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Original Research Article

Antagonistic Potential of Locally Isolated *Trichoderma spp.* on Different Species of *Fusarium*

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A B S T R A C T

In the present investigation twenty locally isolated microorganisms including nineteen *Trichoderma spp.* and one *Penicillium spp*. were tested for their antagonistic property against three different species of the *Fusarium viz. Fusarium oxysporum* f. sp. *Lycopersici*, *Fusarium oxysporum* f. sp. *Cicero*, *Fusarium fujikuroii.* Antagonistic activities results showed that Tharz-6, Tviri-1, Thama-1, and Tviri-5, Tpoly -2 were suppressed higher mycelia growth of *F. o.* f. sp. *ciceri* at 48 hrs and Tharz-1 and Tpoly-1 were superior inhibitor of mycelia growth of pathogen at 96 hrs, time interval. Likewise, Thama-1 and Tpoly-2 were suppressed higher growth of *F. o.* f. sp. *lycopersici* at 48 hrs and Tpoly-1 and Thama-1 were superior inhibitor of mycelia growth of this pathogen at 96 hrs. It indicates the Tpoly and Thama were effective at every time interval. Though, the antagonistic activity of Tharz-2, Tviri-1, Tvire-2 and Tharz-6 were suppressed higher mycelia growth of *F. fujikuroii* at 48 hrs and Tharz-1, Tpoly-1, Tviri-4, and Tvire-2 were superior inhibitor of mycelia growth of this pathogen at 96 hrs, its indicates the Tharz and Tviri were effective at every time interval.

Keywords

Trichoderma spp., *Fusarium* spp. and Bio-control

Introduction

Soil borne plant pathogens are the major problem among the disease causing agents. *Fusarium spp*. are the one of most important soil borne fungal pathogen of plants it causes economic loss to more than 200 host plants.

The *Fusarium spp*. used in the present investigation are soil borne which caused wilt and rot in various crop (Chakraborty, 2005 and Chakraborty and Chatterjee 2008). Therefore, it is for reach to control by chemical or fungicides as soil and seed application. The alternative approach, for the control these pathogens is the use of bio-agents. Though, the biocontrol agent is necessary to control effectively of such soil borne plant pathogens.

In the present communication, an attempt was made to study the antagonistic activity of these fungal antagonist of *Trichoderma spp* and *Penicillium spp*. against soil borne pathogen *fusarium spp* which caused wilt and rot in various crop.

Scenarios of biological control of soil-borne plant pathogens using most encouraging bio-control agent, the genus *Trichoderma* has

been described (Elad & Kapat, 1999; Morsy *et al.,* 2009; Papavizas, 1985; Sabalpara *et al.,* 2009).

Materials and Methods

Collection, Isolation and identification of antagonistic fungi

Twenty isolates of antagonist in which 19 isolates belonging *Trichoderma spp.* and one isolate belonging to *Penicillium spp*. was isolated from different crop rhizosphere and non rhizosphere soil (Table 1).

Collection of the soil sample

Soil samples were collected from experimental fields at CRC, of S.V.P. University Agriculture & Technology Modipuram, Meerut, IIFSR Modipuram Meerut, Moradabad and farmer's fields in the vicinity of Meerut. These soil samples were collected from non-rhizosphere and rhizosphere of healthy and diseased crop plants, the soil samples were collected in sterilized polyethylene bags and brought in the laboratory for the isolation of the antagonistic fungi.

Isolation of antagonistic fungi from soil samples

The antagonist *Trichoderma* were then isolated using dilution plate techniques (Sangle and Bambawale, 2004) on potato dextrose agar medium. Purified and identified culture of *Trichoderma spp*. was maintained on PDA by sub culturing at two months interval.

Identification

The isolated fungus was identified on the basis of culture and morphological characteristics. The cultural characteristics of the fungus were recorded on solid PDA in Petri plates. Temporary slides were prepared in lactophenol cotton blue and examined under the light microscope or morphological characteristics. The identified *Trichoderma spp*. are listed in the Table.

Collection, isolation of pathogen and maintenance of cultures

Collection of sample

Three pathogenic fungi viz., *Fusarium oxysporum* f.sp. *lycopersici* cause wilt of tomato, *Fusarium oxysporum* f.sp. *ciceri* causes the wilt of chickpea, *Fusarium fujikuroi* causes the foot rot of rice, were isolated from wilted/diseased plants of tomato, chickpea and rice respectively. The specimen was collected in the sterilized polyethylene bags.

Isolation and identification of pathogens

The diseased sample cut into the small pieces, sterilized with $HgCl_2$ solution (1.0%) and washed with sterilized distilled water thrice, and blotted with the blotter paper and inoculates in the poured Petri dishes and incubated on 25$\pm$1ºC for 2-3 days.

The isolated pathogens were purified by single spore or hyphal tip method (Rangaswami 1958). The fungus was used as a test fungus. The cultures were maintained on PDA medium by sub culturing at two month interval.

The isolated fungus was identified on the basis of culture and morphological characteristics. The cultural characteristics of the fungus were recorded on solid PDA in Petri plates. Temporary slides were prepared in lacto phenol/cotton blue and examined under the light microscope or morphological characteristics.

Evaluation of antagonistic fungi against *Fusarium spp.*

Dual culture technique

The isolates of *T. viride, T. harzianum, T. koningii, T. polysporum, T. hamatum T. virens* and *Penicillium spp.* were evaluated against three *Fusarium spp.* in laboratory by dual culture technique as described by Morton and Stroube (1955) to screen out the most efficacious strain.

Petri dishes (90 mm) containing PDA (20 ml in each) were inoculated with 3 mm diameter mycelial disc of 4 days old culture of *Fusarium spp.* and *Trichoderma* spp. placed at equal distance apart 5cm each other and 2cm from the periphery. Inoculated plates were incubated at 25±1ºC in BOD incubator and the radial growth of pathogen was measured 24, 48, 72, and 96 hours after inoculation. *Fusarium spp.* without *Trichoderma spp.* were maintained which sereve as control (check). Three replications were maintained for each treatment. Per cent inhibition of radial growth of pathogen was calculated using the formula given below:

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

I = Per cent growth inhibition;

C = Colony diameter of pathogen in control; and

T = Colony diameter/radial growth of pathogen in treatment (dual culture)

From the zone of interaction between the antagonist and pathogen in dual culture plate, the mycelial mats were gently lifted with a needle and put in a drop of cotton blue on a microscopic slide and spread with a needle and observed under microscope for hyphal interaction of both *Fusarium spp*. and *Trichoderma spp*.

Results and Discussion

Isolation identification and maintenance of *Trichoderma* and *Penicillium spp.*

Many researcher have reported that *Trichoderma spp.* are readily isolated from soil by all conventional methods, due to their rapid growth and abundant sporulation *Trichoderma spp*. are also readily obtained by soil washing method. To increase the recovery of the fungus selective method has been devised by Askew and Laing (1993), Elad *et al.*, (1981), Elad and Chet (1983). *Trichoderma spp*. and *Penicillium spp.* were isolated from crop rhizosphere and non rhizospheric soil samples of different crops (Table-1) on PDA. The cultures of *Trichoderma spp*. and *Penicillium spp.* were maintained on PDA slants. The identification of *Trichoderma spp*. on the basis of cultural and morphological characteristics as observed in the present study and with the help of identification manuals. Its characteristics were akin to the description given by several workers (Bissett 1991a, Nagamani and Mew, 1987). Isolates T_3, T_4, T_5, T_7, T_8, and T_{16}were identified of *T. harzianum* which were fluffy in mycelia growth and on sporulation were whitish green, pigmentation was present in some isolates. Isolates T_1, T_6, T_9, T_{12} and T_{12} were identified, of *T.viride* having initially suppressed mycelial growth and yellowish green pigmentation, however in some in isolates absent they gave the darkness of PDA after 120 hours. Isolates T_{15}, T_{18} and T_{20} were identified of *T. koningii* initially grew as fluffy mycelium and growth was fast, the yellowish green in colour. Isolates T_{11} was identified as *T. hamatum* which produced the fluffy growth of mycelium and yellowish

green colonization sporulation, isolates T_2 and T_{10} were identified as *T. polysporum*, the fungus grew fast with the fluffy growth of mycelium with poor sporulation, T_{13} and T_{17} was identified as *T. virens* which were slow growing and suppressed mycelia growth, and pigmentation absent. Only isolate was as matching of the characteristics of *Penicillium spp.* Which was grow slowly. The different soil sample having different *Trichoderma spp* in various crop rhizosphere and non-rhizosphere were also reported supported by Harman *et al.,* (2004). They also reported that *Trichoderma spp.* a present in nearly all soils and other diverse habitats are most prevalent cultivable fungi.

Antagonistic activity of *Trichoderma spp.* and *Penicillium spp* by dual culture

Fusarium oxysporum f. sp. *lycopersici*

As it is shown in figure number one, All the isolates of *Trichoderma* in dual culture inhibited the growth of *F. o.* f. sp. *lycopersici*, at 48 h, the inhibition ranged from 2.60 to 23.98%. Thama-1 suppressed the maximum growth of the pathogen, followed by Tpoly-2, Tvire-1, Tviri-5, and Tkoni-1. However, Tharz-5, Tkoni-3, Penici-1, showed the minimum inhibition of mycelial growth of the *F.o.* f. sp. *lycopersici* which was at par with the control.

At 96 h, the suppression of *F. o.* f. sp. *lycopersici* ranged from 13.47 to 50.68%. Tpoly-1 showed the maximum suppression of the mycelia growth of *F. o.* f. sp. *lycopersici*, followed by Tharz-5, Tviri-5, Thama-1, Tharz-1, and Tkoni-1. However, Penici-1, Tvire-2 and Tvire-1, were less effective for the inhibition of colony growth of the *F. o.* f. sp. *lycopersici* at 96 hours. It was interesting to that Thama-1 and Tpoly-2 were suppressed higher mycelia growth of *F. o.* f. sp. *lycopersici* at 48 h and Tpoly-1 and Thama were superior inhibitor of mycelia growth of pathogen at 96 h. It indicated the Tpoly and Thama were effective at every time interval. This is also supported by Yehia *et al.,* 1981 and Grondon *et al.,* 1997, though they have reported the effectivity of *Trichoderma harzinum* on *F. o.* f. sp. *Lycopersici*.

Padmodya and Reddy (1996) also indicated that *T. viride* was found more effective than *T. harzianum* on *F. o.* f. sp. *lycopersici.* Therefore, Tpoly and Thama may be new effective *Trichoderma spp*. *F. o.* f. sp. *lycopersici.*

Fusarium oxysporum f. sp. *ciceri*

At 48 h, suppression of mycelia growth of *F. o.* f. sp. *ciceri* ranged from 8.52 to 25.93%. Tharz-6 exhibited maximum suppression of the *F.o.* f. sp. *ciceri* followed, by Tkoni-3, Thama-1, Tviri5, Tpoly-2 *and* Tvire-2. However, Tpoly-1, Tharz-4 showed the minimum inhibition of the mycelia growth of the *F. o.* f. sp. *ciceri* at the same exposure duration as showed in figure number two.

At 96 h, the of suppression of mycelia growth ranged between 1.80 to 56.92 per cent and Tharz-1, exhibited the maximum suppression of the mycelia growth of the *F. o.* f. sp. *ciceri,* followed by Tpoly-1, Tviri-1, Tharz-3. However, Tvire-2, Penic-1 showed the minimum suppression of the mycelia growth of the *F. o.* f. sp. *ciceri* at the same exposure duration.

It was interesting to that Tharz-6, Tviri-1, Thama-1 and Tviri-5, Tpoly-2 suppressed higher mycelia growth of *F. o.* f. sp. *ciceri* at 48 hrs, and Tharz-1 and Tpoly-1 were superior inhibitors of mycelia growth of pathogen at 96 h. It indicated the Tpoly and Tharz effective at every time interval.

Table.1 Details of isolates of *Trichoderma* species used in present study

SI. No.	Isolate No.	Place of collection	Source/ host plant/place	species
1	T_1	IIFSR, Meerut	Chickpea non rhizosphere	*Trichoderma viride*
2	T_2	SVP Uni. Meerut	Chickpea rhizosphere	*Trichoderma polysporum*
3	T_3	SVP Uni. Meerut	Tomato rhizosphere	*Trichoderma harzianum*
4	T_4	SVP Uni. Meerut	Perl millet rhizosphere	*Trichoderma harzianum*
5	T_5	SVP Uni. Meerut	Perl millet non rhizosphere	*Trichoderma harzianum*
6	T_6	SVP Uni. Meerut	Urd bean non rhizosphere	*Trichoderma viride*
7	T_7	Moradabad	Sugarcane rhizosphere	*Trichoderma harzianum*
8	T_8	SVP Uni. Meerut	Pegionpea non rhizosphere	*Trichoderma harzinum*
9	T_9	SVP Uni. Meerut	Pegionpea rhizosphere	*Trichoderma viride*
10	T_{10}	SVP Uni. Meerut	Tomato non rhizosphere	*Trichoderma polysporum*
11	T_{11}	SVP Uni. Meerut	Rice rhizosphere	*Trichoderma hamatum*
12	T_{12}	SVP Uni. Meerut	Rice non Rhizosphere	*Trichoderma viride*
13	T_{13}	SVP Uni. Meerut	Maize rhizosphere	*Trichoderma virens*
14	T_{14}	SVP Uni. Meerut	Maize non rhizosphere	*Trichoderma viride*
15	T_{15}	SVP Uni. Meerut	Sugarcane non Rhizosphere	*Trichoderma koningii*
16	T_{16}	SVP Uni. Meerut	Departt. of plant pathology	*Trichoderma harzianum*
17	T_{17}	SVP Uni. Meerut	Sorghum rhizosphere	*Trichoderma virens*
18	T_{18}	SVP Uni. Meerut	Sorghum non rhizosphere	*Trichoderma koningii*
19	T_{19}	SVP Uni. Meerut	Potato non rhizosphere	*Penicillium spp.*
20	T_{20}	SVP Uni. Meerut	Potato rhizosphere	*Trichoderma koningii*

Fig.1 Growth inhibition of *F. oxysporum* f. sp. *lycopersici* by antagonistic fungi using dual culture technique

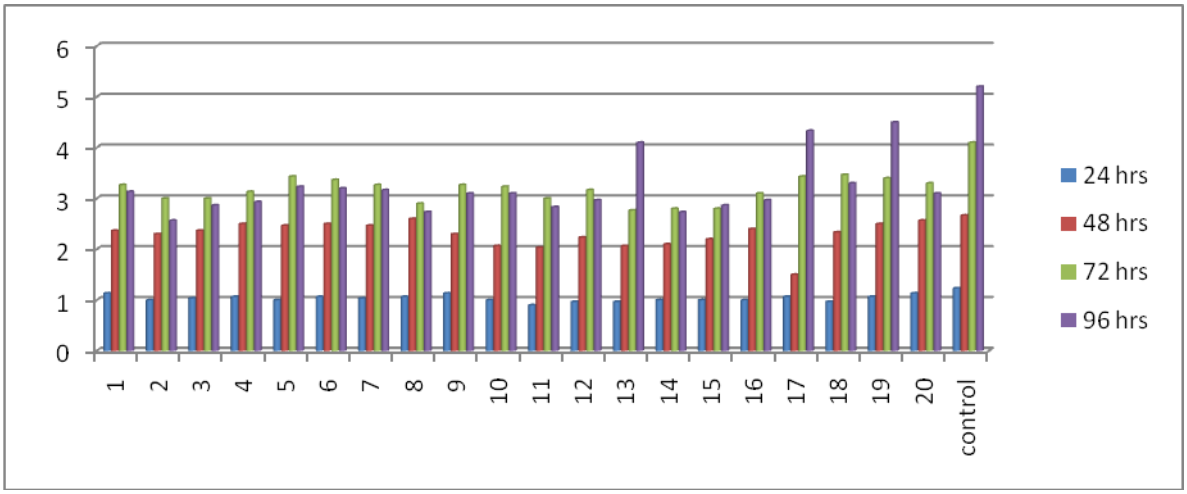


Fig.2 Growth inhibition of *F.oxysporum* f. sp. *ciceri* by antagonistic fungi using dual culture technique

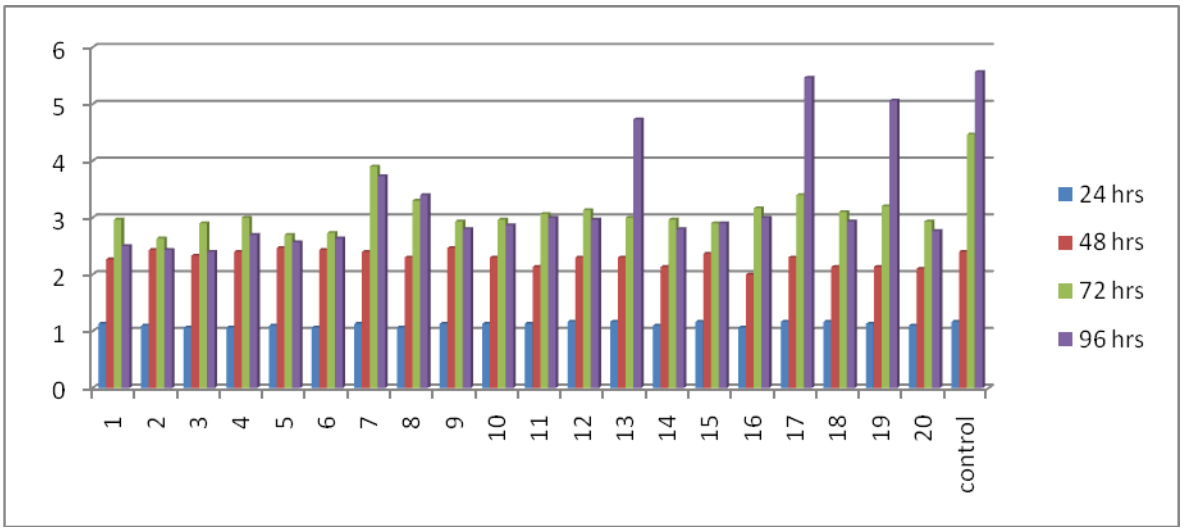
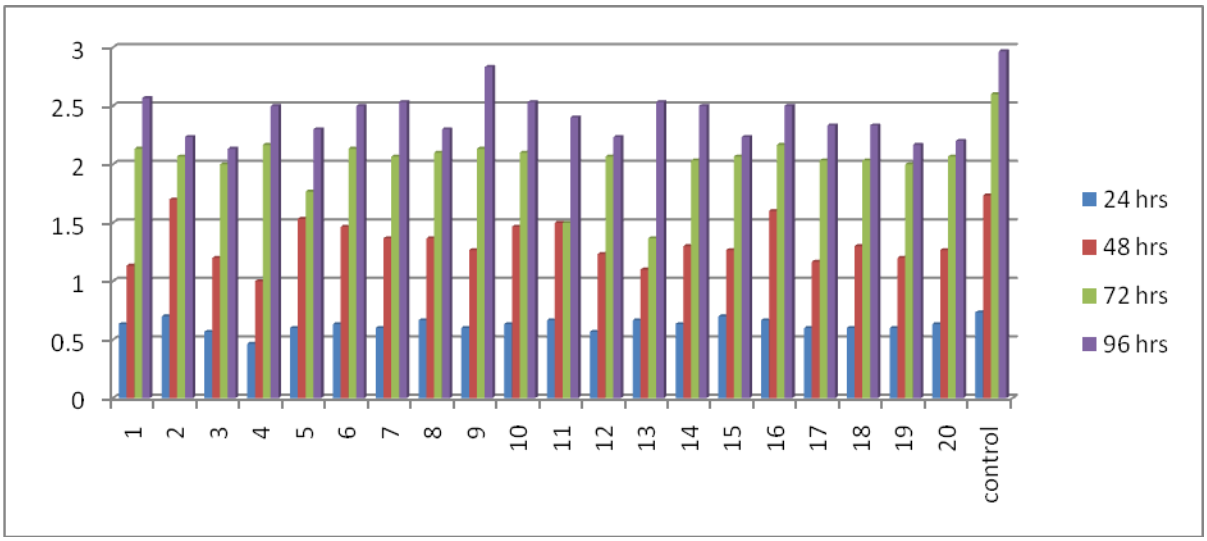


Fig.3 Growth inhibition of *Fusarium fujikuorii* by antagonistic fungi using dual culture technique



T_1=*Trichoderma viride* (Chickpea non-rhizosphere),
T_2=*T. polysporum* (Chickpea rhizosphere)**, T_3=*T. harzianum*** (Tomato rhizosphere),
T_4=*T.harzianum* (Perlmillet rhizosphere), **T_5= *T. koningi*** (Perlmillet non-rhizosphere),
T_6= *T. viride* (Urd bean non-rhizosphere)**, T_7= *T.harzianum***(Sugarcane rhizosphere)**,**
T_8= *T. harzianum* (Pigeonpea non-rhizosphere)**, T_9= *T. viride*** (Pigeonpea rhizosphere),
T_{10}= *T. polysporum* (Tomato non-rhizosphere)**, T_{11}= *T.hamatum*** (Rice rhizosphere)**,**
T_{12}= *T. viride* (Rice non rhizosphere)**, T_{13} = *T. virens*** (Maizerhizosphere)**,**
T_{14}= *T. viride* (Maize non rhizosphere), **T_{15}= *T. koningii*** (sugarcane nonrhizosphere),
T_{16}= *T.harzianum*, T_{17}= *T. virens*(Sorghum rhizosphere)**, T_{18} = *T. koningii*** (potato non-rhizosphere)**,**
T_{20}= *T. koningii* (potato rhizosphere); **and T_{19}= *Penicillium spp.*** (potato rhizosphere).

Similar results also have been reported by Sonwane and Pawar, (20001) they reported that *T. harzianum* was found very effective in controlling vegetative growth of *F. o.* f. sp. *ciceri* followed by *T. hamatum*. Our results are also in supported Silva- Hanlin and Menezes (1997) who observed variation in antagonistic activity of five species of *Trichoderma* during their evaluation in vitro against *F.* o. f. sp. *vasinfectum* and reported that *T. polysporum* was most effective followed by *T. harzianum*. Poddar *et al.*, (2004) also reported *T. harzianum* isolated from rhizosphere and non- rhizosphere soil of chickpea and found *T. harzianum* isolated from rhizosphere, exhibiting highest antagonistic activity against *F.o.* f. sp. *ciceri*. Waghmare and Kurundkar (2007)also supported these results. It was also observed, in dual culture, that growth three *Fusarium spp.* was stopped when they come in contact with the most of the *Trichoderma* isolate. There was a prominent contact line at the meeting point of the paired microorganisms. There isolates not only initiated the growth of the *Fusarium spp* but also after making contact, the whole surface of the Petri dish growing over the Fusarium mycelia. This shows that these isolates of *Trichoderma spp*. is more effective in the fight for the colonized area winning the competition for space and nutrition.

Fusarium fujikuroii

As the observations are represented in figure number three. At 48 h, the suppression of mycelia growth of the *F. fujikuroii* ranged from 1.74 to 42.19 per cent and Tharz-2, suppressed maximum growth of *F. fujikuroii* followed by Tvire-1, Tvire-2, Tharz-6, Tviri-4, Tkoni-1. However, Tpoly-1, Thama-1 showed the minimum suppression of the mycelia growth of the *F. fujikuroii* at 48 hrs. At 96 hours, the suppression of mycelia growth of the *F. fujikuroii* ranged from 4.27 to 28.29 per cent. Tharz-1 exhibited the maximum suppressive of mycelia growth of the *F. fujikuroii* followed byPenici-1, Tkoni-3, Tpoly-1, Tviri-4, Tharz-3, and Tvire-2. However, Tviri-3, Tviri-5 showed the minimum suppression of the mycelia growth of the *F. fujikuroii* at 96 hrs.

It was interesting to that Tharz-2, Tviri-1, Tvire-2 and Tharz-6 were suppressed higher mycelia growth of *F. fujikuroii* at 48 hrs, and Tharz-1, Tpoly-1, Tviri-4 and Tvire-2 were found superior inhibitor of mycelia growth of pathogen at 96 hrs. It indicated that the Tharz and Tvire were effective at every time interval.

The mechanism of inhibition may be comptetion for food and space, production of antibiotics and mycoperasitism. *Trichoderma spp*. may exist direct biocontrol by parasitizing a range of fungi, sequential expression of cell wall degrading enzymes, mostly chitinases, glucanases and proteases (Harman *et al.*, 2004). Morsad (1985) studied antagonism between *Trichoderma spp*. and *Fusarium oxysporum* and observed that the growth of *T. viride* was vigorous in dual culture and it was an effective hyperparasite penetrating and coiling the hyphae around the host hyphae. Though, the interesting observation was recorded as Thama-1 and Tpoly-1 suppressed the mycelium growth of *F. o.* f. sp. *lycopersici* and *F. o*. f. sp. *ciceri*. While, *T. harzianum* and *T. viride* were effective against all tested *Fusarium spp.F. o.* f. sp. *lycopersici*, *F. o.* f. sp. *ciceri* and *F. fujikuroii*.

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