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Antagonistic Activities of Various *Trichoderma* spp. against the isolates of *Fusarium oxysporum* f.sp. *ciceri*

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops grown in the Indian subcontinent and known to cope with the protein demand of the major vegetarian population of our country. Besides its significance, the crop faces prodigious ignorance due to a range of biotic and abiotic stresses. However, Fusarium wilt caused by *F. oxysporum* f. sp. c*iceri* is one of the significant catastrophes to chickpea cultivation in every Indian state. The present study explored the potential of some indigenous *Trichoderma* isolates against two isolates of *Fusarium oxysporum* f.sp. *ciceri* viz., *FOC*UP1 (Uttar Pradesh) and *FOC*RJ1 (Rajasthan). Interestingly, all the tested *Trichoderma* isolates significantly inhibited the radial growth of both *Fusarium* isolates. However, more inhibition of indigenous *Fusarium* isolates (*FOC*UP1) (ranged between 71.85 to 80.37%) was recorded against all the tested *Trichoderma* isolates when compared to nonindigenous isolate, i.e., *FOC*RJ1 (ranged between 55.19 to 67.41%). Among all the tested isolates of *Trichoderma* spp., the highest inhibition in *FOC*UP1 was exhibited by *T. viride* (80.00%), followed by *T. hamatum* (77.78 %). However, in the case of *FOC*RJ1, *T. hamatum* (67.41%) was superior to other tested *Trichoderma* isolates, followed by *T. viride* (62.96%). This *in-vitro* study gave a clue to further exploitation of indigenous bioagents to mitigate losses incurred by this pathogen at a large scale.

Key Words: Fusarium wilt, *Trichoderma* spp., *Fusarium oxysporum* f.sp. *ciceri, in-vitro*

INTRODUCTION

Chickpea or Bengal gram is one of the most important annual grain legumes, cultivated mainly for its protein-rich dried grains (Didinger and Thompson, 2021). These grains are comparable cheap protein sources to animal protein sources, predominantly consumed as dal or snacks (Iriti and Varoni, 2017; Venkidasamy *et al,* 2019). However, the green leaves and seeds are also utilised as vegetables or consumed raw (Amulya *et al,* 2020). India is the largest producer of chickpeas around the globe and accounts for nearly 90 per cent of total global production. Not only production but the Indian subcontinent also covers the highest area under cultivation (10.74mha) of this crop *i.e.*, 72.51 per cent of total global coverage (Sharma and Singh, 2024). However, the productivity of this crucial pulse crop is still marginalised *i.e.,* 1261 kg/ha, which is quite lower than Russia, Australia, Myanmar, and Turkey. A range of abiotic and biotic factors has been reported to hamper the stable production of chickpea all around the globe. The losses caused by individual insect pests and diseases in temperate and tropical regions range between 5-10 per cent and 50-100 per cent, respectively (Dubey *et al,* 2006). However, among various biotic stresses affecting global production of chickpea, Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Synd. and Hans. (*Foc*) is one of the most important threat (Jimenez-Diaz *et al,* 2015; Bhar *et al,* 2021).

This soil-borne and internally seed-borne (Farhana *et al,* 2022) fungus is well known for its global occurrence (at least 33 countries) and significant loss of up to 10-40 per cent worldwide (Sharma *et al,* 2014), however, in India it is reported to lead an annual loss of 10-15 per cent, which may also result in total crop failure under favourable conditions (Sharma *et al,* 2014). The

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symptoms of this disease may be classified as early wilt and late wilt according to the stage of plant infection. The loss incurred in late wilt is comparatively less than to that of in early wilt *i.e.,* 24-65 per cent and 77-94 per cent, respectively (Jiménez-Díaz *et al,* 2015). The disease may affect any stage of the crop and results in development of characteristic symptoms of yellowing and sudden drooping of leaves and petioles. Although, there is no external rotting symptom on root or stem but internal discolouration of xylem and pith can be clearly seen when the root or stem is split opened.

The cultural management practices fail to manage this notorious pathogen due to its prolonged saprophytic survival ability (up to 7 years, due to chlamydospores), use of host-resistance is quite recommended but fails due to limited availability of resistant cultivars. Ultimately, chemical management is the only left option, but this nether economical, nor environment friendly (causes soil and water pollution). At the same time, the rising global concern against environment and human health also leads to adaptation of some alternatives to chemical pesticides. Hence, the alteration of soil micro-flora is one of the most promising options. *Trichoderma* spp. in recent past attracted the attention of researchers due to its multiprong action against several phytopathogens and growth promoting activities to plants (Thaware *et al,* 2017; Rathore *et al,* 2020).

MATERIALS AND METHODS

Isolation, identification and maintenance of *F. oxysporum* f.sp. *ciceri*

The test pathogen was isolated from infected chickpea plants showing characteristics symptoms, i.e., yellowing, drooping and internal discolouration of xylem. The infected chickpea plants were collected from two locations, i.e., Jhalawar (Rajasthan) and Aligarh (Uttar Pradesh). The pathogen was isolated by chopping the infected materials in small pices of 1cm. these small pieces were surface sterilised in NaOCl for 1 minute, followed by two washing in distilled water, and then blot dried for removal of excess moisture. These pieces were then placed in Petri plates containing solidified PDA and incubated in BOD at 25±2°C. the plates were observed regularly for mycelial growth (if any) and purified by single spore culture technique; however, the culture was maintained on PDA slants at 4°C (Belabid and Fortas, 2002). The pathogen was identified on the basis of cultural and morphological characteristics such as, colony growth, pigmentations, more specifically size and shape of micro and macro conidia, septation observed under compound microscope. The Species of Trichoderma were isolated on Trichoderma selective medium (TSM) from the soil collected from the chickpea rhizosphere (all from Aligarh locality) (Elad *et al,* 1981).

Bio-efficacy of *Trichoderma* spp. on radial growth of *F. oxysporum* f.sp. *ciceri* (*Foc*):

Total five isolates of *Trichoderma*, namely, *T. harzianum (isolate1), T. harzianum isolate2, T. viride, T. longibrachiatum,* and *T. hamatum* were evaluated using Dual culture technique, against two isolates of *Foc*, i.e., *FOC*RJ1 and *FOC*UP2, isolated from, Jhalawar (Rajasthan), and Aligarh (Uttar Pradesh), respectively (Dhingra and Sinclair, 1985). Appropriate controls were maintained for monoculture and inoculated plates were incubated at 25±2°C. Observations on radial growth of interacting test fungi, overgrowth, and zone of inhibition, were recorded and % growth inhibition of test fungi was calculated by the formula suggested by Dubey *et al* (2007).

RESULTS AND DISCUSSION

In-vitro experiment was conducted to ascertain the efficacy of various *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceri.* The observation was recorded in terms of radial growth and its inhibition and are presented in Table 1, Fig. 1 and Plate 1.

Perusal of data (Table 1) revealed that all the species of *Trichoderma* had expressed their potential against the test pathogen and significantly inhibited the radial growth of *Foc,* over control, however, a variation in terms of inhibition was also recorded, with more inhibition in Uttar Pradesh isolate of *Foc* (*FOC*UP1) was recorded in compare to Rajasthan isolate

Table1. Bio-efficacy of various Trichoderma spp. against the isolates of *Fusarium oxysporum* f.sp. *Ciceri.*

Trichoderma strains	Per cent growth inhibition of *Fusarium oxysporum* f.sp. *ciceri* isolates (%)	
	***FOC*RJ1**	***FOC*UP1**
T. harzianum isolate1	55.19	72.96
T. harzianum isolate 2	67.04	77.41
T. longibrachiatum	61.11	71.85
T hamatum	67.41	77.78
T. viride	62.96	80.37
Control	0.00	0.00
LSD (P<0.05)		
Fusarium isolates	0.47	
Trichoderma isolates	0.82	
Fusarium: Trichoderma	1.16	

(*FOC*RJ1). Meanwhile, in case of both isolates of fusarium *i.e., FOC*RJ1, and *FOC*UP1, highest inhibition was reported in case of *T. viride viz.,* 62.96 and 80.37 %, followed by *T. hamatum* (67.41 and 67.04 %) and *T. hamatum* (isolate 2) (77.78 and 77.41 %), which were statistically at par to each other. *T. harzianum* isolate 1 was least effective to inhibit the growth of *Foc* isolate of Rajasthan (*FOC*RJ1), i.e., 55.19 %; meanwhile, in case of *FOC*UP1, *T. longibrachiatum* was least effective with only 71.85 % of growth inhibition.

It was clear from Fig.1 and Plate1, that the all the *Trichoderma* spp. under evaluation have a great inhibitory effect on radial growth of *F. oxysporum* f.sp. *ciceri* causing chickpea wilt. Moreover, there was also a significant variation in response among all these species of *Trichoderma* (isolated from different locations). The *Trichoderma* spp. have more inhibitory effect on the *Foc* isolated from same locations. Moreover, in case of both tested isolates of *Foc, T. viride* was the most effective to inhibit the radial growth, *in-vitro,* followed by *T. harzianum* and *T. hamatum.*

Present study signifies the utilization of various species of *Trichoderma* in inoculum reduction and radial growth inhibition of *F. oxysporum* f.sp. *ciceri,* which were also earlier reported by several other researches (Dubey *et al,* 2007; Kumar *et al,* 2019; Moutassem *et al,* 2020; Younesi *et al,* 2021). Amulya *et al.* (2020) evaluated 24 sympatric isolates of *Trichoderma* from six different chickpea growing mandals of Prakasam district (Andhra Pradesh) and one isolate from Bapatla against their inhibitory potential against Bapatla isolate of *Fusarium oxysporum* f. sp. *ciceri (Foc).* They also recorded a variation among *Trichoderma* isolates tested in terms of their antagonistic potential. Screening of *Trichoderma* isolates was done based on the radial growth of interacting test fungi, overgrowth, zone of inhibition, pigmentation of *Foc* and sporulation of *Trichoderma.* In this experiment they identified, 5 potential isolates of *Trichoderma i.e.,* T 19001, T 19007, T 19012, T 19020, T 19023 which were fast in growth and overgrowing the *Foc*. Whereas, T 19001, T 19007 and T 19012 isolates were found to overgrow and sporulate on *Foc*. Media pigmentation in *Foc* changed from light colour in monocultured *Foc* plates to dark in dual cultured plates in interactions involving T 19020 and T 19023 isolates, however, without sporulation on the test pathogen.

CONCLUSION

Fusarium wilt in chickpea, is a great problem in all the chickpea cultivation areas around the globe. However, managing this disease in farmers filed is still a great challenge. The use of chemical fungicides is neither a permanent solution nor a healthy option for the environment and human health. In this case the soil borne

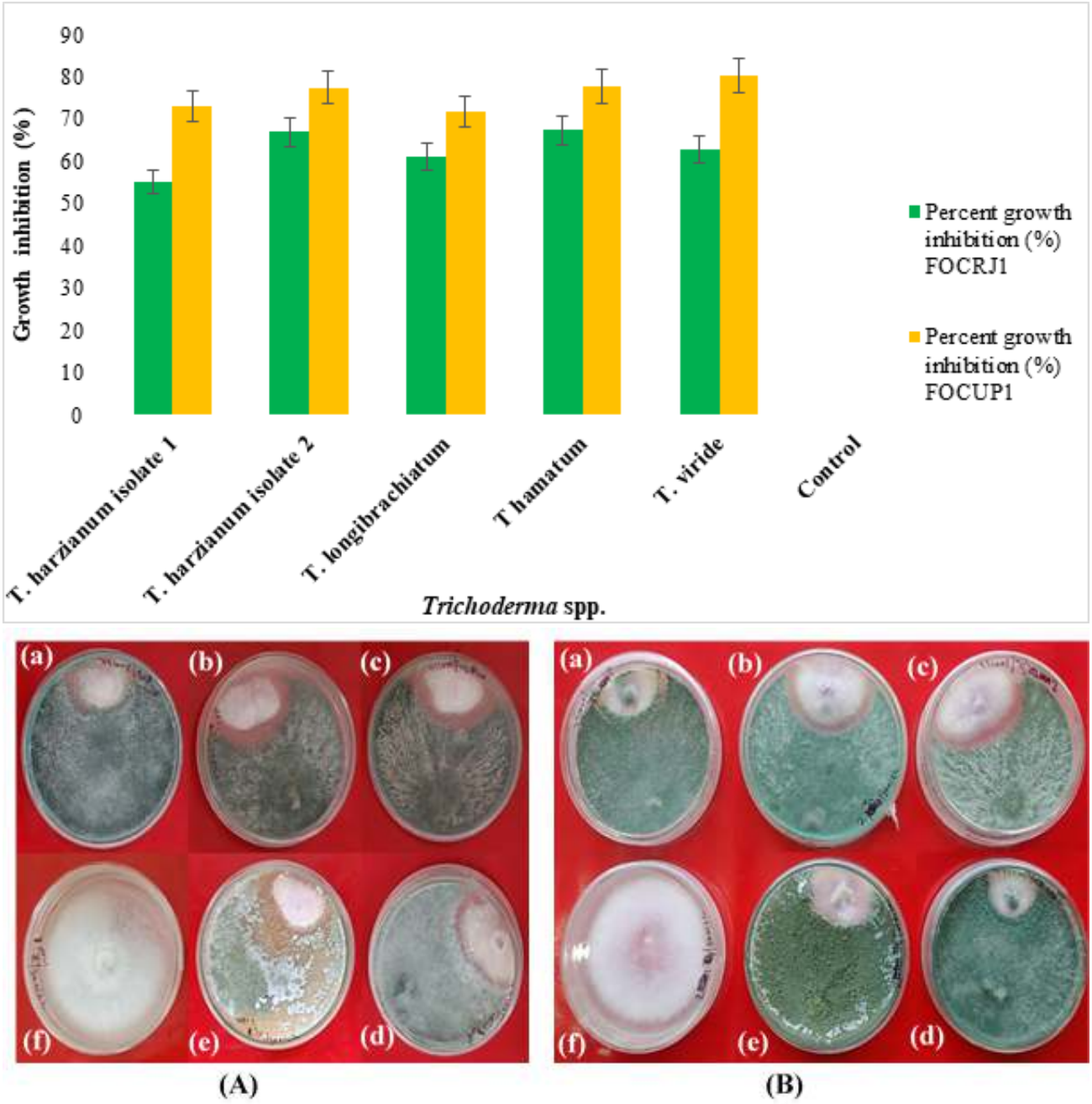

Plate1. Radial growth inhibition of *Foc* isolates; FOCRJ1 (A) and FOCUP1 (B), by various *Trichoderma* spp., i.e., *T. viride* (a), *T. harzianum (isolate1)* (b), *T. harzianum (isolate2)* (c), *T. longibrachiatum* (d), *T. hamatum* (e) and control

antagonistic fungi, *Trichoderma,* which were found to be effective against a range of soil borne phytopathogens provides a great potential to mitigate the losses caused by this pathogen. The present study also signifies the efficacy of the *Trichoderma* spp., against chickpea wilt pathogen, i.e., *F. oxysporum* f.sp. *ciceri.* However, it was also recorded that the isolates of Trichoderma, isolated from the rhizosphere of the same locality, have more inhibitory potential against the *Foc* isolates. Hence care should be taken while selecting the *Trichoderma* isolates.

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