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BIOEFFICACY OF *TRICHODERMA* SPP. AGAINST *SCLEROTIUM ROLFSII* SACC., AN INCITANT OF COLLAR ROT OF CHICK PEA *IN VITRO*

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ABSTRACT
To study the bioefficacyof *Trichoderma* spp. on mycelial growth and sclerotia production of *Sclerotiumrolfsii,* five species of *Trichoderma viz., Trichoderma viride, T. harzianum, T. virens, T. asperellum and T. atroviride* were selected and screened by dual culture, pathogen at center and poison food (Liquid Culture Filtrate) technique against *S.rolfsii* inciting collar rot of chickpea. Among the *Trichoderma* spp. tested through dual culture technique, *T. asperellum* showed maximum mycelial growth inhibition *i.e.* 61.48, 75.00 and 73.33 per cent at 4,6 and 8 days of incubation, respectively. Whereas, *T. harzianum* gave significantly highest per cent inhibition in sclerotia production *i.e.* 98.44, 85. 22 and 78.56 per cent at 4,6 and 8 days of incubation, respectively. In pathogen at center method, *T. asperellum* gave 75.74, 78.33 and 100.00 per cent mycelial growth inhibitionat 4, 6 and 8 days of incubation, respectively. While, *T. harzianum, T. asperellum and T. viride* showed complete inhibition in sclerotia production. However, the LCF study of *T. harzianum* and *T. viride* after 4, 6 and 8 days of incubation revealed complete inhibition of mycelial growth and sclerotia production of *S. rolfsii*. In present *in vitro* study, *T. asperellum* found effective in inhibiting the mycelial growth as well as sclerotia production of *S. rolfsii* followed by *T. harzianum*.

INTRODUCTION

Chickpea is grown in India since ancient times. India stands 1st in production of chick pea followed by Australia. It is widely grown major pulse crop in India and accounts nearly68.1 per cent of the total pulse production in the world (Anonymous, 2013). Chickpea crop is prone to many diseases *viz*., Fusarium wilt, dry root rot, collar rot, Ascochyta blight, Verticillium wilt, black root rot, Phytophthora root rot, wet root rot and foot rot etc. Among these, collar rot incited by *Sclerotium rolfsii* Sacc. is most predominant. *Sclerotium rolfsii* has wide host range, abundant growth and its capability of producing enormous sclerotia which persist in soil for several years and which act as primary source of inoculums (Chet and Henis, 1972; Punja, 1985). Darvin *et al*. (2013) recorded 56.25 per cent mycelial growth inhibition of *S. rolfsii* by *T. harzianum* through dual culture technique and also recorded complete inhibition of *S. rolfsii* by *T. viride* isolate through poisoned food technique. Biological control of soil borne diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. is well documentedas effective biological control agent of plant diseases (Harman *et al*. 1980, Sivan *et al*.1984 and Coley-Smith *et al*. 1991). There fore the present investigation was carried out to evaluate the bioefficacy of *Trichoderma* spp. against *S. rolfsii* an incitant of collar rot of chickpea *in vitro*.

MATERIALS AND METHODS

Collection and isolation of pathogen

Chick pea plants showing typical symptoms of sclerotium rot were brought to the laboratory and pathogen was isolated by tissue isolation method under aseptic conditions. The pure culture of pathogen was subcultured by hyphal tip method and maintained on Potato Dextrose Agar (PDA) medium. The pathogen was identified by morphological characteristics. Various *Trichoderma* spp. *viz*., *Trichoderma harzianum, T. viride, T. virens*, *T. asperellum* and *T. atroviride* which were available in the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat screened to test their efficacy against *S. rolfsii*.

Dual Culture method

A dual culture technique (Dennis and Webster, 1971) was used to test the antagonistic potential of the *Trichoderma* spp. against *S. rolfsii*. The antagonists and pathogen were grown on PDA medium. Sterilized PDA medium (20 mL) was poured aseptically in 90 mm diameter sterilized Petri plate. Mycelial disc of 5 mm size from seven days old actively growing culture of the *Trichoderma* spp. transferred to one side of the Petri plate. In the same way the other side of the Petri plate was inoculated with the seven days old culture of *S. rolfsii*. The test pathogen was grown alone in Petri plate for comparison. All the inoculated plates were incubated in BOD incubator at 27 ± 2^{o}C. Each treatment was repeated three times and the

per cent mycelial growth inhibition as well as inhibition of sclerotia production of the pathogen was recorded after 4, 6 and 8 days of incubation.

The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by following formula (Asalmol *et al*., 1990).

$$I = \frac{C - T}{C} \times 100$$

Where

I = Per cent mycelial growth inhibition

C = Colony diameter (mm) in control plate

T = Colony diameter (mm) in treated plate

Pathogen at centre method

The pathogen at centre method for the interaction between pathogen and bioagent was carried out as method followed by Patel *et al*. (2014). In which Petri plate containing PDA medium was inoculated aseptically in the centre with a 5 mm disc of the test pathogen and four discs of 5 mm size of the bioagents (*Trichoderma* spp.) placed equidistantly in the same Petri plate around the pathogen.The plates with only pathogen kept at the centre were maintained as control. All inoculated plates were incubated in BOD incubator at 27 ± 2ºC. Each treatment was repeated three times and the per cent mycelial growth inhibition as well as inhibition of sclerotia production of the pathogen was recorded after 4, 6 and 8 days of incubation.

Effect of liquid culture filtrate (LCF) of *Trichoderma* spp. against *S. rolfsii*

Bioefficacy of liquid culture filtrate of different *Trichoderma* spp. were evaluated by "Poisoned food technique" suggested by Nene and Thapliyal (1979) against *S. rolfsii in vitro*. Conical flask containing 100 mL of sterilized Potato Dextrose Broth (PDB) medium was inoculated with 5 mm mycelial discs cut from margin of 7 days old culture of bioagents.The flasks containing individual *Trichoderma* spp. were incubated in an orbital shaker cum BOD incubator at 27 ± 2ºC for 15 days. After 15 days of incubation the culture filtrates were separately filtered through Whatman filter paper No. 1 and seitz filter to remove the mycelial mat and the spores**.**The culture filtrates of *Trichoderma* spp. (300 µL/mL of PDA)were added to PDA medium separately and after solidification; 5 mm discs of *S. rolfsii* were inoculated in the center of each Petri plates separately. Petri plates inoculated with*S. rolfsii*, without amendment of filtrate served as a control.The inoculated Petri plates were incubated in BOD incubator at 27 ± 2ºC. Each treatment was repeated three times and the per cent mycelial growth inhibition as well as inhibition of sclerotia production of the pathogen was recorded after 4, 6 and 8 days of incubation.

RESULTS AND DISCUSSION

Dual culture technique

The highest per cent mycelial growth inhibition of *S. rolfsiii i.e.* 61.48, 75.00 and 73.33 was recorded in *T. asperellum* after 4, 6 and 8 days of incubation, respectively followed by *T. harzianum* (63.70, 63.70 and 62.77%). However, maximum per cent inhibition of sclerotia production was observed in *T. harzianum i.e.* 98.44, 85.22 and 78.56 after 4, 6 and 8 days of incubation, respectively. Results of present findings corroborate with the results reported by Rasu *et al*. (2013). They screened different *Trichoderma* spp. against *S. rolfsii*, among all *T. asperellum* (TTH1) exhibited 64.40 per cent mycelial growth inhibition. Further, Darvin *et al*. (2013) recorded 56.25 per cent mycelial growth inhibition of *S. rolfsii* through *T. harzianum*. Sab *et al*. (2014) observed 70 per cent mycelial growth inhibition of *S. rolfsii* by *T. harzianum* (55IIHR),

Figure 1: Effect of *Trichoderma* spp. against *S. rolfsii* through dual culture technique

Table 1: Evaluation of *Trichoderma* spp. against *S. rolfsii* through dual culture technique

Average mycelial growth (mm) and sclerotia production of *S. rolfsii*												
Treatments	4 DAI				6 DAI				8 DAI			
	Mycelial growth	PGI	Sclerotia* production	PSI	Mycelial growth	PGI	Sclerotia* production	PSI	Mycelial growth	PGI	Sclerotia* production	PSI
T. harzianum	32.67	63.70	01.00	98.44	32.67	63.70	21.67	85.22	33.50	62.77	49.67	78.56
T. viride	32.67	63.70	13.00	79.79	35.00	61.11	25.33	82.72	36.67	59.25	61.67	73.38
T. atroviride	40.67	54.81	00.33	99.47	40.33	55.18	35.33	75.90	36.67	59.25	39.00	83.16
T. virens	43.00	52.22	01.00	98.44	40.00	55.55	31.00	78.86	40.00	55.55	54.33	76.55
T. asperellum	34.67	61.48	02.67	95.85	22.50	75.00	46.67	68.18	24.00	73.33	40.33	82.59
Control	90.00	-	64.33	-	90.00	-	146.67	-	90.00	-	231.67	-
S. Em.±	0.84				1.64				1.35			
C.D. (0.05)	1.83				3.57				2.94			
C.V.%	1.25				4.65				3.81			

*Average of three repetitions; PGI = Percent Growth Inhibition (%); PSI = Percent Sclerotia Inhibition (%); DAI = Days after incubation

followed by *T. harzianum* (NBAII) (63%). Rai *et al.* (2014) studied antagonistic effect of *T. harzianum* against *S. rolfsii*. According to them *T. harzianum* (PBT 23) gave 65.70 per cent mycelial growth inhibition of *S. rolfsii*.

Pathogen at centre method

The highest per cent mycelial growth inhibition of *S. rolfsii*, *i.e.* 75.74, 78.33 and 100 was recorded in*T. asperellum* after 4, 6 and 8 days of incubation, respectively. Whereas, *T. virens, T. harzianum, T. atroviride and T. viride* were found at par. However, complete inhibition ofsclerotia production was recorded in *T. harzianum, T. viride* and *T. asperellum* treatments after 4, 6 and 8 days of incubation. Patel *et al.* (2014) reported 63.89 and 51.39 per cent mycelial growth inhibition of *Pythiumaphanidermatum* by *T. harzianum* and *T. viride,* respectively after 3 days of incubation through pathogen at center method.

Effect of liquid culture filtrate (LCF) of *Trichoderma* spp. on mycelial growth inhibition of *S. rolfsii*

Table 2: Evaluation of *Trichoderma* spp. against *S. rolfsii* through pathogen at center method

Average mycelial growth (mm) and sclerotia production of *S. rolfsii*												
Treatments	4 DAI				6 DAI				8 DAI			
	Mycelial growth	PGI	Sclerotia* production	PSI	Mycelial growth	PGI	Sclerotia* production	PSI	Mycelial growth	PGI	Sclerotia* production	PSI
T. harzianum	26.83	70.18	00.00	100.0	32.67	63.70	00.00	100.0	00.00	100.0	00.00	100.0
T. viride	25.00	72.22	00.00	100.0	24.83	72.41	00.00	100.0	00.01	99.89	00.00	100.0
T. atroviride	26.83	70.18	03.00	95.85	33.50	62.78	08.33	95.60	00.53	99.41	09.33	96.54
T. virens	33.50	62.78	09.67	86.63	33.00	63.33	06.33	96.66	00.33	99.63	07.67	97.16
T. asperellum	21.83	75.74	00.00	100.0	19.50	78.33	00.00	100.0	00.00	100.0	00.00	100.0
Control	90.00	-	72.33	-	90.00	-	189.67	-	90.00	-	270.0	-
S. Em.±	1.73				2.62				0.64			
C.D. (0.05)	3.77				5.71				1.39			
C.V.%	5.68				8.86				4.71			

*Average of three repetitions; PGI = Percent growth inhibition (%); PSI = Percent sclerotia inhibition (%); DAI = Days after incubation

Table 3: Evaluation of liquid culture filtrate of *Trichoderma* spp. against *S. rolfsii*

Average mycelial growth (mm) and sclerotia production of *S. rolfsii*												
Treatments	4 DAI				6 DAI				8 DAI			
	Mycelial growth	PGI	Sclerotia* production	PSI	Mycelial growth	PGI	Sclerotia* production	PSI	Mycelial growth	PGI	Sclerotia* production	PSI
T. harzianum	00.00	100.0	00.00	100.0	00.00	100.0	00.00	100.0	00.00	100.0	00.00	100.0
T. viride	00.00	100.0	00.00	100.0	00.00	100.0	00.00	100.0	00.00	100.0	00.00	100.0
T. atroviride	43.33	51.85	02.00	98.48	44.00	51.11	04.67	96.88	51.33	42.96	06.33	95.95
T. virens	52.33	41.85	12.33	90.63	73.00	18.89	14.33	90.45	82.33	08.52	17.67	88.72
T. asperellum	39.00	56.67	04.67	96.45	45.67	49.26	08.33	94.45	47.33	47.41	09.00	94.25
Control	90.00	-	131.67	-	90.00	-	150.0	-	90.00	-	156.67	-
S. Em.±	2.25				2.55				2.45			
C.D. (0.05)	4.91				5.55				5.34			
C.V.%	7.37				7.38				6.64			

*Average of three repetitions; PGI = Percent growth inhibition (%); PSI = Percent sclerotia inhibition (%); DAI = Days after incubation

Figure 2: Effect of *Trichoderma* spp. against *S. rolfsii* through pathogen at center method

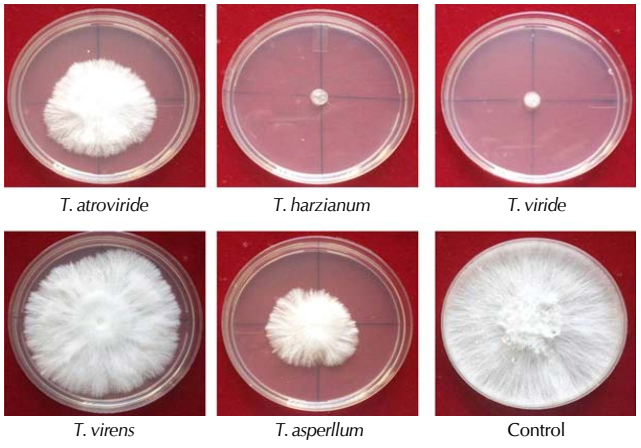

Figure 3: Effect of liquid culture filtrate of *Trichoderma* spp. against *S. rolfsii*

Complete mycelial growth inhibition of *S. rolfsii* was recorded in LCF of*T. harzianum* and *T. viride* after 4, 6 and 8 days of incubation. Whereas, growth inhibition of *S. rolfsii* in culture filtrates of *T. atroviride* (51.85, 51.11 and 42.96 %) and *T. asperellum* (56.67, 49.26 and 47.41 %) found at par after 4, 6 and 8 days of incubation, respectively. However, cent per cent inhibition of sclerotia production was recorded in LCF of *T. harzianum* and *T. viride* after 4, 6 and 8 days of incubation. Results similar to the present findings have been reported by Darvin *et al*. (2013). They reported that *T. viride* isolate completely inhibited the mycelial growth of *S. rolfsii* through poisoned food technique. Further, Sab *et al*. (2014) reported highest mycelial growth inhibition of *S. rolfsii* by *T. harzianum* (70.00%) and *T. viride* (59.00%) after 8 days of incubation through poisoned food technique.

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