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Eco-friendly Management of Collar Rot of Lentil by Introduced Native Rhizobacterial Candidates

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ABSTRACT

Background: Collar rot is an important disease of lentil in India and causes significant yield loss annually. Considering the recent focus on the development and use of environmentally feasible management strategies, the objectives of the study was to identify resistant sources and evaluation of native antagonists as well as plant growth promoting rhizobacteria (PGPRs) in yield improvement and disease management of lentil.

Methods: Eleven popular lentil varieties were screened for resistance/susceptibility reaction against collar rot *in vivo*. The efficacy of two bacterial and fungal biocontrol agents (BCAs) was tested against a virulent isolate of *Sclerotium rolfsii*. Four PGPRs were also evaluated to study their influence on the growth parameters as well as their ability to manage *S. rolfsii.*

Result: Three genotypes were found to be tolerant, four genotypes were moderately susceptible, while four genotypes were highly susceptible. Among the BCAs, the highest average inhibition % was observed in treatment with *Bacillus* sp. Among the PGPR treatments, *Rhizobium* in combination with phosphate solubilizing bacteria and *Trichoderma* or *Bacillus* was the most effective in controlling the collar rot when used as seed treatment and hence can be used for disease management.

Key words: Biocontrol agents, Disease resistance, Lentil, PGPR, *Sclerotium rolfsii.*

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is a popular and important legume in India (Kumar *et al*. 2016), with more than 50 varieties being cultivated and consumed in different states of the country (Koshariya *et al*. 2020). It is vulnerable to a plethora of seed and soil borne diseases, among which collar rot is a growing concern. *Sclerotium rolfsii*, the causal organism of collar rot, is a necrotrophic fungus with a broad host range. It produces sclerotia during unfavorable conditions, which serves as primary source of inoculum for establishment of disease during the next cropping season. Fluffy white fan like fungal mycelia can be seen on the collar region of infected plants, which results in wilting.

Due to its high competitive saprophytic ability, the pathogen has growing significance throughout the country (Singh *et al*. 2012). Resistant varieties are the most effective solution to this problem, but inadequate availability of resistant sources is a limiting factor. Considering the hazardous nature of fungicides, the use of bio-agents is rendered as the most suitable alternative for management of the fungus (Jegathambigai *et al*. 2010; Chakraborty *et al*., 2021; Rayanoothala *et al*., 2021). The efficacy of BCAs have been previously reported in various studies. The siderophore producing bacteria *Alcaligenes sp.* and *Pseudomonas fluorescens* have been reported to be antagonsistc to *Rhizoctonia solani* (Solanki *et al*. 2012). Promising results have also been recorded by *Trichoderma sp*. against tomato root rot (Rai *et al*. 2016; Kashyap *et al*. 2020), *Bacillus sp*. against *Rhizoctonia solani* in tomato (Solanki *et al*. 2015), *Bacillus sp*. against soil borne diseases of chick pea (Sharma *et al.* 2019; Kushwaha *et al*. 2021), *Trichoderma sp*. against *Phomopsis vexans* (Jakatimath *et al*. 2017). The present study was therefore conducted to evaluate lentil varieties against *S. rolfsii.* Experiments were conducted to determine the efficacy of previously reported BCAs on *S. rolfsii* and to assess the influence of plant growth promoting rhizobacteria (PGPR) on the growth attributes of lentil as well as its role in the management of collar rot disease.

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MATERIALS AND METHODS

Isolation and purification of the pathogen

Infected lentil plants were collected from different districts of West Bengal. Sections of 1-2 mm diameter were cut from infected collar region and washed with distilled water. It was then surface sterilized by dipping in mercuric chloride solution (0.1%) for 30 seconds followed by washing in sterile distilled water for 2-3 times. The sections were then placed

in sterilized potato dextrose agar (PDA) media and incubated at 28±1°C in incubator for 7 days for the pathogen growth. The culture was maintained on PDA slants at 5±1°C and preserved for further studies.

Screening of different lines against virulence pathogen *in vivo*

Seeds of eleven lentil varieties (*viz.*, PL -639, LL-56, NDL-11-1, PL-406, KLS-218, KLS-107, RANJAN, WBL-77, NATALIA, RL-12-176 and WBL-8) were procured from Department of Biotechnology, BCKV, Mohanpur, Nadia. The varieties were grown in pots in randomized block design (RBD) with three replications, under greenhouse conditions during the *rabi* season of 2018-19. After 15 days, the soil was inoculated with a 10 day old culture of SRC-7 isolate of *S. rolfsii* (Mondal *et al*. 2020). Disease incidence and mortality percentage were recorded from 5 days after inoculation. Disease incidence (DI) was calculated by the formula:

$$DI\ \% = \frac{\text{Total number of infected plants}}{\text{Total number of healthy plants}} \times 100$$

Mortality percentage was calculated by the formula:

$$\text{Mortality percentage (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of seedlings}} \times 100$$

The plants were classified on the basis of their % mortality and disease incidence as highly resistant (less than 1% mortality), resistant (1-10% mortality), tolerant (11-20% mortality), moderately susceptible (21-50% mortality) and highly Susceptible (mortality 50% or more) (Akram *et al*. 2008).

In vitro* evaluation of biocontrol agents (BCAs) against *S. rolfsii

In-vitro evaluation of two fungal BCAs, *viz.*, *Trichoderma* spp (Accession no. MT107908) and bacterial BCAs, *Bacillus subtilis* (Accession no. MZ900916) and *Pseudomonas* sp. (Accession no.MZ919354) was carried out using dual culture technique with 5 replications for each treatment (Morton and Straube 1955). Colony diameter of BCAs and test fungus was recorded after 48 hours and sclerotia production after 15 days. The plates were incubated at 28 ± 1°C for 4 days, after which the diameter of the test fungal culture was recorded. Percent inhibition of *Sclerotium rolfsii* was calculated by the following formula (Vincent 1947):

$$\text{Percent growth inhibition (I)} = \text{C-T/C} \times 100$$

Where
C = Colony diameter of *Sclerotium rolfsii* in control plate (mm).
T = Colony diameter in the treated plate (mm).

Effect of plant growth promoting rhizobacteria (PGPR) against *Sclerotium rolfsii in vivo*

Seeds of Moitree, a resistant variety of lentil was collected from seed farm, Bidhan Chandra Krishi Viswavidyalaya and treated with four PGPRs (*viz., Rhizobium lentis* (Accession no. MZ956773), phosphate solubilizing bacteria [PSB: *Paraburkholderia caribenis* (Accession no.MZ956803)], *Bacillus subtilis* and *Pseudomonas* sp) (Table 1) and cultured in their respective media. The strains were incubated at 35°C for 48 hours after which the broth media was centrifuged at 10,000 rpm for 20 minutes to obtain a bacterial suspension.

Seven treatments, *i.e.*, *Rhizobium* + phosphate solubilizing bacteria + *Pseudomonas* isolate (R + PSB + P), *Rhizobium* + Phosphate solubilizing bacteria + *Bacillus* isolate (R + PSB + B), *Rhizobium* + Phosphate solubilizing bacteria + *Trichoderma* isolate (R + PSB + T), *Rhizobium* + Phosphate solubilizing bacteria (R + PSB), *Rhizobium* + *Trichoderma* (R + T), *Rhizobium* + *Bacillus* isolate (R + B) and *Rhizobium* + *Pseudomonas* isolate (R + P) were taken. Before using the combinations of treatment, their compatibility was studied (Das *et al*. 2017). The lentil seeds were inoculated with the seven treatments and dried in shade overnight. The next day, perforated plastic pots of 8 cm diameter were filled with unsterilized soil and the inoculated seeds were sown in them with three replications. Un-inoculated seeds served as a control. The experiment was conducted in the net house of Bidhan Chandra Krishi Viswavidyalaya. The plant growth attributes, *viz.,* seedling fresh wt. (g), seedling dry wt. (g), no. of Nodules, Nodule dry wt. (g), nodule fresh wt. (g), were recorded. The collar rot disease incidence and mortality percentage were recorded to evaluate the PGPR against *S. rolfsii.*

RESULTS AND DISCUSSION

Screenings of lentil varieties against virulent isolate of *S. rolfsii*

In the present investigation, the disease incidence and mortality percentage varied significantly among the varieties studied (Fig 1). Maximum disease incidence was recorded in WBL-81 (37.89%) while minimum disease incidence was observed in LL-56 (3.33%) at 5 days after inoculation (DAI). Similar trend was recorded at 10 and 15 DAI, with disease incidence increasing gradually with increase in DAI (Fig 2). Highest disease incidence was observed at 20 DAI, with WBL-81 (47.62%) and LL-56 (10.0%) exhibiting maximum and minimum disease incidence, respectively (Table 2).

Table 1: List of bacterial antagonists used along with their location.

Bacterial bioagents	Location	Accession no.
Rhizobium sp. *(Rhizobium lentis)*	23.50° (N)87.49° (E)	MZ956773
PSB (*Paraburkholderia caribenis*)	23.65° (N)88.13° (E)	MZ956803
Pseudomonas sp. (Pseudomonas putida)	22.90° (N)88.39° (E)	MZ919354
Bacillus spp. (Bacillus subtilis)	23.61° (N)87.69° (E)	MZ900916
Trichoderma sp. (Trichoderma viride)	22.90° (N)88.39° (E)	MT107908

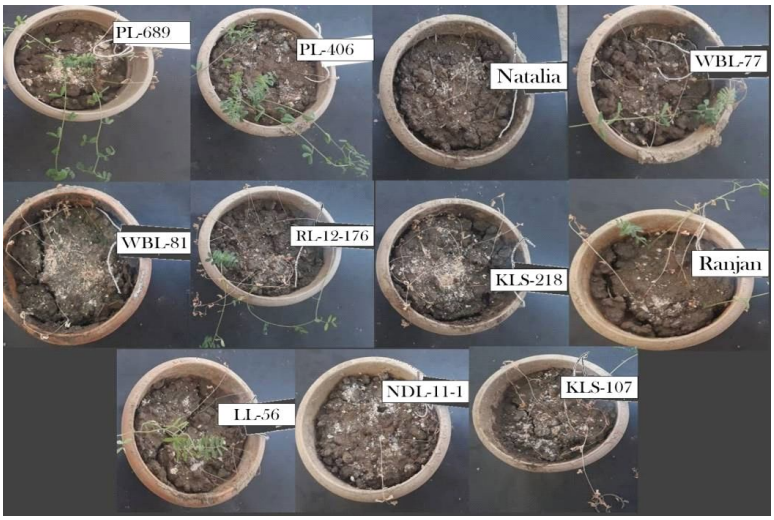

Fig 1: Screening of lentil varieties under controlled conditions.

Table 2: Screening of lentil varieties against *Sclerotium rolfsii.*

Variety	Disease incidence (%) at DAI				Mortality (%) at DAI			
	5 DAI	10 DAI	15 DAI	20 DAI	15 DAI	20 DAI	25 DAI	30 DAI
PL 639	15.28	15.28	19.44	23.61	18.453	18.45	24.80	33.69
LL 56	3.33	6.67	10.00	10.00	3.70	7.41	11.11	11.11
NDL11-1	13.69	13.69	21.19	28.69	18.10	18.10	28.15	41.67
PL 406	11.43	11.43	27.30	27.30	13.89	13.89	38.33	38.33
KLS 218	3.70	3.70	19.91	27.78	4.17	4.17	25.40	38.89
KLS 107	25.18	35.92	47.04	47.04	39.17	57.62	68.00	80.95
Ranjan	15.08	15.08	15.08	15.08	20.00	20.00	20.00	20.00
WBL 77	5.56	16.99	16.99	16.99	6.67	20.56	20.56	20.56
Natalia	25.00	25.00	29.17	38.69	44.44	44.44	53.33	66.67
RL12-176	37.41	37.41	41.11	41.11	65.08	65.08	69.84	69.84
WBL 81	37.90	37.90	47.62	47.62	61.67	61.67	91.67	91.67
	S.E(m) ±		C.D at 5%		S.E(m) ±		C.D at 5%	
Variety	3.38		9.53		7.35		20.69	
DAI	2.04		5.74		4.43		12.48	
Variety × DAI	6.77		NA		14.69		NA	

Maximum mortality percentage was recorded in RL-12-176 (65.08%), while LL-56 (3.70%) exhibited minimum mortality at 15 DAI. A similar trend was observed at 20, 25 and 30 DAI (Fig 1). With increasing DAI, there was a significant increase in mortality percentage, indicating the effect pathogen growth on the plants.

Previously, Koshariya *et al*. (2020) screened 132 lentil lines, among which 3 were found to be highly resistant, 10 were found to be resistant while 14 were found to be tolerant. In the present study, 3 varieties exhibited tolerant reactions, 4 varieties were moderately susceptible and 4 varieties were highly susceptible (Table 3).

Effect of different bio control agents (BCAs) against *S. rolfsii* (*in vitro* assay):

Four BCAs (*viz.*, *Bacillus* spp., *Pseudomonas* spp., *T. harzianum* and *T. viride*) were screened for antagonism against four virulent isolates of *Sclerotium rolfsii in vitro* (Fig 3 and 4).

Among the isolates, maximum inhibition was recorded in SRC-1 (32.55%), whereas SRC-6 (26.95%) reported minimum inhibition irrespective of BCAs used (Table 4). The differential inhibition among the isolates is in keeping with the studies of Sahni *et al*. (2019), who observed differential sensitivity in different isolates of *S. rolfsii* towards the same biocontrol agent.

Among the BCAs, maximum inhibition was done by *B. subtilis* (47.88%) for SRC-1, while minimum inhibition was achieved by *Pseudomonas* spp. (24.51%) (Table 4). This might be due to the ability of *B. subtilis* to produce a range of lipopeptides and antibiotics such as fengycins (Mora *et al*. 2015), Bacillomycin (Luo *et al*. 2015), Iturin and Surfactin (Dimkić *et al*. 2015) and plipastatins A and B which directly inhibit the growth of pathogens (Shafi *et al*. 2017).

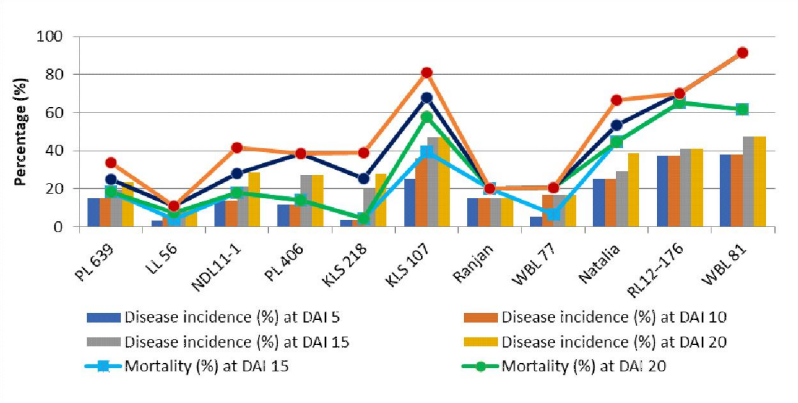

Fig 2: Progress in disease incidence and mortality percentage with Days after Inoculation (DAI).

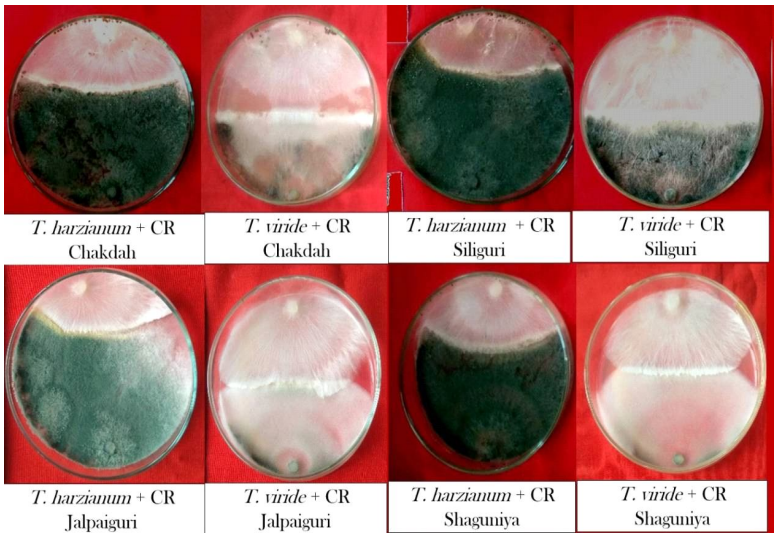

Fig 3: Interaction between *T. harzianum* and *T.viride* with *S. rolfsii* isolates.

Table 3: Screening of different lentil germplasm against *Sclerotium rolfsii.*

Grading	Mortality (%)	No. of varieties	Name of varieties
Highly resistant	NIL	NIL	NIL
Resistant	NIL	NIL	NIL
Tolerant	11-20%	3	LL-56, Ranjan, WBL-77
Moderately susceptible	21-50%	4	PL-639, NDL-11-1, PL-406, KLS- 218
Highly susceptible	70-75%	4	KLS-107, Natalia, RL-12-176, WBL-81
Total		11	

The interaction among isolates, BCAs and days after inoculation was statistically significant. It indicated that all the BCAs were able to inhibit the pathogen isolates and it increased with increase in the age of inoculation and their differences were statistically significant (Fig 5).

Effect of PGPR as a plant growth promoter and as biocontrol agent against *Sclerotium rolfsii*

Different PGPR combinations were assessed for improving the growth parameters. The disease incidence and mortality percentage were recorded to assess their effectiveness in managing collar rot of lentil (Table 5).

Maximum seedling fresh weight was recorded in the *Rhizobium* + *Bacillus* treatment (3.55 g) followed by *Rhizobium*+ *Pseudomonas* (3.13 g) whereas it was minimum in *Rhizobium* + Phosphate solubilizing bacteria (PSB) treatment (2.24 g) (Fig 6). The enhanced fresh weight in *Rhizobium* + *Bacillus* treatment might be due to the increase in nodulation and better nutrient supply (Kumar and Chandra, 2008).

Number of nodules produced in different plants was statistically significant. Maximum nodules were produced in *Rhizobium* + *Bacillus* treated seeds (40.0) followed by *Rhizobium* + PSB (Table 5), while lowest number of nodules were produced in *Rhizobium* + PSB + *Bacillus* (24.00).

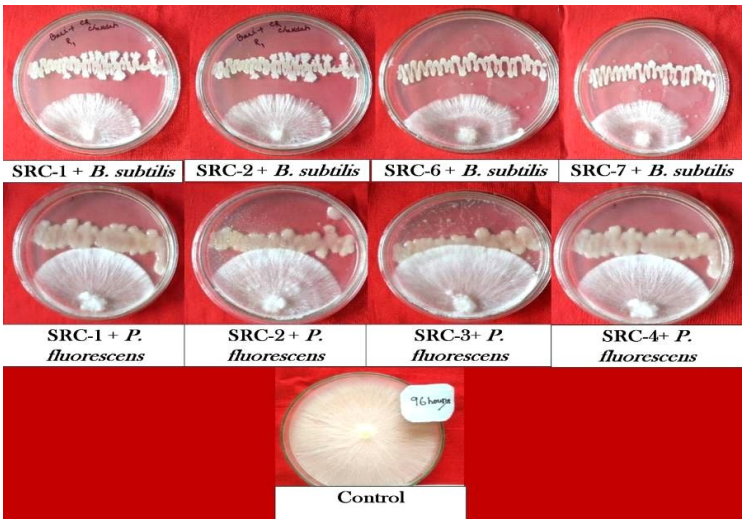

Fig 4: Interaction between *B.subtilis* and *P. fluorescens* with *S. rolfsii* isolates.

Table 4: Inhibition percentage of *Sclerotium rolfsii* by Bio-Control Agents (BCAs) at different days after inoculation.

Isolates	Treatments	Inhibition %			Mean
		2 DAI	3 DAI	4 DAI	
SRC- 1	*Bacillus spp.*	33.507	46.37	63.76	47.88
	Pseudomonas spp.	13.45	36.39	39.84	29.89
	T. harzianum	53.12	39.80	42.45	45.12
	T. viride	32.65	39.77	47.12	39.85
	Control	0.00	0.00	0.00	0.00
	Mean	26.54	32.47	38.63	32.55
SRC- 2	*Bacillus spp.*	38.54	44.18	57.77	46.83
	Pseudomonas spp.	15.59	37.51	31.33	28.15
	T. harzianum	21.83	22.42	45.00	29.75
	T. viride	24.93	35.79	51.42	37.38
	Control	0.00	0.00	0.00	0.00
	Mean	20.18	27.98	37.11	28.42
SRC- 6	*Bacillus spp.*	40.15	40.87	60.59	47.21
	Pseudomonas spp.	15.68	34.65	23.19	24.51
	T. harzianum	9.52	30.38	41.37	27.09
	T. viride	20.52	36.35	46.48	34.45
	Control	0.00	0.00	0.00	0.00
	Mean	17.68	28.37	34.79	26.95
SRC- 7	*Bacillus spp.*	39.19	41.43	57.74	46.12
	Pseudomonas spp.	19.39	35.83	30.66	28.63
	T. harzianum	34.63	29.08	38.11	33.94
	T. viride	24.93	8.92	44.58	26.14
	Control	0.00	0.00	0.00	0.00
	Mean	23.63	23.05	34.22	26.97
			SEm (±)		**CD at 5%**
Isolates			0.98		2.74
Day after inoculation (DAI)			0.85		2.37
Isolates × DAI			1.69		4.74
BCAs			0.98		2.74
Isolates × BCAs			1.95		5.48
BCA × DAI			1.69		4.74
Isolates × BCAs × DAI			3.38		9.49

Table 5: Effect of PGPR combination on growth characteristic, disease incidence percentage and mortality percentage of lentil against *S. rolfsii.*

Treatment	Seedling fresh wt. (g)	Seedling dry wt. (g)	No. of nodule (nos.)	Nodule fresh wt. (g)	Nodule dry wt. (g)	Disease incidence (%)	Mortality (%)
R+PSB+P	2.27	1.23	31.00	0.05	0.00	32.99	32.81
R+PSB+B	2.55	1.42	24.00	0.03	0.00	26.55	29.42
R+PSB+T	2.79	1.55	25.00	0.04	0.00	25.85	27.25
R+PSB	2.24	1.05	28.33	0.04	0.00	35.20	34.23
R+T	3.06	1.25	25.00	0.03	0.00	30.98	30.90
R+B	3.55	0.87	40.00	0.05	0.00	37.21	34.73
R+P	3.13	0.95	29.33	0.04	0.00	39.13	39.03
Control	2.84	0.65	19.00	0.03	0.00	46.90	53.59
S.E(m)±	0.40	0.20	0.91	0.00	0.00	1.65	4.13
C.D at 5%	N/A	N/A	2.74	0.01	N/A	5.05	12.64

Where, R + PSB + P = *Rhizobium* + Phosphate solubilizing bacteria + *Pseudomonas* isolate.
R + PSB + B = *Rhizobium* + Phosphate solubilizing bacteria + *Bacillus* isolate.
R + PSB + T = *Rhizobium* + Phosphate solubilizing bacteria + *Trichoderma* isolate.
R + PSB = *Rhizobium* + Phosphate solubilizing bacteria.
R + T = *Rhizobium* + *Trichoderma*.
R + B = *Rhizobium* + *Bacillus* isolate.
R + P = *Rhizobium* + *Pseudomonas* isolate.

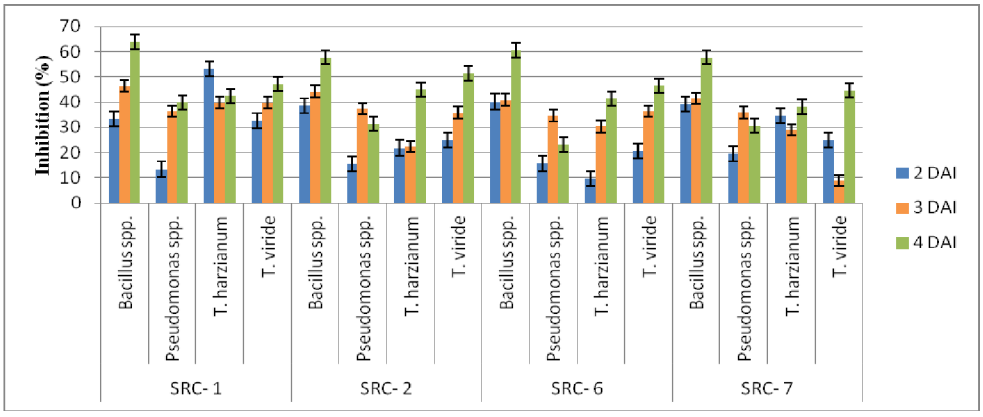

Fig 5: Effect of different Bio agents on inhibition percentage of *S. rolfsii* in different days after inoculation.

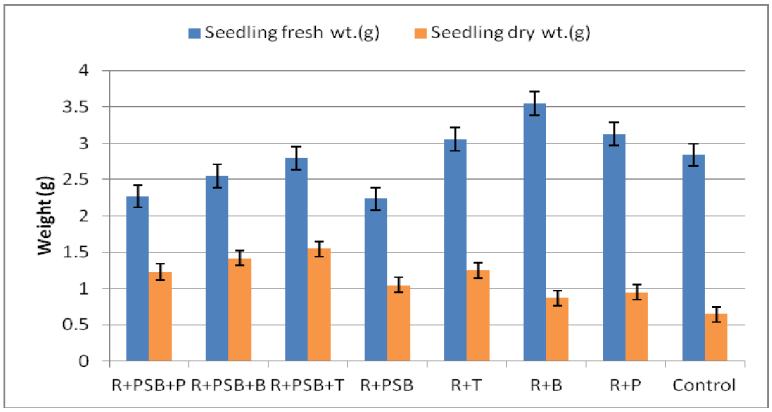

Fig 6: Effect of PGPR combinations on seedling dry and fresh weight (g).

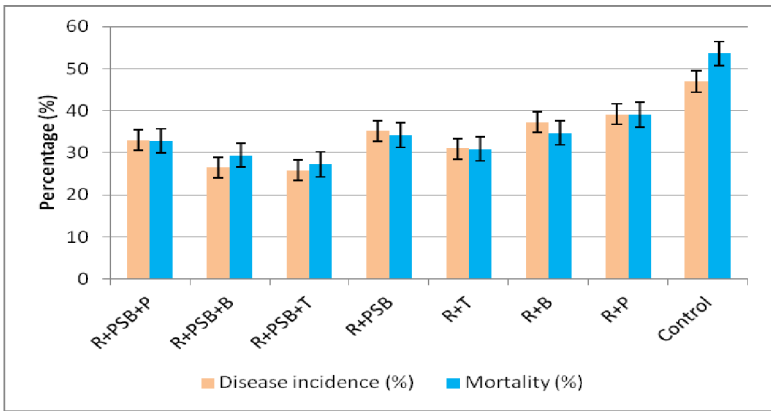

Fig 7: Effect of PGPR combinations on mortality (%) and disease incidence (%) of Lentil against *S. rolfsii.*

Maximum nodule fresh weight was recorded in *Rhizobium* + *Bacillus* (0.052 g) while minimum fresh weight (g) was recorded in *Rhizobium* + PSB + *Bacillus* (0.0288 g) and their difference was statistically significant. Dry weight of nodules in different treatments differed significantly. The increased nodulation is attributed to the influence of PSB on the native *Rhizobium* strain as well as enhanced survival of inoculated *Rhizobium* strain in the presence of PGPRs as reported by Prasad and Chandra (2003) in urd bean, Kumar and Chandra (2008) in lentil and Solanki *et al*. (2012) in tomato.

Jetiyanon and collaborators (2003) observed enhanced resistance in plants upon treatment with a mixture of PGPR. In the present study, the disease incidence was significantly low in all treatments as compared to untreated control and the differences were statistically significant. Minimum disease incidence was recorded in *Rhizobium* + PSB + *Trichoderma* (25.84%), while *Rhizobium* + *Pseudomonas* (39.13%) recorded maximum disease incidence (Table 5).

Minimum mortality was recorded in *Rhizobium* + PSB + *Trichoderma* (27.25%), whereas maximum mortality was recorded in *Rhizobium* + *Pseudomonas* (39.02%) and their differences were statistically significant (Fig 7). PGPRs have been previously reported to produce a plethora of chitinolytic enzymes that inhibits the growth of plant pathogenic fungi and incite resistance in host plants (Sahni *et al*. 2019; Singh *et al*. 2013). The results indicate that *Rhizobium* in combination with phosphate solubilizing bacteria and *Trichoderma* or *Bacillus* is most effective in controlling the collar rot of lentil when used as a seed treatment.

CONCLUSION

The results of present study can be used as for the development of a module for collar rot diseases control in lentil. First, varietal resistance screening, clearly indicates that the varieties L-56, Ranjan and WBL-77 can be used as tolerant sources against collar rot of lentil. Second, on the basis of evaluation of the BCAs, it can be concluded that *Bacillus* sp. had highest fungistatic ability followed by *Trichoderma harzianum.* Third, the PGPR combination of *Rhizobium* + PSB +*Trichoderma* or *Bacillus* was the most effective among the treatments in controlling the diseases as well as promoting other growth characters which are directly related to yield and yield attributes of lentil. This study can be used as a base for conducting quantitative and qualitative assay of PGPRs those directly or indirectly influencing the induced resistance and growth enhancement of lentil.

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