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Antagonistic Variability among the Isolates of *Trichoderma* against *Fusarium oxysporum* f.sp. *ciceri*

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A B S T R A C T

Trichoderma has attained importance as a substitute of chemical pesticides all over the world. Hence, an attempt was intended to corroborate the positive relatedness of antagonistic ability. Among different isolates of *Trichoderma* isolated from rhizospheric soils has brought attention due to its highly antagonistic activity. The study aimed to determine the potency of native *Trichoderma* isolates against *Fusarium oxysporum f.sp. ciceri* under *in vitro* condition. Maximum per cent inhibition was recorded in isolate T_3, followed by T_{15}, T_7 and T_5 in dual culture. All native rhizospheric isolates of *Trichoderma* were found significant in reducing mycelial growth of *Fusarium oxysporum f.sp. ciceri.* The significane of 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 the highest antagonism was found in isolate T_3 (*T. harzianum*) against chickpea wilt.

Introduction

Chickpea (*Cicer arietimum* L.) is one of the most important pulse crop grown in semi and tropical climate. In India, chickpea is ranked first in terms of production and consumption in the world. About 65% of global area with 68% of global production of chickpea is contributed by India (Amarender and Devraj, 2010). It covers an area of 82.18 Lakh ha with a production of 77.02 Lakh tons and productivity of 937 kg per ha. It occupies an area of 28.84 Lakh ha with the production of 30.12 Lakh tons, productivity being 1044 kg per ha in Madhya Pradesh (DPD, 2016-17). Low yield of chickpea is attributed to its susceptibility to several fungal, bacterial and viral diseases. *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo & K. Sato, is the most important soil borne disease of chickpea throughout the world and particularly in the Indian Subcontinent, the Mediterranean Basin and California (Nene *et al.*, 1987).

At the national level, chickpea yield losses encounter due to wilt may vary between five to ten percent (Dubey *et al.*, 2007). Since the pathogen is both seed and soil borne, drenching with fungicides is very expensive

and impractical. Therefore, integrated disease management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen pollution, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre *et al.,* 1998).

Among fungi, the most widely used biofungicides are *Trichoderma sp.* (Mukherjee *et al.,* 2013). India alone is having more than 250 commercial formulations which are being used against many crops for sustainable agriculture (Mukherjee *et al.,* 2013, Singh *et al.,* 2012). Major mechanisms which are responsible for biocontrol potential of *Trichoderma sp*. are mycoparasitism, antibiosis, competition through rhizosphere competence and production of cell wall degrading enzymes. *Trichoderma sp.* is found in all climates over different geographically regions. Even though most *Trichoderma sp*. found on wild mushrooms and trees; soil or rhizospheric soil has been viewed as its main habitat (Mukherjee *et al.,* 2013, Druzhinina *et al.,* 2011). The study was conducted to find out the most effective isolates of *Trichoderma* against chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri.*

Materials and Methods

Collection, Isolation, Purification and identification of Pathogen and *Trichoderma*

F. oxysporum f. sp. c*iceri* was isolated from the infected roots of chickpea plants collected at *Fusarium* infested chickpea field in the Department of Plant Pathology, JNKVV, Jabalpur, M.P. India. Samples were brought in the laboratory of Plant Pathology for examination and isolation. For isolation of *Trichoderma,* soil samples were collected from rhizosphere of different plants (Table 1).

The infected root portion of chickpea variety JG-62 was used for isolation of *F. oxysporum. f.sp. ciceri*. The isolation was made by following standard tissue isolation procedure. The infected specimens were cut into small bits with help of scalpel and washed in running water. These bits were surface sterilized with 1 per cent of sodium hypochlorite solution for one minute then aseptically transferred to Petri plates containing the sterilized PDA medium and incubated at 25±2ºC.

For isolation of *Trichoderma* soil sample of 10 gm weight was weighed and placed in a beaker containing 45ml of sterile distilled water. After shaking thoroughly, it was allowed to stand for a few minutes. From the suspension, 1.0ml was taken out by using glass pipette and added to 9.0ml distilled water in a test tube and shaken well, this gives 10^{-4} dilution. One ml from 10^{-4} dilution is drawn and spread over the PDA plate. These plates were incubated in B.O.D incubator at 25±2 ºC. The plates were monitored regularly for the development of colonies. After three days of incubation, colonies were picked from periphery of the plates and transferred aseptically to another PDA plate.

The test pathogen and *Trichoderma* were purified by hyphal tip method. To obtain the sparse growth, the test pathogen and antagonist were inoculated on sterilized water agar in Petriplates from the original culture Petriplates. After two days the growth of the fungus was carefully examined under low power (10X) of microscope from the reverse side of Petriplates.

A single hyphal strand was located and its location was marked with a marker on bottom of Petriplates. Agar disc corresponding to the marked area was cut with a sterilized cork borer and transferred aseptically on to PDA in Petriplates and incubated at 25±2°C.

The cultures were identified on the basis of the descriptions given in the monograph on the genus *Fusarium* (Booth, 1971). *F. oxysporum. f.sp. ciceri* showed circular, raised, cottony creamy white colony with entire margins. The microconidia was oval to cylindrical, straight or curved and measure 2.5-3.5×5-11mm.

The green colour colonies of *Trichoderma* were identified on the basis of branching of conidiophores, shape of phialides, emergence of phialides and spore characters (Gams and Bisset, 1998).

Antagonistic Variability

The antagonistic variability in twenty isolates of *Trichoderma* was studied by Dual culture method (Morton and Stroube, 1955).

A mycelial disc (2 mm) was cut aseptically from the margins of actively grown region of five day old cultures of *Trichoderma isolates* and inoculated at one end of petriplate (1cm away from edge of petriplate) with sterilized PDA medium and simultaneously, 2 mm disc of test pathogen at opposite end.

For each treatment three replicates were maintained and were incubated at 25±2°C. Plate was kept without antagonist to serve as control. Observations were recorded for linear growth of *F. oxysporum. f.sp. ciceri* and per cent inhibition by antagonist.

Per cent inhibition of the pathogen over control was measured by using following formula.

$$I (\%) = (C-T)/ C \times 100$$

Where,

I = Percent growth inhibition
C= Growth in control.
T=Growth in Dual culture.

Results and Discussion

Data presented in the Table 2 (Plate 1) clearly indicated that, the linear growth of pathogen in presence of antagonist varied from 10.26 mm to 25.33 mm as compared to control (75.50 mm). The minimum linear growth of *F. oxysporum f.sp. ciceri* was recorded with T_3 (10.26 mm) isolate, followed by T_{15} (11.63 mm) and T_7 (12.5 mm). Whereas, maximum linear growth of *F. oxysporum f.sp.ciceri* of 25.33 mm recorded with T_{11} isolate, followed by 24.00mm and 23.00 mm in T_{10} and T_4 isolates respectively. Mycelial growth of *Fusarium. oxysporum f.sp cicer* was significantly inhibited by all the isolates of *Trichoderma*. The per cent inhibition ranged from 66.43% to 86.4%. Maximum inhibition per cent (86.4%) was recorded with isolates T_3. The minimum inhibition per cent was recorded in T_{11} (66.43%) which was at par with T_4 (69.53%), T_{10} (68.21%) and T_{11} (66.43%). Further, all twenty *Trichoderma* isolates were grouped into five classes most efficient, efficient, moderately efficient, poor and very poor based on the overgrowth of *Trichoderma* on the pathogen, as described by Bell *et al.*, (1982) (Table 3). Out of twenty *Trichoderma* isolate T_3 was found highly effective and exhibited maximum inhibition per cent against *Fusarium* oxysporum *f.sp. ciceri*. 14 isolates fell into antagonism class '2', four *Trichoderma* isolates into class '3' and only 1 isolate into class '4'.

The potential of *Trichoderma sp.* had been recognized as biocontrol agent against soil borne pathogen by Weindling (1932), Upadhyay and Mukhoupadhaya (1986), Jha and Jalali (2006) and Singh *et al.*, (2007). Variations in antagonism among different isolates of *Trichoderma* were found significant against *F. oxysporum. f.sp. ciceri*. Maximum percent inhibition was found in T3 isolate followed by T15, T5, T7 and T8 as compared to other isolate (Table 2).

Table.1 List of *Trichoderma* isolates collected from different locations of Jabalpur

S. No.	Isolate code	Crop	Location
1	T_1	Red gram	Forestry field
2	T_2	Green gram	Talab area
3	T_3	Soybean	Dusty area
4	T_4	Soybean	IFS, college campus
5	T_5	Maize	Adhartal
6	T_6	Soybean	Farmer's field
7	T_7	Rice	Farmer's field
8	T_8	Chilli	Maharajpur
9	T_9	Mango	Iemalia
10	T_{10}	Rice	Soil science field
11	T_{11}	Rice	BSP, field
12	T_{12}	Neem	Maharajpur
13	T_{13}	Banana	Iemalia
14	T_{14}	Rice	IFS
15	T_{15}	Betel vine	College campus
16	T_{16}	Soybean	Sehora
17	T_{17}	Okra	BSP,field
18	T_{18}	Soybean	BSP,field
19	T_{19}	Okra	Horticulture field
20	T_{20}	Citrus	Iemalia

Table.2 Average linear growth and percent inhibition in growth of *Fusarium* oxysporum *f.sp ciceri* by isolates of *Trichoderma* after five days of incubation period

Isolate Code	*Linear growth (mm) of *F. oxysporum f.sp. ciceri*	*Per cent inhibition
T_1	15.00	80.13
T_2	18.66	75.27
T_3	10.26	86.40
T_4	23.00	69.53
T_5	12.33	83.66
T_6	15.00	80.13
T_7	12.5	83.44
T_8	13.10	82.64
T_9	19.00	74.83
T_{10}	24.00	68.21
T_{11}	25.33	66.43
T_{12}	15.76	79.11
T_{13}	20.00	73.53
T_{14}	13.33	82.33
T_{15}	11.63	84.59
T_{16}	16.66	77.92
T_{17}	17.50	76.82
T_{18}	14.66	80.57
T_{19}	13.66	81.90
T_{20}	13.16	82.55
Control	75.50	0
SE(m)±	0.81	1.06
CD	2.32	3.04

*Average of three replications

Table.3 Classification of *Trichoderma* isolates antagonistic against *Fusarium* oxysporum *f.sp ciceri* based on Bell *et al.*, (1982)

Antagonism class*	Isolates	No. of isolates
1	T_3	1
2	T_1, T_2, T_4, T_5, T_6, T_7, T_8, T_{10}, T_{14}, T_{15}, T_{16}, T_{18}, T_{19}, T_{20}	14
3	T_9,T_{11}, T_{13}, T_{17}	4
4	T_{12}	1
5	Nil	Nil

Antagonism class*	Isolates	Remark
1	*Trichoderma* overlapped the colony of fusarium and whole surface of media	Most efficient
2	*Trichoderma* grew and it covered 2/3 of the surface of the media	Efficient
3	*Trichoderma* and *Fusarium* colonized each one half of the surface of the media	Moderately efficient
4	*Fusarium* grew and it covered 2/3 of the surface of the media	Poor
5	*Fusarium* grew and covered entire surface of media	Very poor

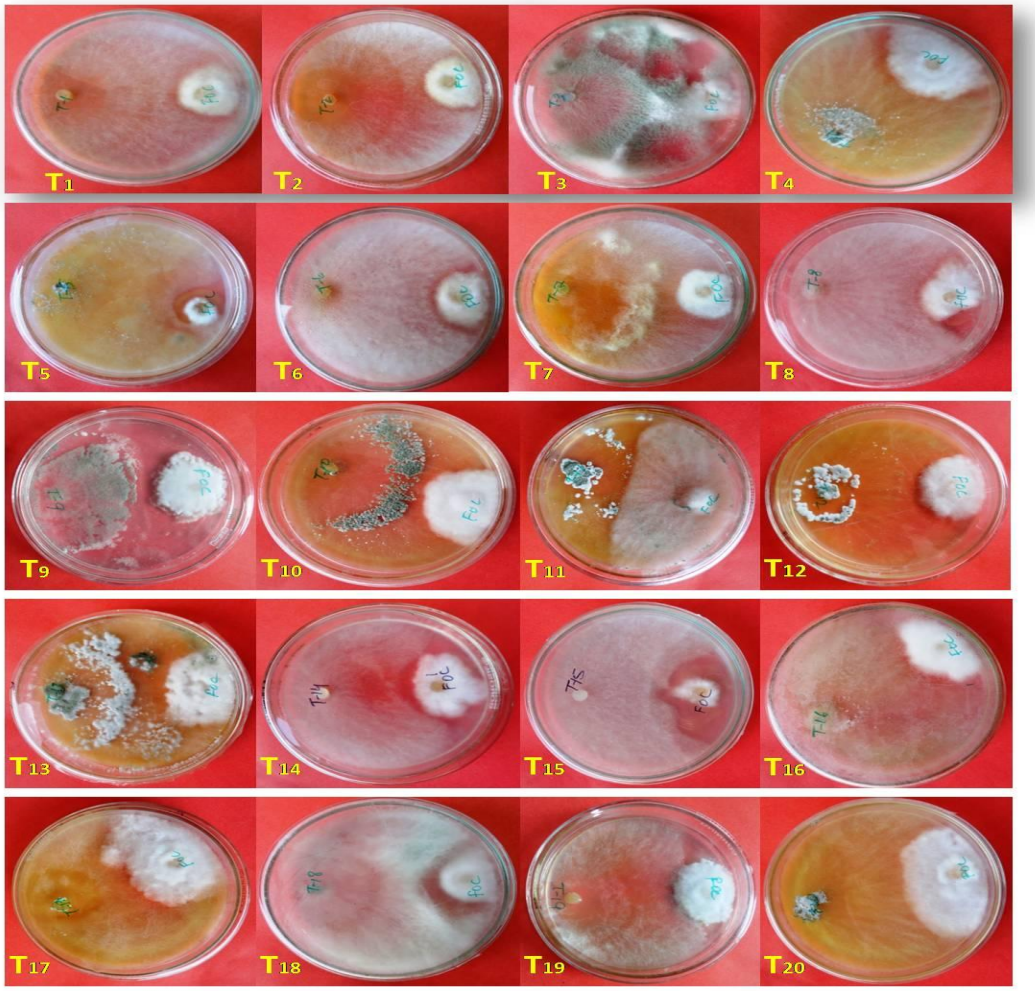

Plate 1: Inhibition of *Fusarium oxysporum f.sp. ciceri* by isolate of *Trichoderma* (T-1 to T-20) *in vitro* by dual culture method

Out of twenty isolates T3 exhibited highest per cent inhibition (86.40%). As all the isolates of *Trichoderma* were found medium to fast growing, it may be due to the maximum reduction in linear growth of *F. oxysporum f.sp. ciceri* attributed due to faster growth of interacting isolates of *Trichoderma* rather than its direct influence. Similar observations had also been reported by Patibanda and Sen (2004) while studying *Aspergillus niger* vs *F. oxysporum. f.sp. melonis*. The variability among different isolates of *Trichoderma* isolates might be due to variation in mycelium coiling rate, sporulation and fungitoxic metabolites (Barkat *et al.,* 2006 and Jegathambigas, 2010). The degree of antagonism (Table 3) between each *Trichoderma* isolate and pathogen in dual culture was scored on scale 1-5 as proposed by Bell *et al.,* (1982). In present study highest antagonism was observed in isolate T3 (*T. harzianum*). However, differences between isolates T5, T7 and T15 with 83.66, 83.44% and 84.59% respectively with T3 (86.4%) were found statistically at par against *F. oxysporum. f.sp. ciceri*. Singh *et al.,* (2013) have also reported the similar findings antagonistic variability among the isolates using dual culture and reported significant reduction in radial growth *F. oxysporum. f.sp. ciceri*.

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