio-agent. This technique boosted the FYM nutritional content while also allowed the bio-agents to proliferate faster on the FYM (Singh *et al.*, 2003).

For testing the combination effect of the value added FYM, bioagents (@83.3g talc formulation each of *Trichoderma asperellum*, *Bacillus subtilis* and *Pseudomonas fluorescens*) were mixed with one quintal of FYM. The mixture was then spread as a 6-10 inch layer under the shade and covered with leaves or rice straw. The supplemented FYM with three bioagents was left for 2 to 3 weeks, with water poured at regular intervals to keep the FYM heap moist as mentioned above. The colonized FYM was ready for further use as it had large population of all the three inoculated compatible fungal and bacterial bioagents.

Table 1: Details of different treatments along with dosage used in the present study under field conditions

S. No.	Symbol	Treatments	Concentration
1.	T ₁	Control (Untreated check)	-
2.	T ₂	Seed treatment with <i>Trichoderma asperellum</i>	@10g/kg seed
3.	T ₃	Seed treatment with <i>Pseudomonas fluorescens</i>	@10g/kg seed
4.	T ₄	Seed treatment with <i>Bacillus subtilis</i>	@10g/kg seed
5.	T ₅	Seed bio-priming with <i>Trichoderma asperellum</i>	@10g/kg seed
6.	T ₆	Seed bio-priming with <i>Pseudomonas fluorescens</i>	@10g/kg seed
7.	T ₇	Seed bio-priming with <i>Bacillus subtilis</i>	@10g/kg seed
8.	T ₈	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i>)	@ 5 kg pre- colonized FYM/plot
9.	T ₉	Soil application of value added FYM (FYM pre-colonized with <i>Pseudomonas fluorescens</i>)	@ 5 kg pre-colonized FYM/plot
10.	T ₁₀	Soil application of value added FYM (FYM pre-colonized with <i>Bacillus subtilis</i>)	@ 5 kg pre-colonized FYM/plot
11.	T ₁₁	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus subtilis</i>)	@ 5 kg pre-colonized FYM/plot
12.	T ₁₂	Seed treatment with Carbendazim	@ 2g/kg of seed

Evaluation of bioagents against the sheath blight disease in barnyard millet under field conditions

The field experiment of both the crop seasons of 2020 and 2021 was comprised of eleven treatments and one control (Untreated check) with three replications. Each treatment was assigned at random in plots of the research field during the course of the investigation.

Details of treatments

The details of different treatments along with their respective concentrations used in the present study are shown in Table 1.

Field preparation

The field was prepared before the sowing of seeds by two ploughings with a power tiller and recommended dose of fertilizer (40: 20: 20) was added in the soil. Thirty-six plots were made of dimensions 2.0 m × 1.35 m along with proper drainage channels, path and with proper space between them.

Seed treatment

Seeds were treated separately with prepared fungal and bacterial biocontrol formulation @10g/kg of seeds and dried in shade before sowing. Seeds that had not been treated served as control (untreated check).

Seed bio-priming

Seeds were treated separately with prepared fungal and bacterial biocontrol formulation @10g/kg of seeds. After seed treatment, seeds were separately kept under warm and moist conditions until prior to radicle emergence to facilitate *Trichoderma asperellum*, *Pseudomonas*

fluorescens and *Bacillus subtilis* colonization on the spermosphere during incubation for a period of 24 hours before radical emergence. Seeds that had not been pre-treated by biocontrol agents served as the untreated check.

Experimental design and layout:

The seeds were planted in Randomized Block Design (RBD) and treated seeds were sown in each plot at a depth of 3 - 4 cm. The seeds were sown in proper rows with spacing of 22.5 cm between row to row and 10 cm from plant to plant was maintained by doing proper thinning after 20 days of germination. The plot size was comprised of 2.00 × 1.35 m² with six rows in per plot.

Observations recorded

The observations were recorded for two consecutive years by randomly selecting five plants in each replication of every treatment and the average of the readings was calculated for the computation of the data. The data were analyzed statistically through OPSTAT programme, described by Gomez and Gomez (1984). The observations were recorded for the following parameters.

Plant height (cm)

Plant height was measured at the time of physiological maturity. Height of five randomly selected plants per plot was measured from the ground level to tip of the fully developed panicle.

Number of tillers per plant

The number of tillers in each plant was counted by randomly selecting five plants from each treatment and each replication at flowering stage.

Number of productive tillers per plant

The number of productive tillers in each plant was counted by randomly selecting five plants from each treatment and each replication.

Ear length (cm)

The ear length was recorded in centimeter with the help of a meter scale.

Five ears grain weight (g)

The ear heads were selected from the 5 tagged plants from each plot and their grains were threshed and winnowed. After that, grains were weighed and expressed as five ear grain weight.

Grain yield (q/ha)

The ear heads were harvested from each and every plant of each plot and their grain was threshed and winnowed. After that, whole grains were weighed and expressed as grain yield per plot.

Per cent yield increase over control

Per cent yield increase over control was evaluated by recording the yield in the treatments and the yield in control. The formula used for calculation of the per cent

yield increase over control is as follow:

$$\% \text{ yield increase over control} = \frac{\text{Yield in treatment} - \text{Yield in control}}{\text{Yield in control}} \times 100$$

Economics

This parameter was met by calculating the Cost Benefit ratio, formula used for which is,

$$\text{Cost benefit ratio} = \frac{\text{Present value of benefit}}{\text{Present value of cost}}$$

Cost of cultivation (rupee/ha):

The cost of cultivation was evaluated by considering the expenditure that incurred on the basis of existing market price of inputs.

Total output (rupee/ha):

The total cost of the output was calculated by multiplying yield per hectare under various treatments with prevailing selling rates of seeds in the market.

Per cent disease incidence

Per cent disease incidence was calculated by using the following formula (Gashaw *et al.*, 2014).

$$\text{Percent disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of infected examined}} \times 100$$

Table 2: Standard Evaluation System (SES) scale for sheath blight disease in barnyard millet

Grade/Score	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)

Table 3: Effect of different treatments on mycelial growth and per cent mycelium growth inhibition of the pathogen *Rhizoctonia solani*

S. No	Treatments	Mycelial growth of pathogen (mm)	Per cent mycelium growth inhibition of pathogen
1.	Control	90.00 ± 0.39	0 ± 0(0.000)
2.	<i>Trichoderma asperellum</i>	13.35* ± 22.25	85.16* ± 0.921(66.574)
3.	<i>Pseudomonas fluorescens</i>	26.67* ± 4.50	70.36* ± 0.407(57.881)
4.	<i>Bacillus subtilis</i>	39.40* ± 0.02	56.22* ± 0.073(48.70)
5.	<i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus subtilis</i>	0* ± 0.12	100* ± 0.000(90.00)
6.	Carbendazim	21.33* ± 13.13	76.30* ± 1.348(61.818)
	S.E (d)	0.046	0.703(0.462)
	C.D. (0.05)	0.101	1.548(1.018)

() Values in parenthesis are angular transformed; * Significant at 5% level of significance as compared with control

Table 4: Effect of different treatments on plant growth parameters in barnyard millet under field conditions

S. No.	Symbol	Treatments	Plant height(cm)	No. of tillers/plant	No. of productive tillers/plant	Ear length (cm)
1.	T ₁	Control (Untreated check)	125.35 ± 1.58	2.36 ± 0.02	1.23 ± 0.02	13.16 ± 0.242
2.	T ₂	Seed treatment with <i>Trichoderma asperellum</i>	133.81* ± 1.23	3.89* ± 0.07	2.73* ± 0.04	14.63* ± 0.14
3.	T ₃	Seed treatment with <i>Pseudomonas fluorescens</i>	130.29* ± 0.98	3.62* ± 0.02	2.60* ± 0.01	14.13* ± 0.05
4.	T ₄	Seed treatment with <i>Bacillus subtilis</i>	129.62* ± 0.76	3.21* ± 0.03	2.17* ± 0.01	13.95* ± 0.11
5.	T ₅	Seed bio-priming with <i>Trichoderma asperellum</i>	138.48* ± 0.92	4.40* ± 0.03	3.45* ± 0.05	16.46* ± 0.26
6.	T ₆	Seed bio-priming with <i>Pseudomonas fluorescens</i>	136.16* ± 1.75	4.33* ± 0.01	3.26* ± 0.01	15.23* ± 0.19
7.	T ₇	Seed bio-priming with <i>Bacillus subtilis</i>	135.18* ± 0.55	3.98* ± 0.07	2.93* ± 0.04	14.87* ± 0.03
8.	T ₈	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i>)	146.13* ± 0.92	4.60* ± 0.02	3.53* ± 0.02	17.27* ± 0.26
9.	T ₉	Soil application of value added FYM (FYM pre-colonized with <i>Pseudomonas fluorescens</i>)	145.42* ± 2.23	4.53* ± 0.06	3.48* ± 0.18	17.21* ± 0.00
10.	T ₁₀	Soil application of value added FYM (FYM pre-colonized with <i>Bacillus subtilis</i>)	141.97* ± 2.62	4.45* ± 0.02	3.35* ± 0.06	16.52* ± 0.40
11.	T ₁₁	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus subtilis</i>)	148.45* ± 0.37	5.20* ± 0.07	4.06* ± 0.01	18.62* ± 0.17
12.	T ₁₂	Seed treatment with Carbendazim	131.53* ± 1.69	3.82* ± 0.02	2.83* ± 0.04	14.49* ± 0.02
	S.E (d)		2.08	0.06	0.09	0.23
	C.D. (0.05)		4.35	0.13	0.19	0.48

*Significant at 5% level of significance as compared with control

Table 5: Effect of different treatments on five ear grain weight, grain yield and per cent yield increase over control in barnyard millet

S. No.	Symbol	Treatments	Five ear grainweight (g)	Grain yield (q/ha)	Per cent yield increase over control
1.	T ₁	Control (Untreated check)	14.77 ± 0.10	15.17 ± 0.13	0.00
2.	T ₂	Seed treatment with <i>Trichoderma asperellum</i>	17.60* ± 0.06	17.27* ± 0.08	12.15
3.	T ₃	Seed treatment with <i>Pseudomonas fluorescens</i>	16.50* ± 0.26	17.24* ± 0.28	12.00
4.	T ₄	Seed treatment with <i>Bacillus subtilis</i>	15.34* ± 0.104	16.32* ± 0.04	7.00
5.	T ₅	Seed bio-priming with <i>Trichoderma asperellum</i>	20.23* ± 0.39	20.13* ± 0.44	24.63
6.	T ₆	Seed bio-priming with <i>Pseudomonas fluorescens</i>	19.60* ± 0.12	19.00* ± 0.16	20.15
7.	T ₇	Seed bio-priming with <i>Bacillus subtilis</i>	18.05* ± 0.01	18.47* ± 0.24	17.86
8.	T ₈	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i>)	23.15* ± 0.56	21.88* ± 0.14	30.65
9.	T ₉	Soil application of value added FYM (FYM pre-colonized with <i>Pseudomonas fluorescens</i>)	22.07* ± 0.55	21.03* ± 0.38	27.82
10.	T ₁₀	Soil application of value added FYM (FYM pre-colonized with <i>Bacillus subtilis</i>)	21.15* ± 0.43	20.16* ± 0.17	24.75
11.	T ₁₁	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus subtilis</i>)	24.70* ± 0.02	22.67* ± 0.24	33.02
12.	T ₁₂	Seed treatment with Carbendazim	16.76* ± 0.39	18.78* ± 0.15	19.22
	S.E (d)		0.41	0.34	-
	C.D. (0.05)		0.85	0.72	-

*Significant at 5% level of significance as compared with control

Per cent disease severity

The percent disease severity was recorded for each treatment in all replications using sheath blight disease 1-

9 SES scale (Patro *et al.*, 2020). The disease rating scale for scoring sheath blight disease severity is presented in Table 2.

Table 6: Effect of different treatments on percent disease incidence, percent disease severity, host reaction and per cent efficacy of disease control (PEDC).

S. No.	Symbol	Treatments	Percent disease incidence	Percent disease severity	Grade/ Score	Host reaction	PEDC
1.	T ₁	Control (Untreated check)	43.36 ± 0.15 (41.172)	32.29 ± 0.38 (33.38)	6	Susceptible (S)	0.00
2.	T ₂	Seed treatment with <i>Trichoderma asperellum</i>	22.58* ± 0.13 (28.36)	12.44* ± 0.11 (20.64)	4	Moderately Resistant (MR)	61.47
3.	T ₃	Seed treatment with <i>Pseudomonas fluorescens</i>	25.65* ± 0.06 (30.42)	13.16* ± 0.02 (21.26)	4	Moderately Resistant (MR)	59.24
4.	T ₄	Seed treatment with <i>Bacillus subtilis</i>	28.40* ± 0.34 (32.19)	16.44* ± 0.09 (23.91)	4	Moderately Resistant (MR)	49.09
5.	T ₅	Seed bio-priming with <i>Trichoderma asperellum</i>	15.74* ± 0.07 (23.37)	9.03* ± 0.14 (17.48)	3	Resistant (R)	72.03
6.	T ₆	Seed bio-priming with <i>Pseudomonas fluorescens</i>	18.43* ± 0.04 (25.41)	10.17* ± 0.21 (18.58)	4	Moderately Resistant (MR)	68.50
7.	T ₇	Seed bio-priming with <i>Bacillus subtilis</i>	19.81* ± 0.18 (26.42)	11.10* ± 0.08 (19.45)	4	Moderately Resistant (MR)	65.62
8.	T ₈	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i>)	5.35* ± 0.02 (13.37)	1.21* ± 0.02 (6.31)	2	Resistant (R)	96.25
9.	T ₉	Soil application of value added FYM (FYM pre-colonized with <i>Pseudomonas fluorescens</i>)	7.51* ± 0.04 (15.89)	2.34* ± 0.01 (8.79)	2	Resistant (R)	92.75
10.	T ₁₀	Soil application of value added FYM (FYM pre-colonized with <i>Bacillus subtilis</i>)	10.85* ± 0.14 (19.22)	6.70* ± 0.14 (15.00)	3	Resistant (R)	79.25
11.	T ₁₁	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus subtilis</i>)	0.00* ± 0.00 (0.00)	0.00* ± 0.00 (0.00)	1	Highly Resistant (HR)	100.00
12.	T ₁₂	Seed treatment with Carbendazim	23.86* ± 0.19 (29.22)	8.72* ± 0.21 (17.16)	3	Resistant (R)	72.99
	S.E (d)		0.17(0.11)	0.23(0.19)			-
	C.D. (0.05)		0.35(0.24)	0.49(0.41)			-

() Values in parenthesis are angular transformed, *Significant at 5% level of significance as compared with control

Per cent efficacy of disease control (PEDC)

The per cent efficacy of the treatments over the control was calculated using the formula given by Chester (1959) and Wheeler (1969).

$$\text{PEDC} = \frac{\text{Disease severity in control} - \text{Disease severity in treatment}}{\text{Disease severity in control}} \times 100$$

RESULTS AND DISCUSSION

Laboratory study (*in vitro*)

Effect of different treatments on the mycelial growth of the pathogen

Mycelial growths of the pathogen varied among the different bioagents under *in vitro* conditions and are presented in Table 3. All the treatments were found significantly effective over control. However, the minimum radial growth of *Rhizoctonia solani* was observed when bioagents were used in combination (*Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus*

subtilis) with 0.00 mm followed by *Trichoderma asperellum* with 13.35 mm, and Carbendazim with 21.33 mm. Mycelial growth of the pathogen was most favored in Control with 90.00 mm.

Effect of different treatments on the per cent mycelium inhibition of the pathogen

The efficacy of different treatments against *Rhizoctonia solani* was evaluated under *in vitro* and the data on per cent mycelium inhibition of *R. solani* is presented in Table 3. The data revealed that the maximum per cent mycelium inhibition (100.00 %) of the pathogen was observed when bioagents were used in combination (*Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by *Trichoderma asperellum* with 85.16 % and Carbendazim with 76.30 %.

Field study

Data presented are the averages of three replicates and pooled data obtained from two independent experiments conducted during Kharif- 2020 and Kharif- 2021. The

Table 7: Effect of different treatments on economics (cost benefit ratio) of barnyard millet crop grown under field conditions

S. No.	Symbol	Treatments	Gross Return/ ha (Rs.)	Cost of Cultivation/ ha(Rs.)	Net Returns (Rs.)	Cost Benefit ratio
1.	T ₁	Control (Untreated check)	91020	54856	36164	1: 1.659
2.	T ₂	Seed treatment with <i>Trichoderma asperellum</i>	103620	55019	48601	1: 1.883
3.	T ₃	Seed treatment with <i>Pseudomonas fluorescens</i>	103440	55019	48421	1: 1.880
4.	T ₄	Seed treatment with <i>Bacillus subtilis</i>	97920	55019	42901	1: 1.779
5.	T ₅	Seed biopriming with <i>Trichoderma asperellum</i>	120780	55019	65761	1: 2.195
6.	T ₆	Seed biopriming with <i>Pseudomonas fluorescens</i>	114000	55019	58981	1: 2.072
7.	T ₇	Seed biopriming with <i>Bacillus subtilis</i>	110820	55019	55801	1: 2.014
8.	T ₈	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i>)	131280	55035	76245	1: 2.385
9.	T ₉	Soil application of value added FYM (FYM pre-colonized with <i>Pseudomonas fluorescens</i>)	126180	55035	71145	1: 2.292
10.	T ₁₀	Soil application of value added FYM (FYM pre-colonized with <i>Bacillus subtilis</i>)	120960	55035	65925	1: 2.197
11.	T ₁₁	Soil application of value added FYM (FYM pre-colonized with <i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus subtilis</i>)	136020	55035	80985	1: 2.471
12.	T ₁₂	Seed treatment with Carbendazim	112680	55404	57276	1: 2.033

Market price of grain = Rs. 60/Kg, Cost of cultivation = [(Cost of land preparation + Cost of fertilizer + Cost of weeding + Cost of harvesting + Cost of labour for all the activities) + Cost of treatment]

experiments were performed in a factorial completely randomized design. Field performance revealed that biocontrol agents applied through seed biopriming and colonized FYM, were found comparatively superior to seed treatment and untreated plot with respect to improving different planting value parameters. The results obtained from the present investigation as well as relevant discussion have been summarized under following heads:

Plant height (cm)

Plant height was significantly influenced by different treatments that ranged from 125.35 cm to 148.45 cm and data is presented in Table 4. The maximum plant height (148.45 cm) was measured in T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 146.13 cm plant height while minimum plant height was measured in T₁ (Control) with 125.35 cm followed by T₄ (Seed treatment with *Bacillus subtilis*) with plant height of 129.62 cm. The enhanced plant height in treatment T₁₁ might be due to rhizobacterial action of auxin production and phosphate solubilization by pre-colonized FYM with combination of different bio-agents which played a key role in better plant growth along with increased plant height. Similar results were reported by Raj *et al.* (2004) in pearl millet and Rawat *et al.* (2019) in finger millet

Number of tillers per plant

A perusal of mean data depicted that the number of tillers

per plant ranged from 2.36 to 5.20. The highest value (5.20) for the number of tillers per plant was observed in the treatment T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 4.60 tillers per plant which was at par with T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 4.53 tillers per plant while minimum tillers per plant was recorded in T₁ (Control) with 2.36 followed by T₄ (Seed treatment with *Bacillus subtilis*) with 3.21 tillers per plant which was statistically at par with treatment T₁₂ (Seed treatment with Carbendazim) with 3.82 tillers per plant. The possible reason for the extent of variability in tillers per plant might be brought about by the production of phytohormones like auxin, cytokinin and gibberellin. Also, microbial inoculants would have enhanced the uptake of nutrients from soil that helped in improved plant growth. These results are also in close agreement with the findings of Raj *et al.* (2005) in pearl millet, Rawat *et al.* (2018) in barnyard millet and Seenivasan (2011) in rice.

Number of productive tillers per plant

The number of productive tillers had significantly differed among different treatments that ranged from 1.23 to 4.06 productive tillers per plant and are presented in Table 4. The highest number (4.06) of productive tillers per plant were counted in T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with

Trichoderma asperellum) with 3.53 and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 3.48 which were statistically at par, while the lowest number of productive tillers were counted in T₁ (Control) with 1.23 productive tillers per plant followed by T₄ (Seed treatment with *Bacillus subtilis*) with 2.17 productive tillers per plant. The significant variation in number of productive tillers per plant might be due to the growth enhancing effect of phytohormones such as auxin, cytokinin and gibberellin produced by the action of bio-agents. Rawat *et al.* (2018) and Raj *et al.* (2005) also reported similar results in barnyard millet and pearl millet respectively.

Ear length (cm)

Ear length was influenced significantly by different treatments which varied from 13.16 cm to 18.62 cm. Maximum ear length (18.62 cm) was measured in T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 17.27 cm and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 17.21 cm which were statistically at par while lowest ear length was measured in T₁ (Control) with 13.16 cm followed by T₄ (Seed treatment with *Bacillus subtilis*) with 13.95 cm ear length (Table 4). The present findings also corroborate with the previous findings of Prasad *et al.* (2009) in wheat and Rawat *et al.* (2019) in finger millet where bioagents incorporation resulted in increased ear length.

Five ear grain weight (g)

Seed weight is the most important qualitative as well as the quantitative parameter that directly affects the seed yield and quality of the seed lot. In the present study, five ear grain weight ranged from 14.77 g to 24.70 g and are presented in Table 5. Maximum five ear grain weight (24.70 g) was recorded in T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 23.15 g and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 22.07 g while minimum was recorded in T₁ (Control) with 14.77 g followed by T₄ (Seed treatment with *Bacillus subtilis*) with 15.34 g. The treatment T₃ (Seed treatment with *Pseudomonas fluorescens*) with 16.50 g five ear grain weight was found statistically at par with the treatment T₁₂ (Seed treatment with Carbendazim) with 16.76 g five ear grain weight. The probable reasons for the enhancement in ear grain weight might be due to incorporation of plant growth promoting microbes. Similar findings were reported by Prasad *et al.* (2009) in wheat and Raj *et al.* (2004) in pearl millet.

Grain yield (q/ha)

Grain yield is perhaps the most essential factor in selecting a crop for commercialization and income production. Significant differences were observed for grain yield which ranged from 15.17 to 22.67 q/ha as depicted in Table 5. Maximum grain yield (22.67 q/ha) was measured in the treatment T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 21.88 q/ha and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 21.03 q/ha which were statistically at par with each other whereas minimum grain yield was recorded in T₁ (Control) with 15.17 q/ha followed by T₄ (Seed treatment with *Bacillus subtilis*) with 16.32 q/ha grain yield. The present findings are in line with the previous findings of other workers viz., Raj *et al.* (2004) in pearl millet, Muis and Quimio (2006) in maize, Prasad *et al.* (2009) in wheat, Kumar (2013) in barnyard millet, Tiwari (2017) in barnyard millet and Patro *et al.* (2020) in little millet. The enhancement in yield and its contributing traits might be due to the synergic result of numerous modes of actions exhibited by consortium of compatible bioagents including suppression of deleterious microorganisms and pathogens, production of HCN and siderophores, phosphate solubilization and promotion of shoot and root growth, and the root growth promotion increases the absorptive surface area of roots for uptake of water and nutrients (Raji *et al.*, 2016; Sivakalai and Krishnaveni, 2017).

Per cent yield increase over control

It is evident from the results that all the treatments were effective in increasing the yield as compared to control. The per cent yield increase over control was found maximum (33.02 %) under the influence of treatment T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 30.65 % and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 27.82 %, whereas the minimum per cent yield increase over control was observed in T₄ (Seed treatment with *Bacillus subtilis*) with 7.00 %. This increase in per cent yield over control might be due to the growth enhancing effect of different bio- inoculants which directly or indirectly affected the growth parameters of plant, suppressed disease and hence enhanced the per cent yield increase over control.

Disease assessment and percent efficacy of disease control

All the treatments were observed superior in reducing the percent disease incidence and per cent disease severity



Fig. 1: Sheath blight disease of barnyard millet (var. PRJ 1) in T₁ (Control) under field conditions.

when compared to the untreated check as presented in Table 6. The highest per cent disease incidence (43.36 %) was recorded in T₁ (Control) followed by the treatment T₄ (Seed treatment with *Bacillus subtilis*) and T₃ (Seed treatment with *Pseudomonas fluorescens*) with 28.40 % and 25.65 %, respectively. Whereas, the minimum per cent disease incidence (0.00 %) was recorded in the treatment T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 5.35 % and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 7.51 %.

The data depicted in Table 6 also revealed that all the treatments were significantly effective in reducing the disease severity when compared to control. The lowest disease severity (0.00 %) with 1G and highly resistant (HR) type of host reaction was recorded in T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas*

fluorescens + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 1.21 % disease severity that occurred in score 2G with resistant (R) host reaction and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 2.34 % disease severity that also occurred in score 2G with resistant (R) host reaction while, maximum disease incidence (32.29 %) was recorded in T₁ (Control) with disease score 6G and susceptible (S) type of host reaction (Fig. 1).

The maximum per cent efficacy of disease control (100.00 %) of sheath blight (Fig. 2) was observed in the treatment T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with 96.25 % and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with 92.75 %, while minimum per cent efficacy of disease control (49.09 %) was recorded in T₄ (Seed treatment with *Bacillus subtilis*) followed by



Fig. 2: Performance of barnyard millet (var. PRJ 1) in treatment T₁₁ (Soil application of value added FYM i.e., FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) showing sheath blight disease free crop under field conditions

T₃ (Seed treatment with *Pseudomonas fluorescens*) and T₂ (Seed treatment with *Trichoderma asperellum*) with 59.24 % and 61.47 %, respectively (Table 6).

It can be concluded that all the treatments were found effective in controlling the sheath blight disease in barnyard millet crop when compared to control (Untreated check). The present findings also corroborate with the earlier work of Rani *et al.* (2013) in maize with lowest disease severity recorded in treatment of *Pseudomonas fluorescens* followed by *Trichoderma viride* and carbendazim against banded leaf and sheath blight disease. Patro *et al.* (2020) observed that the application of *Pseudomonas fluorescens* and *Trichoderma viride* resulted in significant reduction of sheath blight incidence in little millet caused by *Rhizoctonia solani*. Several reports in the literature suggested that the production of siderophores by biocontrol agents sequester iron in the root environment, making it less available to competing deleterious microflora and making the pathogenic populations weak and thus, easier lysis of pathogens by rhizospheric biocontrol agents (Bholay *et al.*, 2012; Deshwal, 2012). Patro and Prusty (2014) has earlier reported that *Pseudomonas fluorescens* showed a significant reduction in sheath blight disease incidence caused by *R. solani* under field conditions in small millets. Similarly, *B. subtilis* was also reported to inhibit the incidence of disease by Morsy and EL-Said (2015) in rice crop. The present results are also supported by the previous work of Wani (2015) who evaluated *Trichoderma* strain against the leaf blight disease in maize with minimum disease incidence (5.40 %).

Economics of the different treatments (Cost benefit ratio)

The cost benefit ratio was calculated for all the treatments and control and the data are depicted in Table 7 and it is evident that all the treatments were potent and effective when compared to untreated check. The cost benefit ratio was found minimum in T₁ (Control) which was measured 1: 1.659 followed by T₄ (Seed treatment with *Bacillus subtilis*) with 1: 1.779. The maximum cost benefit ratio (1: 2.471) was obtained in T₁₁ (FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis*) followed by T₈ (FYM pre-colonized with *Trichoderma asperellum*) with cost benefit ratio 1: 2.385 and T₉ (FYM pre-colonized with *Pseudomonas fluorescens*) with cost benefit ratio 1: 2.292. Singh *et al.* (2019) has previously reported the highest cost benefit ratio in rice crop by the use of *Pseudomonas fluorescens* followed by *Trichoderma viride* and *Trichoderma harzianum* against eco-friendly management of sheath

blight disease caused by *Rhizoctonia solani* Kuhn.

CONCLUSION

Based on the results of the present experiments, it could be concluded that the bio-agents when applied through different modes of application *viz.*, seed treatment, seed biopriming and value added FYM, had significant influence on plant growth parameters, yield, disease suppression, and economics of barnyard millet crop. The soil application of value added FYM pre-colonized with *Trichoderma asperellum* + *Pseudomonas fluorescens* + *Bacillus subtilis* (@ 5 kg pre-colonized FYM/plot) was found most effective and promising for enhancing morpho-physiological growth, yield, economics and at the same time provided resistance against sheath blight disease in barnyard millet incited by *Rhizoctonia solani* under present materials and environmental conditions. The present research, thus, offers a novel approach and merits further attention and may also pave the way for the use of bioagents application (in combination) through value added FYM for improving growth, yield and enhanced disease tolerance in hills of Uttarakhand.

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