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Variation in antagonistic effects of *Trichoderma* species on *Fusarium oxysporum* f. sp. ciceri

Mohammad Faisal, Shashi Tiwari and Umesh Tiwari

Abstract
Fusarium wilt caused by *Fusarium oxysporum* is the most devastating soil-borne disease of chickpea where the yield loss reaches from 10 to 90% at distinguished geographical locations. Hence, a study was taken up *in vitro* to evaluate the variation in inhibition percent of locally isolated *Trichoderma viride* and *T. harzianum* against thirty-five isolates of *Fusarium oxysporum* f. sp. *ciceri* procured from different locations of Prayagraj and adjacent districts. It was found that there was a huge deviation in the inhibiting effects. *Trichoderma viride* inhibition ranged from 31.25 to 44.12 % where best inhibited isolate was FOC-34 (44.12%) followed moderately inhibited FOC-28 (29.76%) and poorly inhibited FOC-33 (3.13%). On the other, inhibition due to *Trichoderma harzianum* ranged from 3.39 to 47.26 % where best inhibited isolate was FOC-13 (47.26 %) followed moderately inhibited FOC-2 (29.66) and poorly inhibited FOC-27 (3.90 %). Hence, it reveals that locally isolated *Trichoderma* sp. had huge variation in their inhibition impacts on *Fusarium oxysporum* f. sp. *ciceri.*

Keywords: Antagonistic, *Trichoderma, Fusarium oxysporum* f. sp. ciceri

Introduction
Chickpea ranks first in perspectives of production and consumption being consumed as a vegetarian source of protein. Chickpea is one of the most important crops in tropical or drought areas of the world. It is also preferred as best crop for unleveled lands as very low amount of water is needed. Globally, India accounts for 65% area under chickpea cultivation and 68% production (Amarender and Devraj, 2010) [1]. In Uttar Pradesh, chickpea was cultivated on 5.14 lakh hectares with yields ranging around 4.6-4.63 lakh tons with productivity 937 kg per hectare (Annual Report, DPD, 2016-17).

Chickpea is an annual legume crop, belonging to family Fabaceae in the tribe *cicerae.* It is a diploid, self-pollinated having deep, hard roots being grown on unlevelled drylands and can reach to their maturity in such conditions which would be unsuitable for most of the other crops (Singh and Reddy, 1991) [15]. Its efficiency to withstand the drought conditions is due to its intensified deep tap-root system. Basically, chickpea is a *rabi* crop being sown in months September – November and ready for harvest around February – April. The productivity of gram is greatly reduced by very cold conditions whereas, cool-climate accompanied by low rainfall favors to increase the production. Chickpea crop grown in sandy, loam soils having perfect drainage systems increases the yields but it is highly sensitive to excess water availabilities.

Chickpea is used vitally by vegetarians due to its high protein content and also easily available to poor's diet being cheaper than other sources. Besides humans, it is also highly used as alternate source of protein instead of animal protein. The people with insulin sensitivity or diabetes use gram as a healthy source of carbohydrate and high fibrous content. Chickpea also has good amounts of available zinc, folate having low polyunsaturated fat. New findings have been reported that chickpea helps in lowering the cholesterol in the bloodstream (Pittaway *et al.* 2008) [14].

The losses in chickpea production is attributed to 67 fungi, 22 viruses, 3 bacteria, and 80 nematodes cause drastic looses (Nene *et al.* 1996) [13] where few are reported to encourage economic losses (Haware, 1990) [8]. In India, it has also reported that number of pathogens causing diseases in chickpea had increased from 35 in 1978 to 89 in 1995 (Nene *et al.* 1996) [13]. In tropical as well as temperate regions, soil-borne wilt disease caused by fungi, *Fusarium oxysporum* f. sp *ciceri* is one of the most economically important destructive diseases.

Fusarium wilt caused by *Fusarium oxysporum*. f. sp. *ciceri* (Padwick), is the most devastating soil-borne disease of chickpea particularly in the Indian Subcontinent, the Mediterranean Basin and California (Nene *et al.*, 1987). The yields losses in early wilt are higher ranging from 77-94% than late wilt which ranges from 24-65%.

The seeds in late wilted plants are lighter and dull in comparison to healthy one. Even the infected seed plants wilt faster than healthy seeds (Haware and Nene, 1980) [7].

As the disease is soil borne which urges towards fungicidal drenching which is an expensive practice and poisons the soil micro-flora. Therefore, use of organic practices should be employed to reduce the harmful effects on soil as well as human health. Also, pathogenic isolates have different effects of a similar isolate of bio agents. In the era of using fungal bio-agents, *Trichoderma* sp. is one of the immensely used bio-fungicide (Mukherjee *et al.*, 2013) [10]. There are more than 250 commercially used bio-formulations in India on wide range of crops for sustainable agriculture (Mukherjee *et al.*, 2013, Singh *et al.*, 2012) [10, 17]. Mostly *Trichoderma sp*. shows phenomena of mycoparasitism, antibiosis, competes at rhizosphere level by producing cell wall degrading enzymes. *Trichoderma sp.* is found in all climates over different geographically regions.

Hence, a study was executed to evaluate the inhibition per cent of locally isolated *Trichoderma viride* and *Trichoderma harzianum* on 35 isolates of *Fusarium oxysporum* f. sp. *ciceri.*

Materials & Methods

The wilted chickpea plants were collected from various locations after diagnosis of the external symptoms were uprooted, were split opened vertically from collar region to upwards. Browning of the xylem tissues thereby confirmed the presence of *Fusarium oxysporum* f. sp. *ciceri* (Nene *et al.* 1979) [11]. *Fusarium oxysporum* f. sp. *ciceri* was isolated from the root portion, exactly where the discolouration of vascular tissues were visible were selected for isolation (Nene *et al.* 1979) [11]. The roots were than washed thoroughly with running tap water to remove the soil particles.

The diseased portion of roots was cut into small pieces along with some healthy tissues. The cut pieces were then subjected to surface sterilization by dipping them in 0.1% Mercuric chloride solution. Further, the pieces were rinsed 2-3 times to remove the left traces of mercuric chloride. The sterilized pieces were transferred aseptically to the petri-plates containing completely cooled, solidified potato dextrose agar. The petri-plates were than incubated at 28±2ºC. Later, after 48 hours, white or dull white cottony growth was observed and then purified by single spore isolation method. The pathogen was then transferred, maintained in potato dextrose agar slants and kept for storage in refrigerator (Dhingra and Sinclair, 1993) [5]. Identification was made on the basis of descriptions given in the literatures on the genus *Fusarium* (Booth, 1971) [2].

Further, isolation of *T. viride* and *T. harzianum* was made from the soil sample collected from the experimental plot to procure the local isolate. One gram of homogeneously mixed soil sample was weighed, mixed in test tube having 10 ml of sterilized distilled water and shaken well (1:10). Further, 1ml of this sample was transferred to 9 ml dilution blank (1:1000). Later, the serial dilution was made to subsequent blanks till they reach the dilution up to 10^{-7}. From the last 10^{-7} suspension, one ml with help of sterilized pipette was transferred to petri-plates containing 20 ml of *Trichodema* selective medium, keeping three replications. The petri-plates were then incubated at 25±2ºC. The plates were monitored regularly for the development of colonies. After three days of incubation, colonies were picked from the periphery of the plates and transferred aseptically to another PDA plate. The bio-control fungus was identified and stored in refrigerator for further studies (Johnson *et al.* 1959) [9].

The variation of *Fusarium oxysporum* f. sp. *ciceri* against *Trichoderma* sp. was studied with the help of dual culture technique and inhibition percent was recorded. The dual culture technique was carried out by having antagonist and pathogen on PDA medium. Potato dextrose agar was poured in lukewarm condition and then allowed to solidify. Simultaneously, 5mm diameter disc was cut in both pathogen (*Fusarium oxysporum* f. sp. *ciceri*) and antagonist (*Trichoderma* sp.) from their actively growing cultures. A disc of pathogen and antagonist were placed in the PDA poured plates on the opposite ends of petri-plate, replicated 3 times. Also, a control with three replicates was made by placing each isolate's mycelial disc in the centre. The petri-plates were incubated at 28 ± 2ºC for 4 days. Each isolate against antagonist was placed in a similar manner and observations were recorded for the radial growth (mm.) of the pathogen after 96 hours interval, post-inoculation. From the observations, percent inhibition was computed with help of following formula (Vincent, 1947) [18]:

$$\text{Percent Inhibition (\%)} = \frac{A_1 - A_2}{A_1}$$

Where, A_1 denotes for 'Radial growth (mm.) by the test fungus in control'

A_2 denotes for 'Radial growth (mm.) by the test fungus in dual culture'

Results

Inhibition by *Trichoderma viride* on different isolates of *Fusarium oxysporum* f. sp. *ciceri*

The overall Inhibition percent variations of *Fusarium oxysporum* f. sp. *ciceri* due to *Trichoderma viride* ranged from 3.13 to 44.12 % at 96 hrs. and the data was found to be significant. The inhibition percent due to *T. viride* on *Fusarium oxysporum* f. sp. *ciceri* at 96 hours with maximum inhibition percent ranged between 31.25 to 44.12 %. Maximum inhibition of the range was recorded in FOC-34 (44.12) followed by FOC-32 (42.32), FOC-25 (37.89), FOC-7 (37.11), FOC-5 (35.51), FOC-18 (33.23), FOC-22 (32.11), FOC-9 (32.72) and FOC-17 (31.25). Similarly, moderate inhibition ranged from 13.44 to 29.76 % which was recorded in FOC-28 (29.76) followed by FOC-10 (29.45), FOC-11 (25.79), FOC-29 (25.78), FOC-2 (25.13), FOC-19 (24.52), FOC-35 (22.51), FOC-13 (21.70), FOC-14 (20.77), FOC-8 (18.82), FOC-15 (18.26), FOC-30 (18.24), FOC-31 (17.06), FOC-20 (15.38), FOC-26 (15.20) and FOC-4 (13.44).It was also observed that some of the isolates of *Fusarium oxysporum* f. sp. *ciceri* had poorest effect of *T. viride* after 96 hours of inoculation which ranged between 3.13 to 9.64 %. Among these poorest inhibited isolates was FOC-33 (3.13) followed by FOC-12 (4.17), FOC-3 (4.45), FOC-16 (5.22), FOC-1 (5.26), FOC-24 (6.67), FOC-27 (9.42), FOC-21 (9.45), FOC-6 (9.62) and FOC-23 (9.64)**.** Hence, from the data obtained it shows that best inhibition of *T. viride* on *Fusarium oxysporum* f. sp. *ciceri* was recorded in FOC-34 (44.12) followed by FOC-32 (42.32). Among moderately inhibited isolates were FOC-28 (29.76) and FOC-10 (29.45) whereas least inhibition was recorded for FOC-33 (3.13) and FOC-12 (4.17).

Inhibition by *Trichoderma harzianum* on different isolates of *Fusarium oxysporum* f. sp. *ciceri*

The overall Inhibition percent of *Fusarium oxysporum* f. sp.

ciceri due to *Trichoderma harzianum* ranged from 3.39 to 47.26 % at 96 hours and the data was found to be significant. The inhibition percent due to *T. harzianum* on *Fusarium oxysporum* f. sp. *ciceri* at 96 hours with maximum inhibition percent ranged between 32.34 to 47.26 %. Maximum inhibition of the range was recorded in FOC-13 (47.26) followed by FOC-17 (38.81), FOC-22 (33.73), FOC-34 (32.40) and FOC-7 (32.34). Similarly, moderate inhibition ranged from 11.20 to 29.66 %. Among moderate inhibition response against isolates, inhibition percent of the range as FOC-2 (29.66) and FOC-19 (29.60) followed by FOC-15 (28.99), FOC-30 (25.79), FOC-14 (25.68), FOC-9 (23.33), FOC-5 (20.34), FOC-35 (20.15), FOC-18 (19.47), FOC-31 (19.28), FOC-20 (19.18), FOC-10 (17.86), FOC-28 (17.68), FOC-4 (17.44), FOC-26 (16.81), FOC-29 (14.25), FOC-8 (13.77), FOC-11 (13.43), FOC-25 (12.56) and FOC-32 (11.20). The poorest effect of *T. harzianum* after 96 hours ranged from 3.90 to 10.26 %. Among these isolates, poorest inhibition was recorded in FOC-27 (3.90) followed by FOC-24 (6.81), FOC-33 (8.36), FOC-16 (9.02), FOC-6 (9.29), FOC-23 (9.62), FOC-21 (9.66), FOC-1 (9.77), FOC-12 (9.88) and FOC-3 (10.26). Hence, from the data obtained it shows that best inhibition of *T. harzianum* on *Fusarium oxysporum* f. sp. *ciceri* was recorded in FOC-13 (47.26) followed by FOC-17 (38.81). Among moderately inhibited isolates, maximum inhibition was recorded for FOC-2 (29.66) and FOC-19 (29.60) whereas least inhibition was recorded for FOC-27 (3.90) and FOC-24 (6.81).

Table 1: Variations in inhibition percent of *Trichoderma viride* on mycelial growth (mm.) of *Fusarium oxysporum* f. sp. *ciceri* isolates

S. No.	Isolate	Mean Inhibition Percent	Reaction
Isolates highly inhibited by *Trichoderma viride*			
1.	FOC 34	44.12	R
2.	FOC 32	42.32	R
3.	FOC 25	37.89	R
4.	FOC 7	37.11	R
5.	FOC 5	35.51	R
6.	FOC 18	33.23	R
7.	FOC 9	32.72	R
8.	FOC 22	32.11	R
9.	FOC 17	31.25	R
Isolates moderately inhibited by *Trichoderma viride*			
10.	FOC 28	29.76	MR
11.	FOC 10	29.45	MR
12.	FOC 11	27.59	MR
13.	FOC 29	25.78	MR
14.	FOC 2	25.13	MR
15.	FOC 19	24.52	MR
16.	FOC 35	22.51	MR
17.	FOC 13	21.7	MR
18.	FOC 14	20.77	MR
19.	FOC 8	18.82	MR
20.	FOC 15	18.26	MR
21.	FOC 30	18.24	MR
22.	FOC 31	17.06	MR
23.	FOC 20	15.38	MR
24.	FOC 26	15.2	MR
25.	FOC 4	13.44	MR
Isolates weakly inhibited by *Trichoderma viride*			
26.	FOC 23	9.64	NR
27.	FOC 6	9.62	NR
28.	FOC 21	9.45	NR
29.	FOC 27	9.42	NR
30.	FOC 24	6.67	NR
31.	FOC 1	5.26	NR
32.	FOC 16	5.22	NR
33.	FOC 3	4.45	NR
34.	FOC 12	4.17	NR
35.	FOC 33	3.13	NR

Table 2: Variation in inhibition percent of *Trichoderma harzianum* on mycelial growth (mm.) of *Fusarium oxysporum* f. sp. *ciceri* isolates

S. No.	Isolate	Mean Inhibition Percent	Reaction
Isolates highly inhibited by *Trichoderma harzianum*			
1.	FOC 13	47.26	R
2.	FOC 17	38.81	R
3.	FOC 22	33.73	R
4.	FOC 34	32.4	R
5.	FOC 7	32.34	R
Isolates moderately inhibited by *Trichoderma harzianum*			
6.	FOC 2	29.66	MR
7.	FOC 19	29.6	MR
8.	FOC 15	28.99	MR
9.	FOC 30	25.79	MR
10.	FOC 14	25.68	MR
11.	FOC 9	23.33	MR
12.	FOC 5	20.34	MR
13.	FOC 35	20.15	MR
14.	FOC 18	19.47	MR
15.	FOC 31	19.28	MR
16.	FOC 20	19.18	MR
17.	FOC 10	17.86	MR
18.	FOC 28	17.68	MR
19.	FOC 4	17.44	MR
20.	FOC 26	16.81	MR
21.	FOC 29	14.25	MR
22.	FOC 8	13.77	MR
23.	FOC 11	13.43	MR
24.	FOC 25	12.56	MR
25.	FOC 32	11.2	MR
Isolate weakly inhibited by *Trichoderma harzianum*			
26.	FOC 3	10.26	NR
27.	FOC 12	9.88	NR
28.	FOC 1	9.77	NR
29.	FOC 21	9.66	NR
30.	FOC 23	9.62	NR
31.	FOC 6	9.29	NR
32.	FOC 16	9.02	NR
33.	FOC 33	8.36	NR
34.	FOC 24	6.81	NR
35.	FOC 27	3.9	NR

Discussion

From the recordings, it is clear that *Trichoderma* sp. had huge variations showing the inhibitory effects against various isolates of *F. oxysporum* f. sp. *ciceri.* Similar findings are also reported by Carter *et al.* (2002) [3] where they screened 188isolates of *Fusarium graminearum* and reported that the isolates had diverse variations in response to the inhibitions by *Trichoderma* sp. Also, Choudhary and Mohanka assessed the indigenous potential of bio-agents and their antagonistic potential against *Fusarium oxysporum* f.sp. *lentis* where they found that isolates of different antagonists had high, moderate and slow inhibition effect. They concluded that isolates had variations in inhibition. Even, Singh *et al.* 2018 [16] attempted to corroborate the relatedness of antagonistic ability to determine the potency of native *Trichoderma* isolates against *Fusarium oxysporum f.sp. ciceri* under *in vitro* condition. All native rhizospheric isolates of *Trichoderma* were found significant in reducing mycelial growth of *Fusarium oxysporum f.sp. ciceri.* The significance of the antagonistic potential of twenty *Trichoderma* isolates was scored on scale (1-5) for degree of antagonism against *Fusarium oxysporum f.sp. ciceri.* The result revealed that there was high level of fluctuation in the inhibition pattern.

Conclusion

From the above study, it can be concluded that the bio-agents have best inhibition effect on the pathogens from a similar type of ecological conditions, else the inhibition effect may vary and the complete management of the disease cannot be achieved.

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