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Bio-Efficacy Of Trichoderma Species Against Lentil Wilt Pathogen

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Abstract: Two biocontrol agent viz., Trichoderma viride and Trichoderma harzianum were evaluated to test the antagonism against Fusarium oxysporum under in vitro conditions. All the two biocontrol agents have the potential of parasitizing the growth of Fusarium oxysporum in vitro. For control of seed-borne infection of *F.oxysporum* the best results were obtained from biological agent *Trichoderma viride*. Maximum control of *F.oxysporum* incidence (71.15%) and infected seedling (85.0%) was obtained when *T.viride* was applied as 80ml concentration which was followed by 20ml concentration. Control of pathogen incidence (42.3%), infected seedlings (60%) and seed germination (76.25%) was low in 120ml concentration. In *F.oxysporum* infected seeds, the maximum control for pathogen incidence 56% to 80% and infected seedling 47.61% to 92.86% was observed in 20ml-240ml dilutions of *T.harzianum*.

Keywords: Bio-efficacy, Fusarium oxysporum, lentil, Trichoderma, Wilt

I. INTRODUCTION

Lentil (Lens culinaris L.) is the second most important cool-season legume crop in India (Ram and Punia, 2018). It covers an area of 1.51 million ha with a production of 1.56 million tons and productivity of 1,032 kg ha−1 (Directorate of Economics and Statistics, 2020). In India, the pulse cultivation occupies nearly about 24.70 million hectare of land every year and the annual production touches over 14.50 million tones and productivity are 587 kg/ha. Lentil is currently an important pulse crop grown widely throughout the Indian subcontinent, Middle East, Northern Africa, Southern Europe, North and South America, Australia and west Asia (Ford and Taylor, 2003; Erskine, 1997).Lentil are among ancient plants known to be cultivated by man carbonized lentil found in Neolithic villages in the Middle East have been dated as being between 8 and 9 thousand years old. After initial cultivation of the crop in the Middle East. Lentil use began to spred around the Mediterranean.By 2200B.C lentil begain to appear in Egyptian tombs (Pooja, 2005). Various fungal and bacteria antagonists have been tried for control of Fusarium wilt in lentil. The most commonly used are *B.subtiles, Rhizobium leguminosorum, Glicladium virens, T.viride, Streptomyces gourgereti, Streptomyces sp.* (Essalmani and Lahlou, 2003; Singh and Mukhopadhyay, 2002; Mehrotra and Cladius, 1972).They observed that isolates of *Pseudomonas, Erwinia, Rhizobium, Pencillium expansum* and *Tricoderma lignorum* were antagonistic to *F. oxysporum* on lentil. *Tricoderma harzianum* and *Tricoderma konigii* showed antagonism against the lentil wilt pathogen in laboratory (Saxena and Mukhopadhyay 1987; Mukhopadhyay et al. 1989).Bojdov'A (1993) found that Trichoderma harzianum RK-1 successfully controlled *Fusarium* infection of Lentil.Bhat et al. (2003) and Singh, Mishra and Vyas (2007) reported that biocontrol agent *T.viride* and *T.harzianum* caused reduction in chickpea wilt and tomato wilt caused by *F.oxysporum*.Srivastava and Mishra (2008) used antagonistic fungi in seed dressing for the management of chickpea and pigeon pea wilt respectively.

In present investigation all the two antagonists were quite effective but *Trichoderma* spp. gave best control of *R.solani* as also observed by Sharma (2003) and Agrawal (2002). The biological agents not only reduced the recovery of pathogen but also showed increase in potential of seed germination.Pandey and Upadhyay (2002) reported that *T.viride* causes loops and coiling of mycelium and rupture of cell wall of the pathogen. *G.virens* resulted in twisting, air bubbling and disintergration of the fungal hyphe, while *T.harzianum* causes severe vacuolation, shrinkage and coagulation of the cytoplasm of the fungal hyphae. Similar results were obtained by Mukherjee and Tripathi (2000) while screening *G.virens* against *Rhizoctonia solani*.Khandelwal (2009) found accumulation of conidia of *G.virens* around the mycelium of the pathogen caused bending shrinkage and breakage of the fungal hyphae. Mycelium of *T.viride* coiled around the mycelium of the pathogen and also caused hyphal bulging T.harzianum showed discontinuity and coagulation of protoplasm.

II. MATERIALS AND METHODS

Trichoderma viride and *T. harzianum* were used as biological agents. Their pure cultures were obtained from National Center of Fungal Taxonomy, IARI, and New Delhi and raised on PDA for seed treatment. Seed samples Id. Nos. 3527, 3529 and 3530, 3538 with *F. oxysporum* and *R. solani* respectively were used (Table 2). For treating the seeds, 10ml of distilled water was added to 12day old culture plate and the suspension was diluted to 20ml. considering 20ml as stock solution 40, 80,120 and 240ml concentrations were made by adding water. 100 seeds per concentration for each pathogens *F. oxysporum* and *R. solani* (naturally infected) were taken at random, surface sterilized with 1% chlorine solution, soaked in suspension of *T.viride* and *T.harzianum* separately for 5h and

sown in petriplates(20 seeds/petriplate) for 8 days. Observations were taken on seed germination and pathogen incidence along with infected seedlings.

III. RESULTS AND DISCUSSION

Pure culture suspension of *T.viride* and *T. harzianum* and their four diluted concentration viz. 20ml, 40ml, 80ml and 120ml were used for seed treatment. Both *T.viride* and *T.harzianum* were antagonistic and inhibited growth of *F.oxysporum* However, the antagonistic effect of *T.viride* was better than *T.harzianum*.

T.viride (Tables-1; Fig.-1, C & D)

The result shows that seed treatment with bio-agents reduced pathogen incidence significantly in all five concentration as compared to control. Maximum control of *F.oxysporum* incidence (71.15%) and infected seedling (85.0%) was obtained when *T.viride* was applied as 80ml concentration which was followed by 20ml concentration. Control of pathogen incidence (42.3%), infected seedlings (60%) and seed germination (76.25%) was low in 120ml concentration (Fig.-1C, D).

***T. harzianum* (Tables -1; Fig.1 A, B & C)**

All the five concentration of *T.harzianum* was significant in controlling the pathogen incidence and seedling infection over the check. The seed germination also enhanced except in 20ml dilution for *F.oxysporum* infected seeds.

In *F.oxysporum* infected seeds, the maximum control for pathogen incidence 56% to 80% and infected seedling 47.61% to 92.86% was observed in 20ml-240ml dilutions of *T.harzianum*. Of these 80ml gave best control showing poor recovery of pathogen and good germination percentage (Fig.-1A,B & C).

Germination was significant in all concentration (81-95%) except in 20 ml (81%) as compared to control (76%) Statistically all the five concentration of *T.viride* proved superior for control of *F.oxysporum*.Various fungal and bacteria antagonists have been tried for control of Fusarium wilt in lentil. The most commonly used are *B.subtiles, Rhizobium eguminosorum,Glicladium virens, T.viride, Streptomyces gourgereti, Streptomyces sp.* (Essalmani and Lahlou, 2003; Singh and Mukhopadhyay, 2002; Mehrotra and Cladius, 1972).In present study the antagonistic effects of *T. harzianum* and *T.viride*, were studied in SBM and all were found antagonists to *Rhizoctonia solani* and *F.oxysporum*. The antagonistic effects of *T.harzianum* was the highest followed by *T.viride* in case of Fusarium wilt. Similar results were found by Ujevic et al. (1970).They observed that isolates of *Pseudomonas, Erwinia, Rhizobium, Pencillium expansum* and *Tricoderma lignorum* were antagonistic to *F. oxysporum* on lentil. *Tricoderma harzianum* and *Tricoderma konigii* showed antagonism against the lentil wilt pathogen in laboratory (Saxena and Mukhopadhyay 1987; Mukhopadhyay et al. 1989).Bojdov'A (1993) found that Trichoderma harzianum RK-1 successfully control led *Fusarium* infection of Lentil.Bhat et al. (2003) and Singh, Mishra and Vyas (2007) reported that biocontrol agent *T.viride* and *T.harzianum* caused reduction in chickpea wilt and tomato wilt caused by *F.oxysporum*. *T.viride, T.harzianum* and VAM fungi *G.mosseae* and *G.fascicutum* brought about significant reduction in pathogenic effect by *F.oxysporum* on brinjal, but *T.viride* and *T.harzianum* gave best control (Abdul Hamid wani, 2005).Srivastava and Mishra (2008) used antagonistic fungi in seed dressing for the management of chickpea and pigeon pea wilt respectively.In present investigation all the two antagonists were quite effective but *Trichoderma* spp. gave best control of *R.solani* as also observed by Sharma (2003) and Agrawal (2002). The biological agents not only reduced the recovery of pathogen but also showed increase in potential of seed germination.Pandey and Upadhyay (2002) reported that *T.viride* causes loops and coiling of mycelium and rupture of cell wall of the pathogen. *G.virens* resulted in twisting, air bubbling and disintergration of the fungal hyphe, while *T.harzianum* causes severe vacuolation, shrinkage and coagulation of the cytoplasm of the fungal hyphae. Similar results were obtained Mukherjee and Tripathi (2000) while screening *G.virens* against *Rhizoctonia solani*.Khandelwal (2009) found accumulation of conidia of *G.virens* around the mycelium of the pathogen caused bending shrinkage and breakage of the fungal hyphae. Mycelium of *T.viride* coiled around the mycelium of the pathogen and also caused hyphal bulging T.harzianum showed discontinuity and coagulation of protoplasm**.**Works on the effect of non-volatile compounds of Trichoderma on some more pathogens such as Botrytis fabae (Barakat et al., 2014, Fusarium moniliforme (Kumar et al., 2012) and other Fusarium species (Sain and Pandey, 2016) have been reported. But very few such works appear to have been done against F. oxysporum wilt pathogen of lentil. Therefore, the antagonist, the T. viride may be chosen to be the most promising biocontrol agent for F. oxysporum.

IV. Declaration

The author declare that they have no competing interests.

V. Acknowledgements

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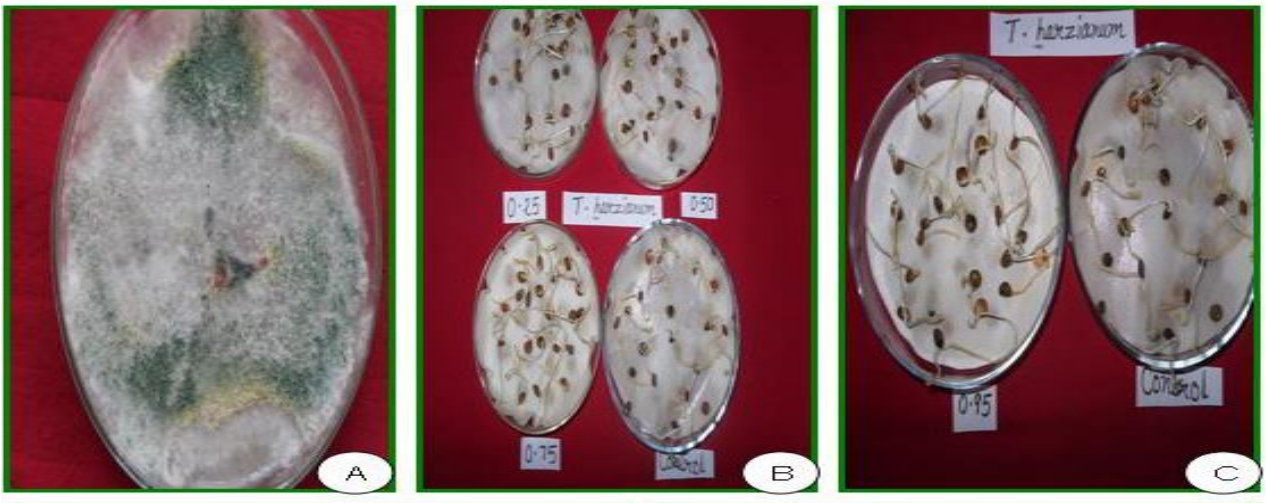
Figure-1 (A, B, C and C, D)



VII. TABLE 1: CONTROL OF SEED-BORNE INFECTION OF *FUSARIUM OXYSPORUM* BY *TRICHODERMA HARZIANUM AND T. VIRIDE*

Concentration	Germination (%)				Seedling Infection Control (%)				Pathogen Incidence Control (%)			
20 ml	87.5	17.5	85	17	76	1.2	73.33	1.2	66.34	1.75	70.83	1.75
40 ml	90	18	81.25	16.25	65	1.75	77.77	1.5	61.53	2	83.33	1
80 ml	93.78	18.6	86.25	17.25	85	0.75	68.88	1.4	71.15	1.5	66.66	2
120 ml	76.25	15.25	81.25	16.25	60	2	55.55	2	42.3	3	58.33	2.5
240 ml	81.25	16.25	90	18	75	1.25	61.11	1.75	66.34	1.75	60	2.4
Control	71	14.2	75	15	-	5	-	4.5	-	5.2	-	6