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Effects of bio-inoculants for the management of collar rot of chickpea

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Abstract
Chickpea (*Cicer arietinum* L.) is an important legume crop in the semi arid tropical countries and its production is second to cereals. Chickpeas provides protein, vitamins and minerals, hence included in many healing diets. Collar rot caused by *Sclerotium rolfsii Sacc*. is one of the fungal disease affecting this crop and is reported almost all over the world wherever chickpea is grown. The present investigation used fungal and bacterial bioagents and with combination applied to chick pea. The observations to be recorded on growth parameters Germination percentage, Pre- emergence mortality, Post- emergence mortality, Total mortality and phenotypic parameters like Plant height, Fresh weight, Dry weight, plant Vigour Index, Vigour Index Mass in pot cultivation and antagonist effect under labaratatory.

Keywords: *Sclerotium rolfsii*, bio-agents, growth parameters and phenotypic parameters

Introduction
Chick pea is a major pulse crop widely grown in India, accounts for nearly 75 percent of the total pulse production in the world. It is a rich source of quality protein (20–22 %) to the predominantly vegetarian population in Indian subcontinent, other South Asian countries and the Middle East. It has the highest nutritional compositions and free from anti-nutritive components com-pared to any other dry edible grain legumes and thus it is considered a functional food or nutraceu-tical. Besides proteins, it is rich in fibre and minerals (phosphorus, calcium, magnesium, iron and zinc), and its lipid fraction is high in unsaturated fatty acids (Williams and Singh 1987) [18]. Chickpea crop is prone to many diseases such as Fusarium wilt, dry root rot, collar rot, Ascochyta blight, Verticillium wilt, black root rot, Phytophthora root rot, wet root rot, foot rot, Pythium rot and seed rot. Among these, collar rot caused by *Sclerotium rolfsii* is of high importance. *S. rolfsii* is a pathogen of high economic impact since it affects numerous crops worldwide. It has an extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers and cucurbits and commonly occur in the tropics, subtropics and other warm temperate regions (Punja ZK 1985) [11]. *S. rolfsii* is a devastating soil-borne plant pathogenic fungus with a wide host range (Punja 1988) [12] has prolific growth and ability to produce persistent sclerotia contributing in high degree of economic losses (Mahen *et al*., 1995) [9]. This fungus can overwinter as mycelium in infected tissues or plant debris. Sclerotia serve as the principal over wintering structure and primary inoculum for disease persisting near the soil surface, sclerotia may exist free in soil or in association with plant debris (Punja, 1985) [11]. The fungus forms differentiated sclerotia and sterile mycelia like other sclerotium-producing fungi. Those characterized by small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus Sclerotium (Punja and Rahe 1992) [10]. However, the teleomorphic state was discovered later (Punja 1988) [12], confirming that the fungus was a basidiomycete. *Sclerotium rolfsii* usually causes collar rot (Singh and Pavgi 1965) [16]. Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* sp. is well documented as an effective biological control agents of plant diseases (Duffy BK *et al* 1996) [6]. *P. fluorescens* mixed with other strains of fungi or bacteria increased the efficacy of biocontrol (Rahber-Bhatti MH 1986) [13]. Hence, present investigation was carried out to screen out the most compatible combinations of biocontrol agents to find out efficient management practices against collar rot of chickpea caused by *S. rolfsii*.

Materials and Methods

The following material and methods were used to "Effects of Bio-inoculants for the Management of Collar rot of Chickpea, Experiment and related studies conducted in the (AICRP Lab on Chickpea, Department of plant breeding and genetics) JNKVV, Jabalpur.

Collection of disease sample

Chickpea plants showing typical symptoms of collar rot at seedling, flowering and pod formation stage were collected from breeder seed production unit, sick plots, Department of Plant Breeding and Genetics, JNKVV, Jabalpur. Samples were brought in the laboratory and symptoms were identified and confirmed.

Seed of crop were used for study: JG 62

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.

Pathogencity of *Sclerotium rolfsii*

Mass culture technique for *Sclerotium rolfsii* obtained from infected plant part were tested pathogenic behaviour. The inoculum of *Sclerotium rolfsii* mass multiplied on sterilized sorghum grains. 250 gm sorghum grains was filled in bags and sterilized in an autoclave. The sterilized mixture was inoculated with 7 days old culture of pathogen (*Sclerotium rolfsii*) and incubated at 25±1^{0}C for 25 days. Thus profuse and dense growth of fungal mycelium and Sclerotia was obtained. The inoculum was thoroughly mixed in sterilized soil (sand + soil) (1:1) @ 10 g/ kg soil (Gupta, 2001) [7]. The inoculated soil filled in pots @ 3 kg/ pot. After inoculation the soil was incubated at room temperature for 15 days. Pots were filled with this infested soil. For determining the pathogenicity, seeds were surface sterilized with mercuric chloride 1:1000 for 30 seconds, were sown in pots. These pots were kept in natural condition, chickpea seeds sown in uninoculated sterilized soil served as control.

Dual culture technique

Bacterial isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA. 9 mm mycelial disc from seven days old PDA culture of *Sclerotium rolfsii* were placed at the opposite side of Petri dishes perpendicular to the bacterial streak respectively and incubated at 27 ± 20C for 5-7 days. Petri dishes inoculated with fungal discs alone served as control. Three replications were maintained for each isolate. Observations on width of inhibition zone and mycelial growth of test pathogen were recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent (1947) [17]. Per cent inhibition (I) = C-T/C ×100 Where, C- mycelial growth of pathogen in control T- mycelial growth of pathogen in dual culture plate

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 vigor index mass and vigor index percentage as follows (Abdul Baki and Anderson 1973) [1];

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Vigour index (%) = Germination × Seedling length on the day of final count and 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. Percent mortality will be calculated by using the following formula;

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

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.

Disease severity index

Determination of disease severity index: The disease severity index was calculated as described by Bhattacharya *et al*., (1985) [3]. The extent of infection by *Sclerotium rolfsii* was indicated by the presence of white mycelial growth and also by the presence of micro sclerotia of the fungus on root systems. Healthy and infected plants were divided into four groups as follows:

- Healthy plants 0 = No root rot symptoms,
- Slightly infected plants 1 = White mycelial growth on collar as well as on primary roots.
- Heavily infected plant 2= Weak and stunted plants with rotting of roots,
- Plants dead 3 = Dead and fallen plants

Lesions on the entire root system and the disease severity index (D.I.) were calculated as follows:

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

Where
(Hn) = Number of healthy plants
(Sn) = Number of slightly infected plants
(Hn*) = Number of heavily infected plants
(Dn) = Number of dead plants (Kumar *et al*., 2007) [8]

Results and Discussions

Evaluation of bio-agents against *Sclerotium rolfsii in-vitro* condition

The bioagents *viz., Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated by dual culture technique for their antagonistic effect against

Sclerotium rolfsii under *in-vitro* conditions. Zone of inhibition (mm) was recorded and the per cent inhibition was calculated. The results, thus obtained are presented in Table 1.

Table 1: Evaluation of bioagents against *Sclerotium rolfsii* in vitro condition

Treatments	Bio agents	Mean colony diameter (mm)	Per cent growth inhibition
T1	*Trichoderma viride*	35.43*	60.64
T2	*Pseudomonas fluorescens*	41.70	53.67
T3	*Bacillus subtilis*	53.85	40.17
T4	Control	90.00	0
	SEm+	0.684	
	C. D.	2.132	

*Avg. of four replication

Data presented in Table 1 indicated that, all the bioagents evaluated significantly inhibited the growth of the pathogen and per cent inhibition ranged from 40.17 to 60.64 per cent. The fungal bioagent shows maximum inhibition of 60.64 per cent growth was recorded in *Trichoderma viride.* Sab *et al.,*(2014) [15] tested eight bioagents against *S. rolfsii*, *Trichoderma harzianum-55* IIHR recorded maximum inhibition of 70% followed by *T. harzianum* NBAII with 63% and least mycelial inhibition was observed in *Pseudomonas fluorescens* and *Bacillus subtilis*.

Table 2: Effect of Bio-inoculants and their combinations on collar rot (*Sclerotium rolfsii)* disease incidence

Treatments	Combination	Germination %	Pre- emergence mortality	Post- emergence mortality	Total mortality
T_1	Treated control	52.38*	47.62	83.33	90.48
T_2	*T. viride*	80.95	19.05	23.33	38.10
T_3	*B. subtilis*	76.19	23.81	31.11	47.62
T_4	*P. fluorescens*	76.19	23.81	25.56	42.86
T_5	*T. viride + P. fluorescens*	90.48	9.52	15.87	23.81
T_6	*T. viride + B. subtilis*	85.71	14.29	15.87	28.57
T_7	*T. viride + B. subtilis + P. fluorescens*	90.48	9.52	10.32	19.05
T_8	Untreated control	76.19	23.81	37.78	52.38
SEm+		2.159	2.552	2.255	2.469
C. D.		6.528	7.716	6.819	7.466

*Avg. of three replication

Germination percentage

Data presented in Table 2 indicated that germination percentage among treatment ranged from 76.19 to 90.48%. All the treatment had higher germination percentage as compared to T_1 (Treated control). Among the treatments minimum germination per cent 76.19% was observed in T_3 (*Bacillus subtilis)* and T_4 *(Pseudomonas fluorescens)* followed by 80.95% in T_2 *(Trichoderma viride),* 85.71% in T_6 *(Trichoderma viride + Bacillus subtilis)* and highest germination per cent 90.48% was recorded in T_5 *(Trichoderma viride + Pseudomonas fluorescens)* and T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens).*

Pre- emergence mortality

All the treatments 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*) and T_4 (*Pseudomonas fluorescens)* followed by 19.05% in T_2 (*Trichoderma viride),* 14.29% in T6 (*Trichoderma viride + Bacillus subtilis)* and minimum pre-emergence mortality 9.52% was recorded in T_5 (*Trichoderma viride + Pseudomonas fluorescens)* and T_7 *(Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens).*

Post-emergence mortality

Disease intensity, at flowering stage, among treatment varied from 10.32 to 31.11% as compared T_1 (Treated control) post-emergence was 83.33%. Maximum PDI of 31.11% was observed in T_3 (*Bacillus subtilis*) followed by 25.56% in T_4 (*Pseudomonas fluorescens*). Minimum PDI of 10.32% was recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens*).

Total mortality (%)

Total mortality per cent among treatments ranged from 19.05 to 47.62% as compared to T_1 (treated control) total mortality was 90.48%. Maximum mortality of 47.62% was observed in T_3 (*Bacillus subtilis*) followed by 42.86% in T_4 (*Pseudomonas fluor*escens) and minimum mortality 19.05% was recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluor*escens).

Begum *et al*. (2010) [2] were evaluated five Trichoderma strains to assay their efficacy in suppressing Alternaria fruit rot disease of chili and promoting chili plant growth and yield and observed that application of T. harzianum IMI 392432 significantly suppressed the disease and improved highest seed germination percentage, vigour index, growth and yield. Studies have been confirmed in case of *T. harzianum* and *T. viridi* to enhanced seed germination root and shoot length (Dubey *et al*. 2007) [5] as well as increasing the frequency of healthy plants, and boosting yield (Rojoa *et al*. 2007) [14].

Table 3: Effect of Bio-inoculants and their combinations on collar 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	36.03*	7.10	2.13	0.38	2259.15	19.90
T_2	*T. viride*	44.07	9.73	2.67	0.68	4355.11	55.05
T_3	*B. subtilis*	40.23	8.63	2.58	0.58	3722.64	44.19
T_4	*P. fluorescens*	41.03	8.97	2.62	0.60	3809.50	45.71
T_5	*T. viride + P. fluorescens*	48.03	11.00	2.83	0.88	5341.03	79.62
T_6	*T. viride + B. subtilis*	46.07	10.60	2.70	0.70	4857.19	60.00
T_7	*T. viride + B. subtilis + P. fluorescens*	50.47	11.93	2.97	1.00	5645.95	90.48
T_8	Untreated control	39.20	8.10	2.32	0.58	3603.79	44.19
SEm±		0.647	0.396	0.047	0.046	-	-
CD		1.955	1.197	0.143	0.140	-	-

*Avg. of three replication

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 36.03 cm and 7.10 cm respectively. Maximum shoot height 50.47 cm, 48.03 cm, 46.07 cm, 44.07 cm, 41.03 cm and 40.23 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 11.93 cm, 11.00 cm, 10.60 cm, 9.73 cm, 8.97 cm and 8.63 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.

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 2.13 g. Higher fresh weight 2.97 g, 2.83 g, 2.70 g, 2.67 g, 2.62 g and 2.58 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 3722.64 to 5645.95 as compared to 2259.15 in T_1 (Treated control). Maximum Vigour index % of 5645.95 and 5341.03 was recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens*) and T_5 (*Trichoderma viride + Pseudomonas fluorescens*) respectively. Minimum 3722.64 and 3809.50 was observed in T_3 (*Bacillus subtilis*) and T_4 (*Pseudomonas fluorescens*) respectively.

Vigour Index Mass

Vigour index mass of all the treatments had increased as compared to T_1 (Untreated control). Maximum of 90.48 and 79.62 was recorded in T_7 (*Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens*) and T_5 (*Trichoderma viride + Pseudomonas fluorescens*) respectively. Minimum 44.19 and 45.71 was observed in T_3 (*Bacillus subtilis*) and T_4 (*Pseudomonas fluorescens*) respectively.

Chien-Yih Lin (2002) [4] screened Trichoderma strains on plant growth and root growth of bitter gourd, loofah and cucumber and noted that Trichoderma strains significantly increased of 26 to 61% in seedling height, 85-209% in root exploration, 27-38% in leaf area and 38 to 62% in root dry weight after 15 days of sowing.

Table 4: Disease severity index of Collar rot

Treatments	Treatment Combination	Collar rot
T_1	Treated control	74.07
T_2	*T. viride*	14.44
T_3	*B. subtilis*	27.65
T_4	*P. fluorescens*	20.98
T_5	*T. viride + P. fluorescens*	12.34
T_6	*T. viride + B. subtilis*	15.87
T_7	*T. viride + B. subtilis + P. fluorescens*	8.81
T_8	Untreated control	8.39
SEm±		1.918
CD		5.799

*Avg. of three replication

Sclerotium rolfsii

Under pot house conditions *Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens* were shown to be effective reduction of the disease severity rate on chickpea plants when applied in mix with *Sclerotium rolfsii* compared to soil infested only with the pathogens. Disease severity of collar rot 8.81% was least in T_7 *(Trichoderma viride + Bacillus subtilis + Pseudomonas fluorescens)* as compared to 74.07% in T_1 (Treated control) followed by 12.34% T_5 *(Trichoderma viride + Pseudomonas fluorescens),* 15.87% in T_6 *(Trichoderma viride + Bacillus subtilis*) and maximum disease severity 27.65% was observed in T_3 *(Bacillus subtilis)* followed by 20.98% in T_4 *(Pseudomonas fluorescens)* and 14.44% in T_2 (*Trichoderma viride*).

References

1. Abdul Baki AA, Anderson JD. Vigour determination in soybean by multiple criteria. Crop Science. 1973; 13(6):630-633.
2. Begum MF, Rahman MA, Alam MF. Biological control of Alternaria fruit rot of chili by Trichoderma species under field conditions. Mycobiology. 2010; 38(2):113-117.
3. Bhattacharya D, Basu S, Chattapadhyay JP, Bose SK. Biocontrol of *Macrophomina* root rot disease of jute by an antagonistic organism, *Aspergillus versicolor*. Plant Soil. 1985; 87(3):435-438.

4. Chaur-Tsuen L, Chien-Yih L. Screening strains of Trichoderma spp. for plant growth enhancement in Taiwan. Plant Pathology Bulletin. 2002; 11:215-220.
5. Dubey SC, Suresha M, Singha B. Evaluation of Trichoderma species against *Fusarium oxysporum f. sp. ciceris* for integrated management of chickpea wilt. Biological Control. 2007; 40:118-127.
6. Duffy BK, Simon A, Weller DM. Combination of *Trichoderma oningii* with *Fluorescent pseudomonads* for control of take-all on wheat. Phytopathology. 1996; 86:188-94.
7. Gupta OM. JNKVV Research Journal 2001, Publ.-2002; 35(1-2):38-42.
8. Kumar V, Kumar A, Kharwar RN. Antagonistic potential of flourocent pseudomonads and control of charcoal rot of chick pea caused by *macrophomina phaseolina* journal of environmental Biology. 2007; 28(1):15-20.
9. Mahen VK, Mayer CD, Brennemen TB, McDonald D. Stem and pod rots of groundnut. Information Bulletin No. 4, ICRISAT1995, India, 28p.
10. Punja ZK, Rahe JE. Methods for research on soilborne phytopathogenic fungi. St. Paul: APS Press. Sclerotium, 1992p. 166-170. In: Singleton, LL, Mihail JD, Rush CM, eds.
11. Punja ZK. The biology, ecology and control of *Sclerotium rolfsii*. Ann Rev Phytopathol. 1985; 23:97-127
12. Punja ZK, Sclerotium (Athelia) rolfsii, a pathogen of many plant species. In: Sidhu GS, ed. Genetics of plant pathogenic fungi. London: Academic Press, 1988 6:23-534p.
13. Rahber-Bhatti MH. Control of *Phakopsora grewia* with plant diffusates. Pak J Bot. 1986; 18:329-33.
14. Rojoa FG, Reynosoa MM, Fereza M, Chulze SN, Torres AM. Biological control by Trichoderma species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop Prot. 2007; 26:549-555.
15. Sab J, Nagaraja A, Nagamma G. Efficacy of bio-pesticides against *Sclerotium rolfsii* sacc causing collar rot of chickpea (*Cicer arietinum* L. An International Quarterly Journal of Life Sciences. 2014; 9(1):335-339.
16. Singh UP, Pavgi MS. Spotted leaf rot of plants, a new sclerotial disease. Plant Dis. Reptr. 1965; 49:58-59.
17. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 159:850-850.
18. Williams PC, Singh U. Nutritional quality and the evaluation of quality in breeding programmes. In: Saxena MC, Singh KB (eds) The chickpea. CAB International, Wallingford, 1987, 329-390p.