Evaluation of effectiveness of different *Trichoderma* spp. in managing Fusarium wilt disease of chickpea

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Abstract: The present study was undertaken to evaluate the biocontrol efficiency of four species of *Trichoderma*, namely, *T. harzianum. T. viride, T. atroviride* and *T. koningii*, in managing the wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri.* The biocontrol agents were applied either as seed treatment or were mixed in the soil at the dose of 4gm/kg seed or soil, respectively. Among all the four *Trichoderma* spp. tested, soil application of biocontrol agent *T. harzianum* was found to be most efficient in managing the wilt disease followed by its seed treatment while application of *T. atroviride* was noticed to be least effective in either of the two forms (significant at P≤0.05).

Keywords - *Trichoderma* spp., wilt disease, *Fusarium oxysporum* f. sp. *ciceri,* seed treatment, soil application.

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important legume crop cultivated all over in the tropics, subtropics and temperate regions in South and West Asia, East and North Africa, southern Europe, North and South America and Australia. India leads the world production by producing 71.95% of the total world produce (FAOSTAT, 2014). Chickpea is a rich source of protein that makes it a highly valued crop to be substituted for animal protein (Jukanti *et al*., 2012).

It is attacked by numerous pests and pathogens (Chattopadhyay *et al*., 2001). Among the different pathogens infesting the chickpea crop, *Fusarium oxysporum* f. sp. *ciceri* that causes wilt disease, is most devastating. The fungus is both seed borne (Pande *et al*., 2007) as well as soil borne (Jiménez-Fernández *et al*., 2011). Even in the absence of the host, the fungus can survive for more than six months, in form of facultative saprophyte in the soil or as chlamydospores (Haware *et al*., 1986). The disease reduces the crop yield and quality and thereby limits the productivity. In India alone 10-15% annual yield losses have been estimated due to wilt disease (Dubey *et al*., 2007). General wilt symptoms include drooping, yellowing and in view the importance of the crop, the following study was carried out to evaluate biocontrol efficiency of different species of *Trichoderma* in managing the Fusarium wilt disease of chickpea.

II. MATERIALS AND METHODS

The pathogenic fungus was isolated from the infected chickpea plants and was identified on the basis of its morphological characters. Pathogenicity test was also done using Koch's postulates and the isolated fungus was compared with the previously isolated fungus and was found identical to it.

Biocontrol agents used were procured from Indian Agricultural Research Institute (IARI) and were mass cultured on sorghum grains and were grinded before applying in the soil @4 g/kg soil. However, for seed treatment, the different *Trichoderma* spp. were coated on chickpea seeds @4 g/kg seed. For better adhesion of the bio-agents, seeds were mixed with 5% jaggery solution.

Seeds were sown in field micro-plots of 2x3m^2 size in the first fortnight of November for the two consecutive years and was harvested in April. Three replicates of each treatment (seed treatment and soil application) were maintained and the mean was calculated and following observations were made:

1. Per cent seed germination was calculated 10 days after sowing using the formula:
   Seed germination (%) = (Total number of seedlings emerged/Total number of seeds sown) x 100
2. Wilt incidence were calculated as follows:
   Wilt incidence (%) = {(Number of wilted plants/ Total number of plants) x 100}
3. Number of functional root nodules/plant
4. Crop yield

Statistical analysis was done using Minitab 11.0 software with Windows 10. L.S.D. was calculated at P≤0.05 for all variables and Tukey's test was also used to mark the significance level.

III. Results

3.1. Per cent seed germination

It was observed that maximum seed germination rate of 89.96% against 79.05% in untreated control in the first year and 87.88% over 77.71% in control plots in the second year of experimentation was achieved when *T. harzianum* was employed as seed treatment @4g/kg followed by its soil application (table 2). Seed germination rate of 88.07% and 86.61%, respectively, was achieved when *T. viride* was applied as seed dresser. *T. atroviride* exhibited least germination rate when applied in either of the two forms and was thus found to be least efficacious at 5% significant level.

3.2. Wilt incidence

Once again *T. harzianum* was noticed as the most efficient bio-agent in managing the wilt incidence among all the different tested *Trichoderma* species. However, in comparison to the seed treatment, soil application of *T. harzianum* was more efficient in reducing the disease as it caused 49.62% and 48.29% reduction in the wilt incidence during the two successive years of crop cultivation. Soil amendment with *T. viride* stood next best option in dwindling the per cent wilt incidence and was followed by seed treatment with *T. harzianum* as can be seen in table 1&2 at P≤0.05.

3.3. Number of functional root nodules/plant

Highest number of functional root nodules/plant were recorded in the plants grown in the micro-plots having soil treated with *T. harzianum* in comparison to control that gave a total nodule count of 21.82 and 20.27 nodules/plant. A maximum of 40.07 and 39.88 root nodules/plant were observed in these plots. it was followed by seed treatment of *T. harzianum* that yielded 36.40 and 35.73 nodule count/plant during the two years of experimental work. It was found to be insignificant to soil application of *T. viride* which gave almost similar number of root nodules/plant, i.e. 35.74 and 35.38number of functional roots/plant, respectively, (P≤0.05).

3.4. Crop yield

1007.20 kg/ha of yield was obtained in the previous year of crop growth from plots having soil mixed with *T. harzianum* which was 35.36% more than the yield obtained from controlled plots that gave a total produce of 744.12kg/ha. In the second year also 32.01% increase in yield (significant at P≤0.05) was recorded when *T. harzianum* was amended in the soil as it gave 978.73 kg/ha of produce against 741.41 kg/ha in controlled plots. Seed treatment with *T. harzianum* gave next best results followed by soil application of *T. viride.* Among all the treatment *T. atroviride* gave lowest produce when applied as seed treatment although it was far better than the total yield collected from the control plots (table 1&2).

IV. DISCUSSION

Soil application of *T. harzianum* was found to be the best option in managing the wilt disease of chickpea followed by its seed treatment among all the tested species of *Trichoderma* followed by *T. viride*. Srivastava *et al*., (2015) also found similar results as they reported *T. harzianum* as the best bio-control agent followed by *T. viride and A. niger* in degenerating the chickpea wilt pathogen. Many other workers like Wani (2005) and Dubey *et al*., (2007) also established the ability of *T. harzianum* in managing soil-borne wilt and root rot causing fungi. Similar to the present study, Altomare *et al.* (1999) found enhanced seed germination rate and seed weight by using *T. harzianum* against the pathogenic fungus. Verma *et al.*, (2014) in their trials observed noteworthy increment in yield attributes and inhibition in pathogen growth due to the use of *Trichoderma* spp.

Table. 1 Effect of seed treatment of bio-control agents on phenological parameters, per cent disease incidence and yield of chickpea plants grown in the field plots.

I Year

S. No.	Treatment	Per cent germination	PDI	Per cent wilt reduction over control	No. of root nodules/ plant	No. of pods/plant	Yield (kg/ha)
1.	***T. harzianum***	89.96±1.63^{a}	19.71±1.48^{e}	40.65±1.64^{a}	36.40±1.74^{a}	27.53±1.35^{a}	978.73±7.02(31.53)^{a}
2.	***T. koningii***	82.50±1.25^{b}	24.17±1.77^{c}	27.23±1.21^{c}	27.23±1.18^{c}	19.87±1.63^{c}	892.34±8.92(19.93)^{c}
3.	***T. viride***	88.07±1.64^{a}	20.69±1.65^{d}	37.73±1.52^{b}	33.47±1.29^{b}	23.84±1.80^{b}	918.20±7.58(23.40)^{b}
4.	***T. atroviride***	82.04±1.12^{b}	27.14±2.02^{b}	18.25±2.15^{d}	25.26±1.56^{d}	19.42±1.24^{c}	864.15±8.45(16.13)^{d}
5.	**Control**	79.05±1.45^{c}	33.20±1.88^{a}	0.00	21.82±1.30^{e}	17.33±1.46^{d}	744.12±5.12^{e}
***F*-value**		40.91	941.47	469.64	315.07	18.59	637.77
LSD		2.71	0.69	2.06	1.52	1.21	15.08

II Year

S. No.	Treatment	Per cent germination	PDI	Per cent wilt reduction over control	No. of root nodules/ plant	No. of pods/plant	Yield (kg/ha)
1.	***T. harzianum***	87.88±1.02^{a}	22.70±2.04^{e}	38.87±1.55^{a}	35.73±1.62^{a}	27.32±1.67^{a}	972.07±7.38(31.12)^{a}
2.	***T. koningii***	82.71±1.57^{b}	27.31±1.27^{c}	26.30±1.87^{c}	29.13±1.03^{c}	20.14±2.23^{c}	884.34±9.94(19.28)^{c}
3.	***T. viride***	86.61±2.08^{a}	24.16±2.08^{d}	34.92±1.50^{b}	32.64±1.09^{b}	22.82±1.81^{b}	909.89±8.97(22.72)^{b}
4.	***T. atroviride***	81.94±1.45^{b}	29.95±2.52^{b}	19.27±1.15^{d}	25.00±1.16^{d}	18.87±1.44^{c}	851.51±6.36(14.85)^{d}
5.	**Control**	77.71±1.3^{c}	37.10±2.58^{a}	0.00	20.11±1.63^{e}	16.22±1.52^{d}	741.41±5.73^{e}
***F*-value**		21.81	805.36	348.95	125.96	18.77	594.21
LSD		3.38	1.28	3.24	1.81	1.32	14.26

Each value is a mean of three replicates. Values with in a column followed by different alphabets are significantly different at P≤0.05 according to Tukey's test. Figures in parenthesis are per cent increase in yield.

Table. 2 Effect of soil application of bio-control agents on phenological parameters, per cent disease incidence and yield of chickpea plants grown in the field plots.

I Year

	Treatment		PDI				Yield (kg/ha)

S. No.		Per cent germination		Per cent wilt reduction over control	No. of root nodules/ plant	No. of pods/plant	
1.	***T. harzianum***	89.17±2.60[a]	16.78±2.34[e]	49.62±2.81[a]	40.07±1.68[a]	29.57±0.94[a]	1007.20±6.37(35.36)[a]
2.	***T. koningii***	81.67±1.91[c]	20.88±2.68[c]	37.24±3.33[c]	32.09±1.81[c]	22.18±1.05[c]	911.64±7.84(22.51)[c]
3.	***T. viride***	86.25±2.54[b]	18.34±2.05[d]	44.83±2.00[b]	35.74±1.12[b]	24.37±0.83[b]	926.55±7.50(24.52)[b]
4.	***T. atroviride***	82.15±2.07[c]	24.24±1.99[b]	26.98±3.09[d]	31.41±1.15[c]	21.97±0.97[c]	892.59±8.31(19.95)[d]
5.	**Control**	78.96±2.37[d]	33.20±2.81[a]	0	21.82±1.30[d]	17.20±0.70[d]	744.12±5.12[e]
***F*-value**		54.74	362.41	554.53	291.11	211.36	1791.43
LSD		2.13	1.03	3.31	1.39	1.93	11.3

II Year

S. No.	Treatment	Per cent germination	PDI	Per cent wilt reduction over control	No. of root nodules/ plant	No. of pods/plant	Yield (kg/ha)
1.	***T. harzianum***	87.21±1.88[a]	19.57±1.92[e]	48.29±3.05[a]	39.88±1.36[a]	29.08±1.14[a]	978.73±7.02(32.01)[a]
2.	***T. koningii***	83.15±1.16[b]	25.29±2.42[c]	33.22±2.21[c]	31.76±1.93[c]	21.93±0.97[c]	892.34±8.92(20.36)[c]
3.	***T. viride***	86.67±1.91[a]	21.73±2.26[d]	42.65±2.10[b]	35.38±1.09[b]	23.71±1.01[b]	918.20±7.58(23.85)[b]
4.	***T. atroviride***	81.25±1.45[b]	27.81±2.59[b]	26.50±2.95[d]	31.05±1.80[c]	20.99±1.37[c]	874.65±6.54(17.97)[d]
5.	**Control**	77.50±2.25[c]	37.84±2.83[a]	0	20.27±1.63[d]	16.15±1.10[d]	741.41±5.73[e]
***F*-value**		43.63	415.81	678.17	266.35	145.35	1068.5
LSD		3.18	1.38	2.87	2.17	2.11	14.4

Each value is a mean of three replicates. Values with in a column followed by different alphabets are significantly different at P≤0.05 according to Tukey's test. Figures in parenthesis are per cent increase in yield.

V. CONCLUSION

The field trial has shown that *F. oxysporum* f.sp. *ciceri* is a highly pathogenic fungus effecting the chickpea crop since early growth stages. Approximately 33-38% of wilt incidence was noticed in uncontrolled non-treated plots. However, selected BCAs established themselves well in the wilt infected fields, and thus produced encouraging results by significantly reducing the wilt incidence to a remarkable level besides boosting the plant growth and increasing the crop produce. Of all the bio-agents tested, application of *T. harzianum* either through soil or seed treatment significantly controlled the per cent wilt incidence and promoted the chickpea yield. *T. atroviride* although gave lowest results for all the parameters taken into consideration but they were significantly far better in contrast to the control.

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