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Rhizoctonia bataticola (Taub.) Butler {Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid} is a soil inhabiting fungus which is a serious threat to more than 500 plant species. Although considerable research related to ecology of Rhizoctonia has been done, it still appears to be a potential pathogen causing severe losses in various crops. Bio-control association also stimulated plant defensive mechanisms induction of resistance metabolism similar to the hypersensitive response, systemic acquired resistance and induced systemic resistance in plants. There is a growing demand for biologically based soil borne pathogen management practices. Experiment was conducted *in vitro* and *in vivo* condition. The effect of bio agents on growth parameters and phenotypic parameters inoculated with *Rhizocotina* in pot culture.

Key words: Bio-inoculants, phenotypic parameters and growth parameters

INTRODUCTION

Chickpea, c L., is a legume crop of great importance worldwide, especially within African and Asian countries due to the nutritional value of its seeds. This legume has been increasingly used as an alternative to animal protein, mostly within developing countries, and has become an important dietary component for people who are vegetarian by choice or due to economic reasons (Wood and Grusak 2007; Jukanti *et al*. 2012). Dry root rot (DRR) of chickpea caused by *R. bataticola* is a serious threat to the global chickpea production. The estimated crop losses due to DRR have been estimated around 10-25% . *Rhizoctonia bataticola* (Taub.) Butler is a very important soil-inhabiting, fungus posing a serious threat to a wide range of crops. It is known to incite different types of diseases viz., stem blight, seedling blight, leaf blight, wilt, seedling decay, root rot, stalk rot, fruit rot, and charcoal rot in several crop plants. It has been reported that root colonization by*Tricho-derma* spp. frequently enhanced root growth and development, crop productivity, resistance to a biotic stress and uptake and use of nutrients. Root fungus association also stimulated plant defensive mechanisms induction of resistance, metabolism, similar to the hypersensitive response (HR), systemic acquired resistance (SAR) and induced systemic resistance (ISR) in plants. Abdel Monaim (2011) has tested seven bio control agents, namely *Bacillus subtilis*, *B. megaterium*, *B. cereus*, *Trichoderma viride*, *T. harzianum*, *Aspergillus* sp., *Penicillium* sp. isolated from chickpea rhizosphere, for their antagonistic action against the tested pathogens. *Trichoderma* may directly kill or suppressed the growth of pathogen by mycoparasitism and antibiosis.It may adversely affect the growth and development of the pathogen either by antibiosis or by competing for nutrient, space for oxygen. Indirectly, it may contribute by promoting plant growth resistance to biotic and abiotic stresses and changes in the nutritional status of the plant. Many soils borne fungal pathogen like, *Rhizoctonia, Sclerotinia, Sclerotium, Macrophomina* etc. form hard resting structure called sclerotia. These sclerotia play vital role in long term survival of pathogen in soil. It is difficult to kill these sclerotia using fungicides.

MATERIALS AND METHODS

The following material and methods were used Experiment and related studies conducted in the (AICRP Lab on Chickpea, Department of Plant Breeding and Genetics) JNKVV, Jabalpur.

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Seed source: Chickpea JG62.

Bio-inoculants source

The bio-inoculants *Trichoderma viride, Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from Microbes Research and Production Center, JNKVV, Jabalpur (M.P.).

Consortia with fungal and bacterial antagonists

A total of eight treatments combinations were designed to study the plant health of chickpea in net house condition.**T1-** Treated control,**T2**- *Trichoderma viride,***T3**- *Bacillus subtilis,***T4**- *Pseudomonas fluorescens,***T5**- *Trichoderma viride* +*Pseudomonas fluorescens,***T6**- *Trichoderma viride* + *Bacillus subtilis,***T7**- *Trichoderma viride* + *Bacillus subtilis* + *Pseudomonas fluorescens* and **T8**- Untreated control.

Pathogenicity test and mass multiplication of Rhizoctonia bataticola

Pathogenicity test was conducted by soil infestation method. The bags containing sorghum seeds were autoclaved at 121^{0}C for 20 min. The pathogen was mass multiplied on sterilized sorghum grains in 250 ml conical flasks. Then the flasks were inoculated with 4 discs of 5.0 mm diameter mycelial growth of three days old culture of *Rhizoctonia bataticola* grown on PDA plate. The flasks were incubated at 28 ± 2°C for seven days. Then the inoculum was mixed with sterilized soil @ 10 g kg^{-1} soil and filled in the pots (30 cm diameter). The seeds of chickpea were sown simultaneously with pathogen inoculation @ 7 seeds per pot and an un-inoculated control was maintained. The plants were observed for root rot symptoms. Each treatment replicated three times.

Dual culture technique

To test the efficacy of antagonistic fungus, twenty ml of sterilized melted PDA was plated in Petri plates (9 cm) and allowed to solidify. Mycelial discs measuring five mm diameter from three day old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile Petri plate containing PDA medium. Growth of antagonists, pathogen and zone of inhibition were measured after recording full growth of the pathogen in control plate. Per cent inhibition of mycelial growth over control was calculated.

Effect of bio-inoculants on growth parameters

Data on germination percentage was recorded after 10 days and at the time of maturity plant height (cms), dry weight and fresh weight (g/plant) was calculated. The vigour index mass and vigour index percentage were determined (Abdul Baki and Anderson 1973)

Vigour index (%) = Germination × Seedling length on the day of final count.

Vigour index mass = Germination percentage × seedling dry weight.

Pre and post-emergence

Germination percentage and pre and post emergence mortality will be recorded up to 90 days. Per cent mortality will be calculated by using the following formula;

$$\text{Per cent mortality} = \frac{\text{Number of diseased plants Total}}{\text{number of seedlings}} \times 100$$

Plant height, fresh and dry weight

Observations were recorded at the time of maturity. Mature plants were carefully uprooted for measuring the height, fresh weight and dry weight. After measuring length and fresh weight, the seedling were placed between blotting paper and kept at 45ºC for 2 - 3 days in an oven for drying. The dry weight was recorded in an electronic balance.

Determination of disease severity index : The disease severity index (D.I.) was calculated following Kumar *et al.* (2007).

$$\text{D.I.} = \frac{0\,(\text{Hn}) + 1\,(\text{Sn}) + 2\,(\text{Hn*}) + 3\,(\text{Dn})}{\text{Total number of plants examined}} \times 100$$

Where -

(Hn) = Number of healthy plants
(Sn) = Number of slightly infected plants
(Hn*)= Number of heavily infected plants
(Dn) = Number of dead plants

RESULTS AND DISCUSSION

Data presented in Table 1 show that in a biological control of *Rhizoctonia bataticola, in vitro* trials

indicated that all the bioagents evaluated signifiantly inhibited the growth of the pathogen and per cent inhibition ranged from 45.61 to 62.31 per cent. The fungal bioagent showed maximum inhibition of 62.31 per cent growth which was recorded in *Trichoderma viride.*Data also revealed that in case of bacterial bioagents, *Pseudomonas fluorescens* inhibited fungal growth to the extent of 49.83 per cent, which was succeeded by *Bacillus subtilis* with 45.61 per cent. Among the bioagents evaluated, fungal bioagents were found more effective in inhibiting the growth of pathogen compared to bacterial bioagents. It was reported that strain of *Trichoderma* inhibited the growth of *Rhizoctonia bataticola* by 51.1 per cent under *in vitro* conditions.

Table 1: Evaluation of bioagents against *Rhizoctonia bataticola in- vitro* condition

Treatments	Bio agents	Mean colony diameter (mm)	Per cent growth inhibition
T1	*Trichoderma viride*	33.93*	62.31
T2	*Pseudomonas fluorescens*	45.15	49.83
T3	*Bacillus subtilis*	48.95	45.61
T4	Control	90.00	
	SEm±	0.634	
	C. D.	1.974	

*Avg. of four replication

Germination percentage

Data presented in Table 2 indicated that germination percentage among treatment ranged from 76.19 to 95.24%. All the treatment had higher germination percentage as compared to T_1 (Treated control). Among the treatments minimum germination 76.19% was observed in T_3 (*Bacillus subtilis)* followed by 80.95% in T_4 *(Pseudomonas),* 85.71% in T_2 *(Trichoderma viride)* and T_6 *(Trichoderma viride + Bacillus subtilis)* highest germination 95.24% was recorded in T_5 *(Trichoderma viride + Pseudomonas fluorescens)* and T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens).*

Pre-emergence mortality

All the treatments (Table2) had significantly reduced the pre-emergence as compared to T_1 (Treated control). Maximum pre-emergence mortality 23.81% was recorded in T_3 (*Bacillus subtilis*) followed by 19.05% in T_4 (*Pseudomonas fluorescens),* 14.29% in T_2 (*Trichoderma viride)* and T_6 (*Trichoderma viride + Bacillus subtilis*). Minimum pre-emergence mortality 4.76% was recorded in T_5 (*Trichoderma viride + Pseudomonas fluorescens)* and T_7 *(Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens)*.

Table 2: Effect of Bio-inoculants and their combinations on dry root rot (*Rhizoctonia bataticola)* growth parameters

Treatments	Combination	Germination %	Pre-emergence mortality	Post-emergence mortality	Total mortality
T_1	Treated control	57.14*	42.86	60.56	76.19
T_2	*T. viride*	85.71	14.29	22.54	33.33
T_3	*B. subtilis*	76.19	23.81	24.44	42.86
T_4	*P. fluorescens*	80.95	19.05	23.41	38.10
T_5	*T. viride + P. fluorescens*	95.24	4.76	9.52	14.29
T_6	*T. viride + B. subtilis*	85.71	14.29	16.67	28.57
T_7	*T. viride + B. subtilis + P. fluorescens*	95.24	4.76	3.56	9.52
T_8	Control	76.19	23.81	37.78	52.36
	SEm±	2.475	1.785	1.881	2.020
	C. D.	7.848	5.397	5.689	6.107

*Avg. of three replication

Post-emergence mortality

Disease intensity, (Table 2) at flowering stage, among treatment varied from 3.56 to 24.44% as compared to T_1 (Treated control) post-emergence was 60.56%. Maximum PDI of 24.44 and 23.41% was observed in T_3 (*Bacillus subtilis*) and T_4 (*Pseudomonas fluorescens*) respectively. Minimum PDI of 3.56% was recorded in T_7 (*Trichoderma viride* + *Bacillus subtilis* + *Pseudomonas fluor*esc*ens*).

Total mortality (%)

Total mortality per cent among treatments **(**Table 2, ranged from 9.52 to 42.86% as compared to T_1 (Treated control) total mortality was 76.19%. Maximum mortality of 42.86% was observed in T_3 (*Bacillus subtilis*) followed by 38.10% in T_4 (*Pseudomonas fluor*esc*ens*) and minimum mortality 9.52% was recorded in T_7 (*Trichoderma viride* + *Bacillus subtilis* + *Pseudomonas fluor*esc*ens*).

Plant height (shoot and root)

Data presented in Table 3 at the time of maturity showed that shoot and root height was significantly increased in all the treatments except T_1 (Treated control) which was 37.20 cm and 7.40 cm respectively.Maximum shoot height 54.00 cm, 51.23 cm, 48.80 cm, 47.30 cm, 46.07 cm and 44.53 cm were recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens),* T_5 (*Trichoderma viride + Pseudomonas fluorescens),* T_6 (*Trichoderma viride + Bacillus subtilis),* T_2 (*Trichoderma viride),*T_4 (*Pseudomonas fluorescens)* and T_3 (*Bacillus subtilis)* respectively.Maximum root height 13.03 cm, 11.53 cm, 11.03 cm, 10.37 cm, 9.73 cm and 9.13 cm were recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens),* T_5 (*Trichoderma viride + Pseudomonas fluorescens),* T_6 (*Trichoderma viride + Bacillus subtilis),* T_2 (*Trichoderma viride),*T_4 (*Pseudomonas fluorescens)* and T_3 (*Bacillus subtilis)* respectively.

Table 3: Effect of bio-inoculants and their combinations on dry root rot disease and phenotypic parameters

Treatments	Treatment Combination	Av. Plant height (cm)		Fresh weight (g/plant)	Dry weight (g/plant)	Vigour Index (%)	Vigour Index Mass
		Shoot	Root				
T_1	Treated control	37.20*	7.40	2.45	0.53	2548.44	30.28
T_2	*T. viride*	47.30	10.37	2.80	0.83	4942.90	71.14
T_3	*B. subtilis*	44.53	9.13	2.70	0.73	4088.36	55.62
T_4	*P. fluorescens*	46.07	9.73	2.72	0.77	4517.01	62.33
T_5	*T. viride* + *P. fluorescens*	51.23	11.53	2.90	0.97	5977.26	92.38
T_6	*T. viride* + *B. subtilis*	48.80	11.03	2.88	0.88	5128.03	75.42
T_7	*T. viride* + *B. subtilis* + *P. fluorescens*	54.00	13.03	3.07	1.15	6383.94	109.53
T_8	Untreated control	40.83	8.83	2.40	0.68	3783.60	51.81
	SEm±	0.648	0.269	0.038	0.034	-	-
	CD	1.960	0.813	0.115	0.104	-	-

*Avg. of three replication

Fresh weight

Data presented in Table 3 at the time of maturity showed that fresh weight was significantly higher in all the treatments as compared to T_1 (Treated control) which was 2.45 g.Higher fresh weight 3.07 g, 2.90 g, 2.88 g, 2.80 g, 2.72 g and 2.70 g were recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens),* T_5 (*Trichoderma viride + Pseudomonas fluorescens),* T_6 (*Trichoderma viride + Bacillus subtilis),* T_2

(*Trichoderma viride),* T_4 (*Pseudomonas fluorescens)* and T_3 (*Bacillus subtilis)* respectively.

Dry weight

Data presented in Table 3 at the time of maturity showed that dry weight was significantly higher in all the treatments as compared to T_1 (Treated control) which was 0.53 g.Higher dry weight 1.15 g, 0.97 g, 0.88 g, 0.83 g, 0.77 g and 0.73 g were recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens),* T_5 (*Trichoderma viride + Pseudomonas fluorescens),* T_6 (*Trichoderma viride + Bacillus subtilis),* T_2 (*Trichoderma viride),* T_4 (*Pseudomonas fluorescens)* and T_3 (*Bacillus subtilis)* respectively.

Vigour index (%)

Data presented in the Table 3. indicated that Vigour index (%) recorded at the time of maturity sowing indicated that all the treatments had higher vigour index % as compared to T_1 (Treated control). At the time of maturity, it varied from 4088.36 to 6383.94 as compared to 2548.44 in T_1 (Treated control). Maximum Vigour index % of 6383.94 and 5977.26 was recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens*) and T_5 (*Trichoderma viride + Pseudomonas fluorescens)* respectively. Minimum 4088.36 and 4517.01 was observed in T_3 (*Bacillus subtilis)* and T_4 (*Pseudomonas fluorescens)* respectively.

Table 4: Disease severity index of Dry root rot

Treatments	Treatment Combination	Dry root rot
T_1	Treated control	75.30
T_2	*T. viride*	15.16
T_3	*B. subtilis*	20.74
T_4	*P. fluorescens*	19.70
T_5	*T. viride + P. fluorescens*	8.37
T_6	*T. viride + B. subtilis*	9.25
T_7	*T. viride + B. subtilis + P. fluorescens*	5.82
T_8	Untreated control	4.19
	SEm±	2.132
	CD	6.445

*Avg. of three replication

Vigour Index Mass

Vigour index mass Table 3 of all the treatments had increased as compared to T_1 (Untreated control). Maximum of 109.53 and 92.38 was recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens*) and T_5 (*Trichoderma viride + Pseudomonas fluorescens)* respectively**.** Minimum 55.62 and 62.33 was observed in T_3 (*Bacillus subtilis*) and T_4 (*Pseudomonas fluorescens*) respectively. Rajasekhar et al. (2016) showed that efficiency was maximum in treatment *Trichoderma viride + Bacillus subtilis + Pseudomonas fluoroscens* as compared to control. The disease severity caused by *Fusarium oxysporum* f. sp *ciceri, Sclerotium rolfsii* and by *Rhizoctonia bataticola* was maximum in treated control and minimum in untreated control and in treatment *Trichoderma viride + Bacillus subtilis + Pseudomonas fluoroscens* as compared to treated control.

Rhizoctonia bataticola

Under net house conditions *Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens* were found to be effective in reducing the disease severity rate on chickpea plants when applied in mix with *Rhizoctonia bataticola* compared to soil infested only with the pathogens (Table 4). Disease severity of collar rot, 5.82% was least in T_7 *(Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens)* as compared to 75.30% in T_1 (Treated control) followed by 8.37% T_5 *(Trichoderma viride + Pseudomonas fluorescens),* 9.25% in T_6 (*Trichoderma viride + Bacillus subtilis*) and maximum disease severity 20.74% was observed in T_3 *(Bacillus subtilis)* followed by 19.70% in T_4 *(Pseudomonas fluorescens)* and 15.16% in T_2 *(Trichoderma viride).*

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