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PLANT PATHOLOGY

Integration of *Trichoderma, Pseudomonas* and fungicides for the control of collar rot disease of chickpea (*Cicer arietinum* L.)

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Abstract

An experiment was conducted on integration of *Trichoderma, Pseudomonas* and fungicides for the control of Collar rot disease of Chickpea during 2013-14 and 2014-15. Results indicated that the most effective treatment was *Trichoderma harzianum* @ 8q/ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} seed with minimum mortality (4.30 and 2.25%) which was at par with treatment *Pseudomonas fluorescens* @ 8q/ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} seed (5.80 and 2.59%) and *Trichoderma harzianum* @ 8q/ha^{-1} (Soil)+Tubeconazole @ 3ml/kg^{-1} seed (6.15 and 4.09%) whereas maximum mortality 15.70 and 12.35%) was recorded in control plot. Maximum no. of pods per plant (41.30 and 49.75) was recorded in treatment T_7 = *Trichoderma harzianum* @ 8q/ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} seed which was at par with T_8 = *Pseudomonas fluorescens* @ 8q/ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} (38.7 and 45.95) and significantly superior over rest of the treatment. In case of grain yield highest grain yield was increased in treatment T_7 = *Trichoderma harzianum* @ 8q/ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} seed (44.85%) followed by T_8 = *Pseudomonas fluorescens* @ 8q/ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} (43.61%) and T_{10} = *Pseudomonas fluorescens* @ 8q/ha^{-1} (Soil) + Tubeconazole @ 3ml/kg^{-1} seed (29.63%).

Highlights

- *Trichoderma harzianum* @ 8q/ha^{-1} (Soil)+Hexaconazole @ 3ml/kg^{-1} seed and *Pseudomonas fluorescens* @ 8q/ha^{-1} (Soil)+Hexaconazole @ 3ml/kg^{-1} was found most effective treatment for the control of collar rot of chickpea under field condition. As compare to alone application *Trichoderma harzianum* or *Pseudomonas fluorescens* or Hexaconazole integrated application of *Trichoderma harzianum* or *Pseudomonas fluorescens* and Hexaconazole showing minimum mortality and maximum plan growth and yield of chickpea under field condition.

Keywords: Chickpea, Collar rot, Integration management, *Pseudomonas fluorescens*, *Trichoderma harzianum*

Chickpea (*Cicer arietinum* L.) is the most important pulse crop in India. India is the largest producer of chickpea with about 63% of the total area under chickpea production lying in India. Gram is the most dominant pulse having a share of around 40% in the total production followed by pigeonpea at 15 to 20% and urdbeen and mungbeen around 8-10%. Area and production and productivity of chickpea in India have been increased during the past decade. In India, chickpea occupies an area of 8 mha and its

production is 7.1 mt with an average productivity of 885 kg·ha^{-1} during 2014-15 (Anonymous 2016) but the production was decreased as compared to 2013-14 (9.5 million tons) due to many biotic and abiotic constraints. Nearly 172 pathogens have been reported so far that infect chickpea (*Cicer arietinum* L.) in different parts of the world but only a few of them have the potential to devastate the crop. Some diseases are persistent problems in chickpea production in wide geographical areas, notably, ascochyta blight, fusarium wilt, dry root rot, botrytis gray mold, collar rot, black root rot, phytophthora root rot, and pythium root and seed rot, while others are sporadic in occurrence or endemic in distribution (Nene *et al.* 2012). Among them soil borne diseases such as fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*), dry root rot (*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii*) and black root rot (*Fusarium solani*) are the major limiting factor in chickpea production (Ghosh *et al.* 2013). Among the above soil borne pathogen *Sclerotium rolfsii* is the major problematic fungus in Chhattisgarh.

Now days it is becoming a serious problem in central and peninsular India due to high soil moisture, the presence of under decomposed organic matter on the soil surface, low soil pH and high temperature (25 to 30°C) which are favor the disease development (Al-Askar *et al.* 2013). It has an extensive host range; at least 500 species in 100 families are susceptible (Nagamma and Nagaraja 2015). The most common hosts are the legumes, crucifers, and cucurbits. *S. rolfsii* commonly occurs in the tropics, subtropics, and other warm temperate regions including India. *Sclerotium rolfsii* Sacc., is a major cause for 55–95% mortality of chickpea seedlings (Gurha and Dubey 1982). This may be due to the prolific growth, extensive host range of the pathogen and having the ability to produce large number of sclerotia that may persist in the soil for several years (Sennoi *et al.* 2013).

Several management strategies such as physical, chemical, biological and growing of resistant varieties were used for the control of collar rot disease caused by *Sclerotium rolfsii* Sacc.. Still there is no resistant or tolerant varieties were identified against this pathogen in India. In case of chemical control large numbers of broad range or spectrum fungicides were tried by the several workers but there is no one fungicides was much effective under field condition. Besides of this fungicides are damaging the biotic and abiotic environments which are problematic for human beings. The control of collar rot of chickpea (*Sclerotium rolfsii*) is not possible by one method of plant disease control. It can be manage by the integration of all available control measures. Therefore, keeping in view the above facts an experiments was conducted on integration of *Trichoderma, Pseudomonas* and fungicides for the control of Collar rot disease of Chickpea.

Materials and methods

An experiment was conducted on integration of *Trichoderma, Pseudomonas* and fungicides for the control of Collar rot disease of Chickpea at S.K. College of Agriculture and Research Station Farm, Kawardha (Kabirdham), C.G. during 2013-14 and 2014-15. Experiment layout under Randomized Block Design (RBD) with ten treatment and three replications. The plot size of experiment was 4x 3 m. Chickpea variety JG-16 is widely grown in Chhattisgarh was selected for this experiment. *Culture of Trichoderma harzianum* and *Pseudomonas fluorescens* were collected from Department of Plant Pathology, TCB college of Agriculture and Research Station (IGKV), Bulaspur, C.G. *Trichoderma harzianum* culture was maintained on PDA media and *Pseudomonas fluorescens* culture was maintained in King's 'B' medium and stored at 5°C. The mass culture of *Trichoderma harzianum* and *Pseudomonas fluorescens* was prepared on Talk powder as per the standard procedure adopted by State Biological Control Laboratory (SBCL), TCB College of Agriculture and Research Station, Chorbhatti, Bilaspur (C.G.). Seeds were treated with Tubeconazole @ 3ml kg^{-1} seed, Hexaconazole @ 3ml kg^{-1} seed, *Trichoderma harzianum* @ 10g kg^{-1} seed and *Pseudomonas fluorescens* @ 10g kg^{-1} seed as per the treatments details given in Table 1.

In case of soil application *T. harzianum* and *P. fluorescens* antagonists were inoculated in the sterilized FYM at 20 days before sowing. This inoculated FYM kept under shade to allow grow of both bio-control agents. After the colonization enriched FYM applied in furrow before sowing of the crop. Then the furrows were covered with soil. Seeds of variety JG-16 were sown with row to row distance 30 cm and plant to plant 10 cm.

Observation on mortality was recorded at weekly interval in which total and rotted plants were recorded from each experimental plot. Plant height, number of pod and grain yield were also recorded from the all experimental plot. The per cent diseased incidence was calculated with following formula:

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

Table 1: Treatment Details

Treatment code	Treatment details
T_1	Control
T_2	Seed treatment with Tubeconazole @ 3ml kg^{-1} seed
T_3	Seed treatment with Hexaconazole @ 3ml kg^{-1} seed
T_4	Seed treatment with *Pseudomonas fluorescens* @ 10g kg^{-1} seed
T_5	Seed treatment with *Trichoderma harzianum* @ 8q ha^{-1} +10g kg^{-1} seed
T_6	Soil application of *P. fluorescens* @ 8q ha^{-1}+ Seed treatment with *P. fluorescens* 10g kg^{-1} seed
T_7	Soil application of *T. harzianum* @ 8q ha^{-1} + Seed treatment with Hexaconazole @ 3ml kg^{-1} seed
T_8	Soil application of *P. fluorescens* @ 8q ha^{-1} + Seed treatment with Hexaconazole @ 3ml kg^{-1} seed
T_9	Soil application of *T. harzianum* @ 8q ha^{-1} + Seed treatment with Tubeconazole @ 3ml kg^{-1} seed
T_{10}	Soil application of *P. fluorescens* @ 8q ha^{-1} + Seed treatment with Tubeconazole @ 3ml kg^{-1} seed

Results and discussion

Experimental data indicated that the minimum mortality recorded was 4.30 and 2.25% in plot treated with *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed followed by plot treated with *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed (5.80 and 2.59 %) during 2013-14 and 2014-15, respectively whereas, untreated plot showed maximum mortality of 15.70 and 12.35% during 2013-14 and 2014-15, respectively. Among the all treatments treatment mean highest reduction in plant mortality was recorded in plots treated with *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed (76.66%) followed by plot treated with *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed (70.10%) (Table 2).

Maximum mean plant height was observed in treatment *Trichoderma harzianum* @ 8q ha^{-1} + 10 g kg^{-1} seed (Soil + Seed) (49.03 cm) followed by *Pseudomonas fluorescens* @ 8q ha^{-1}+10g kg^{-1} seed (Soil + Seed) (48.50 cm) and *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed (47.73 cm). There is no significantly difference recorded among the all treatment (Table 3). Data pertaining to no. of pods per plant have been presented in Table 3 reveal that the maximum no. of pods per plant (41.30 and 49.75) was recorded in treatment T_7 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed which was at par with T_8 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml/ kg^{-1} seed (38.7 and 45.95) and significantly superior over rest of the treatment. While, minimum number of pods per plant (25.40 and 36.70) was observed in untreated control. In case of grain yield maximum grain yield (14.269 and 19.095 q ha^{-1}) was observed in T_7 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed followed by T_8 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} (14.127 and 18.948 q ha^{-1}) while minimum grain yield (14.135 q ha^{-1}) was obtained in untreated control. Maximum grain yield was increased in treatment T_7 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed (44.85%) followed by T_8 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} (43.61%) and T_{10} = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Tubeconazole @ 3ml kg^{-1} seed (29.63%) (Table 4).

Result indicated that the combine application fungicides as seed treatment and bio-control agents as soil treatment was found effective in controlling of collar rot disease of chickpea. This might be due to initial effect of fungicides and long time affectivity of bio-control agents. Sugha *et al.* (1993) also reported that conidial coating of the antagonistic *Trichoderma harzianum* and *T. viride* on seeds significantly reduced seedling mortality (47-65%) infected by *Sclerotium rolfsii* compared with the untreated controls. Khan and Javaid (2015) tested Tegula

Table 2: Efficacy of *Trichoderma, Pseudomonas* and fungicides against collar rot disease of chickpea

Treatments	Plant mortality (%)			Reduction in plant mortality (%)
	2013-14	2014-15	Mean	
T_1 = Control	15.70 (23.27)	12.35 (20.53)	14.03 (21.90)	-
T_2 = Tubeconazole @ 3ml kg^{-1} seed	8.08 (16.30)	5.47 (13.34)	6.78 (14.82)	51.71
T_3 = Hexaconazole @ 3ml kg^{-1} seed	8.41 (16.66)	5.72 (13.67)	7.07 (15.17)	49.64
T_4 = *Pseudomonas fluorescens* @ 10g kg^{-1} seed	12.35 (20.47)	9.60 (17.98)	10.98 (19.23)	21.77
T_5 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + 10g kg^{-1} seed	9.54 (17.83)	7.22 (15.47)	8.38 (16.65)	40.27
T_6 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + 10g kg^{-1} seed	10.82 (19.07)	8.95 (17.33)	9.89 (18.20)	29.54
T_7 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @3ml kg^{-1} seed	4.30 (11.87)	2.25 (8.53)	3.28 (10.20)	76.66
T_8 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @3ml/kg seed	5.80 (13.87)	2.59 (8.32)	4.20 (11.10)	70.10
T_9 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Tubeconazole @3ml kg^{-1} seed	6.15 (14.30)	4.09 (11.35)	5.12 (12.83)	63.51
T_{10} = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Tubeconazole @3ml kg^{-1} seed	7.32 (15.66)	5.06 (12.79)	6.19 (6.19)	55.88
CV (%)	11.50	13.46	12.48	
SEm±	0.71	0.95	0.83	—
CD at 5%	1.86	2.48	2.17	—

Control

***Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml/kg^{-1} seed**

***Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed**

Overall view of experiment

Table 3: Efficacy of *Trichoderma, Pseudomonas* and fungicides against collar rot disease of chickpea

Treatments	Plant height (cm)			No. of pods per plant		
	2013-14	2014-15	Mean	2013-14	2014-15	Mean
T = Control	40.25	45.60	42.93	25.40	36.70	31.05
T_1 = Tubeconazole @ 3ml kg^{-1} seed	41.30	46.75	44.03	32.00	43.50	37.75
T_2 = Hexaconazole @ 3ml kg^{-1} seed	42.00	47.65	44.83	35.50	43.75	39.63
T_3 = *Pseudomonas fluorescens* @ 10g kg^{-1} seed	44.45	49.50	46.98	29.30	40.90	35.10
T_4 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + 10g kg^{-1} seed	46.25	51.80	49.03	30.50	41.40	35.95
T_5 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + 10g kg^{-1} seed	46.00	51.00	48.50	26.24	37.70	31.97
T_6 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Hexaconazole @ 3ml kg^{-1} seed	45.50	49.95	47.73	41.30	49.75	45.53
T_7 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil)+Hexaconazole @ 3ml/kg seed	45.00	49.15	47.08	38.70	45.95	42.33
T_8 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) + Tubeconazole @ 3ml kg^{-1} seed	44.65	48.00	46.33	36.20	44.65	40.43
T_9 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) + Tubeconazole @ 3ml kg^{-1} seed	43.60	47.60	45.60	36.80	45.05	40.93
CV (%)	12.52	14.52	—	14.50	12.46	—
SEm±	2.40	2.57	—	3.23	2.89	—
CD at 5%	NS	NS	—	8.40	7.52	—

Table 4: Efficacy of *Trichoderma, Pseudomonas* and fungicides against collar rot disease and grain yield of chickpea

Treatments	Grain Yield (q/ha)			Increased yield over control (%)
	2013-14	2014-15	Mean	
T = Control	8.896	14.135	11.516	-
T_1 = Tubeconazole @ 3ml kg^{-1} seed	11.019	16.065	13.542	17.59
T_2 = Hexaconazole @ 3ml kg^{-1} seed	11.117	16.245	13.681	18.80
T_3 = *Pseudomonas fluorescens* @ 10g kg^{-1} seed	10.179	15.338	12.759	10.80
T_4 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil) +10g kg^{-1} seed	10.971	16.043	13.507	17.29
T_5 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil) +10g kg^{-1} seed	10.244	15.635	13.303	15.52
T_6 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil)+Hexaconazole @ 3ml kg^{-1} seed	14.269	19.095	16.682	44.85
T_7 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil)+Hexaconazole @ 3ml/ kg seed	14.127	18.948	16.538	43.61
T_8 = *Trichoderma harzianum* @ 8q ha^{-1} (Soil)+Tubeconazole @ 3ml kg^{-1} seed	11.838	16.965	14.402	25.06
T_9 = *Pseudomonas fluorescens* @ 8q ha^{-1} (Soil)+Tubeconazole @ 3ml kg^{-1} seed	12.431	17.425	14.928	29.63
CV (%)	10.28	9.87	10.08	-
SEm±	0.89	0.86	0.88	-
CD at 5%	2.33	2.24	2.29	-

(tebuconazole), Thiophanate Methyl, Ridomil Gold (metalaxyl + mancozeb) and Mancozeb fungicides at 50, 100, 150, 200, 250 ppm concentrations. Fungicides Thiophanate methyl, Tegula and Mancozeb were found effective against *Sclerotium rolfsii*. Kumar *et al.* (2008) evaluated two fungicides (carboxin and thiram) and two bio-control agents (*Pseudomonas fluorescens* and *Trichoderma harzianum*) as seed treatment in different combinations against *Sclerotium rolfsii*, the causal organism of collar rot of chickpea (*Cicer arietinum*).

Seed treated with *T. harzianum* (4g/kg seed) + carboxin (0.5g/kg seed) provided maximum protection to the crop by giving maximum seedling emergence (495.0/20 m^2), final plant stand (480.4/20m^2) and grain yield (18.2q/ha). Nagamma and Nagaraja (2015) revealed that the maximum inhibition of mycelial growth (71.67%) was noticed in *T. harzianum* (Bacteriology lab isolate) which was followed by *T. viride* (Microbiology lab) (63.33%). Least inhibition was observed in *T. harzianum* GKVK isolate (31.67%). Sab *et al.* (2014) tested eight bioagents tested 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*. Veena and Reddy (2016) revealed that the seed treatment with Copper oxychloride + soil application of potential fungal (*Trichoderma isolate-7)* and bacterial bio-control agent (CREB-16) was found to be superior as it recorded the highest germination percentage (100 %), highest initial (10.00) and final population of chickpea (9.66), least PDI (16.00 %), maximum plant height (25.61 cm), root length (13.40 cm) and maximum shoot (0.49 g) and root dry weights (0.11 g). The population dynamics of both antagonists and pathogen were estimated at two different intervals initially at 7 DAS and then on 45 DAS in pot culture experiment.

With the increase in antagonists population, the population dynamics of pathogen were significantly reduced from 7 DAS to 45 DAS over control in all the treatments with the maximum reduction in T8 treatment. Singh *et al.* (2013) revealed that the *Trichoderma harzianum* NBRI-1055 significantly suppress the seedling blight of sunflower caused by *Rhizoctonia solani* and induce defence mechanism in host. Singh *et al.* (2014) revealed that the seed and furrow application of the formulation of Trichoderma viride 2% W.P. formulation significantly reduced the wilt disease of tomato and damping-off of chilli. The yields of tomato and chilli were also significantly enhanced. Further, the formulation did not have any phyto-toxic effect either on tomato or chilli plants at all the doses levels tested for field bio-efficacy. The *T. viride* formulation also did not have any adverse effect on the beneficial rhizospheric microbes, like Arbuscular Mycorrhizae (*Glomus spp.*) in tomato and chilli rhizosphere at all dosages as confirmed with microscopic observations. Based on the above findings, the *T. viride* 2% W.P. formulation is found safe and effective for using as an efficient and ecologically-safe alternative to chemical fungicides for the management of wilt of tomato and damping-off of chilli as well as for obtaining higher yields.

Conclusion

The main objective of the present study was to manage the collar rot disease of chickpea by integration of fungicide and *Trichoderma harzianum* or *Pseudomonas fluorescens*. The results of the present study concluded that the collar rot disease of Chickpea can be minimizing by the integration of *Trichoderma harzianum* @ 8q ha^{-1} (Soil application) or *Pseudomonas fluorescens* 8q ha^{-1} (Soil application) followed by the application of Hexaconazole @ 3ml kg^{-1} seed as seed treatment under field conditions.

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