

***Trichoderma koningiopsis* a New and Strong Antagonist against Soil Borne Pathogens of Chickpea**

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Trichoderma spp. are biocontrol agents extensively used in management of fungal diseases of crop plants exhibiting mycoparasitism against a wide range of plant pathogens. Five *Trichoderma* spp. viz; *T. aggressivum*, *T. citrinoviride*, *T. erinaceum*, *T. harzianum* and *T. koningiopsis*, isolated from different locations of India were characterized for their antagonistic activity against *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia bataticola* causing wilt complex in chickpea. All the species revealed differential reaction pattern against all the test pathogens. However, *T. koningiopsis* was the only species which show high level of tolerance most effective in percentage inhibition of mycelial growth of test pathogen. Scanning Electron microscopic investigations about hyphal interactions between antagonists and test fungi revealed that the mycoparasitic hyphae were usually attached longitudinally to the hyphae of the pathogen. Coiling of hyphae, short contact branches, hyphal depression and pincer shaped structures were seen during observations through SEM indicated the mode of action in biological control of the test pathogen.

Key words: *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia bataticola*, *Trichoderma* spp., Antagonism, Hyphal relationship, Wilt Complex, Chickpea, Biological management.

Chickpea (*Cicer arietinum* L.) is one of the major grain legumes widely grown in India as well as other parts of the world. Among various factors attributing to low productivity of chickpea, susceptibility to diseases is very important. It is estimated that yield loss due to insects and diseases ranges from 5 to 10% in temperate and 50 to 100% in tropical regions (Van Emden *et al.* 1988). Chickpea is prone to many diseases and among them wilt and dry root rot caused by *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia bataticola*

are the major constraints in chickpea production and causing 10-20% annual loss. Wilt complex caused by *Fusarium oxysporum* f.sp. *ciceri* (*Foc*) and *Rhizoctonia bataticola* (*Rb*) causes more severe damage to the crop. (Vishwa Dhar and Chaudhary 2001).

In chickpea, chlamydospores and sclerotia surviving in the soil are the major sources of primary inoculum. Since 75% cultivation of chickpea in India is under rainfed, the crop faces severe moisture stress which predisposes the crop to wilt and dry root rot development. The saprophytic survival ability of the pathogens in soil makes chemical control and crop rotation ineffective. Cultivation of resistant varieties is an economical approach for the management of wilt and dry root rot but up to now effective resistant

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cultivars are not available to combat with the diseases. The preference for biological control method is justified also by the undesirable side effects of pesticides. The technology that seems promising to manage the diseases without disturbing the equilibrium of harmful and useful composition of environment and ecosystem is the use of more and more biological control agents. Use of *Trichoderma* spp. as biological agents has been very much successful against soil borne diseases for which no resistant sources have been identified. (Mukhopadhyay 1994, Mukherjee et al, 2012).

However, there is still considerable interest in finding more efficient mycoparasitic fungi especially within *Trichoderma* spp., which differ considerably with respect to their biocontrol effectiveness. It is important to isolate *Trichoderma* spp. having potentially higher antagonistic efficiency by the selection of isolates with high potential of mycoparasitic activities. The aim of this study was screening of *Trichoderma* spp. for their antagonistic ability, higher survivability as well as their capability of interaction and hyphal depression to the test pathogens.

MATERIALS AND METHODS

Soil samples and Isolation

Soil samples were collected from the rhizosphere soil of different crop niches. Five-fold serial dilutions (Singh, 1970) of each soil sample was prepared in sterilized distilled water and 0.5 ml diluted sample was poured on the surface of *Trichoderma* Specific Medium (TSM). Plates were incubated at $25 \pm 2^\circ\text{C}$ for 96 h. Morphologically different colonies appearing on the plates were purified in the Potato Dextrose Agar (PDA) (HiMedia, India) and send to ITCC, New Delhi for identification.

Cultural, Morphological and Physiological Characterization

Cultural and morphological observations of colony were based on *Trichoderma* isolates grown on PDA for 7 days in an incubator at $25 \pm 2^\circ\text{C}$ with altering 12h/12h fluorescent light/ darkness. Characters of the conidium- bearing structures and conidia were assessed for each isolate. Growth-rate trials were done in 9 cm diam petridishes with 20 ml PDA at 15, 20, 25, 30, 35, 40 and 45°C .

Measurements of colony radius, the greatest distance from the edge of the plug of inoculum to the edge of the colony were taken daily upto 72h. Trials were replicated thrice. Physiological observations of *Trichoderma* spp. were based on mycelial growth on different pH ranged from 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.

Antagonistic Activity of *Trichoderma* Isolates

The dual culture technique described by Morton and Stroube was used to test the antagonistic ability of 5 *Trichoderma* spp. viz; *T. aggressivum* (ITCC 7277), *T. citrinoviride* (ITCC 7283) *T. erinaceum* (ITCC 7287), *T. koningiopsis* (ITCC 7291), and *T. harzianum* (ITCC 6796) against *Fusarium oxysporum* f.sp.ciceri and *Rhizoctonia bataticola*. The pathogen and *Trichoderma* spp. were grown on PDA for a week at $25 \pm 2^\circ\text{C}$. 5mm disc of the target fungi cut from the periphery was transferred to the Petri dish previously poured with PDA. *Trichoderma* spp. was transferred aseptically in the same plate of opposite end and were incubated at room temperature with alternate light and darkness for 7 days and observed periodically. Control plates were maintained without *Trichoderma*. The experiment was replicated thrice and percent growth inhibition was calculated by the formula of $I = (C''T)/C \times 100$, where C is mycelial growth in control plate, T is mycelial growth in test organisms inoculated plate and I is inhibition of mycelial growth. Vincent et al (1999)

Scanning electron microscopy (SEM)

Small pieces of agar (approx. 2 mm²) were taken from the dual culture plates at the point of interaction between *Trichoderma* spp. and test fungi. The samples were fixed in 2.5% glutaraldehyde dissolved in 0.5M phosphate buffer at pH 7.2 and stored overnight at 4°C , then rinse with the same buffer. After dehydration using a graded ethanol series, samples were critical-point dried in carbon dioxide after a graded transition from ethanol to acetone. Sections (5×5mm) were mounted on stubs, coated with gold-palladium, and examined with a JEOL-JSM-T300 SEM operating at 15 kV.

RESULTS

Microscopic examinations on morphological characters of all *Trichoderma* species revealed that the asexual states of all

species have typical *T. harzianum*-like morphology except *T. koningiopsis* which forms less conidia but more chlamydospores. Phialides arise in whorls at the tips of secondary branches and from the tip of the main axis. The average dimensions of phialides ranged between 3.1-7.6 x 2.4-3.4. Longest phialides are found in *T. harzianum* while shortest in *T. koningiopsis*. Widest phialides were seen in *T. citrinoviride* while narrowest in *T. koningiopsis*. Conidia did not vary in shape and most were globose to subglobose or broadly ovoidal. Optimum temperature for the growth of all species was between 25 to 30 °C. On increasing the temperature up to 45°C *T. erinaceum* did not grow and rest of the species grew very poorly except *T. koningiopsis* with 0.270 cm. mecelial growth . Similarly, pH range 6.5 to 7.0 supported best growth for all the species. *T. koningiopsis* is the only species which grow well at 7.5 pH. Observations on all these characters indicated that *T. koningiopsis* is the only species which can grow well at variable temperature and pH indicating its tolerance against adverse conditions (Table 1, Fig. 1).

Macroscopic examination of the fungal dual cultures revealed that most of the strains made hyphal contact with test pathogen within two days

after inoculation. *T. koningiopsis* was the most inhibiting antagonist that grew over the pathogen. Other *Trichoderma* spp. acted only as a barrier against *Fusarium oxysporum* f.sp. *ciceri* and *R. bataticola*.

Antagonistic potential of *Trichoderma* spp. through dual culture indicated that colony growth after 72 h was 13.5-20.0 mm in *Foc* and 35.0-42.5 mm in *Rb* as compared to control. Colony growth of test pathogens was appressed and after coming in contact, the antagonists grew and sporulated over the pathogen colony due to their prolific growth habit and mycoparasitic character. Inhibition percent of growth by different *Trichoderma* spp. ranged between 33.3 – 55.0 percent and 22.7 – 36.3 in *Foc* and *Rb* respectively (Table 2, Fig 2 a & b). These findings corroborate the findings by earlier workers. (Dennis and Webster, 1971; G.J. Samuels, 1996; Sumeet and Mukherjee, 2000; Golve and Kurundkar, 2002; and Jagathambigai *et al.*, 2009.)

A similar behavior for each antagonist-pathogen combination was observed by SEM. There were similarities and differences in the antagonistic ability of all species of *Trichoderma* to invade the pathogen in dual culture. Direct contact with the pathogen was always followed

Table 1. Comparison of morphology of *Trichoderma* spp.

Characters/ Species Habitat	<i>T. aggressivum</i> Soil	<i>T. citrinoviride</i> Soil	<i>T. erinaceum</i> Soil	<i>T. koningiopsis</i> Soil	<i>T. harzianum</i> Soil
Conidium length (µm)	3.1 -3.8	2.7-3.1	3.1 -3.4	4.3-6.8	2.8-3.2
Conidium width (µm)	2.8-3.1	2.1-2.8	2.5-3.1	2.4-3.4	2.5-2.9
Phialid length (µm)	4.3-5.9	6.2-6.8	4.3-6.2	3.1-3.4	3.4-7.6
Phialid width (µm)	2.4-3.1	3.1-3.4	2.4-3.1	2.4-2.8	2.5-3.4
Growth after 72h at 15°C (cm)	4.8	3.1	3.8	6.6	-
Growth after 72h at 20°C	6.2	6.8	5.8	7.1	4.4
Growth after 72h at 25°C	6.8	7.2	6.6	7.5	5.5
Growth after 72h at 30°C	7.5	7.7	7.2	7.8	6.9
Growth after 72h at 35°C	8.1	8.2	8.2	5.2	4.7
Growth after 72h at 40°C	7.5	7.5	7.6	3.2	2.9
Growth after 72h at 45°C	0.115	0.122	No growth	0.135	0.270
Mycelial growth at pH4.0	0.232	0.107	0.032	0.296	0.269
Mycelial growth at pH4.5	0.608	0.116	0.170	0.296	0.298
Mycelial growth at pH5.0	0.151	0.212	0.303	0.266	1.08
Mycelial growth at pH5.5	0.276	0.475	0.111	1.710	1.22
Mycelial growth at pH6.0	0.164	0.184	0.277	0.217	1.24
Mycelial growth at pH6.6	0.926	0.917	0.188	1.105	1.22
Mycelial growth at pH7.0	0.601	0.121	0.196	0.322	1.20
Mycelial growth at pH7.5	0.171	0.131	0.110	0.196	0.287

by various types of hyphal aggression. SEM investigations revealed that mycoparasitic hyphae were usually attached longitudinally to the hyphae of the pathogen. Hyphal coiling, hooks, pincer shaped structures, short contact branches and hyphal depression were also observed.

In case of conidial and sclerotial inoculum of *Foc* & *Rb* it was observed that percent conidia and sclerotia killed ranged from zero to 100 percent depending upon the antagonistic potential of the

Trichoderma species. *T. harzianum* kill all the conidia and sclerotia while other species killed some of the inoculum (Fig. 4a-h). All *Trichoderma* sp. were effective in reducing conidial and sclerotial viability. These observations revealed that penetration and multiplication of antagonist inside the conidia and sclerotia is dependent on the ability of the biocontrol agent to attack and establish on the wall of conidia and sclerotia. As the studies done so far on biological control of *F. oxysporum*

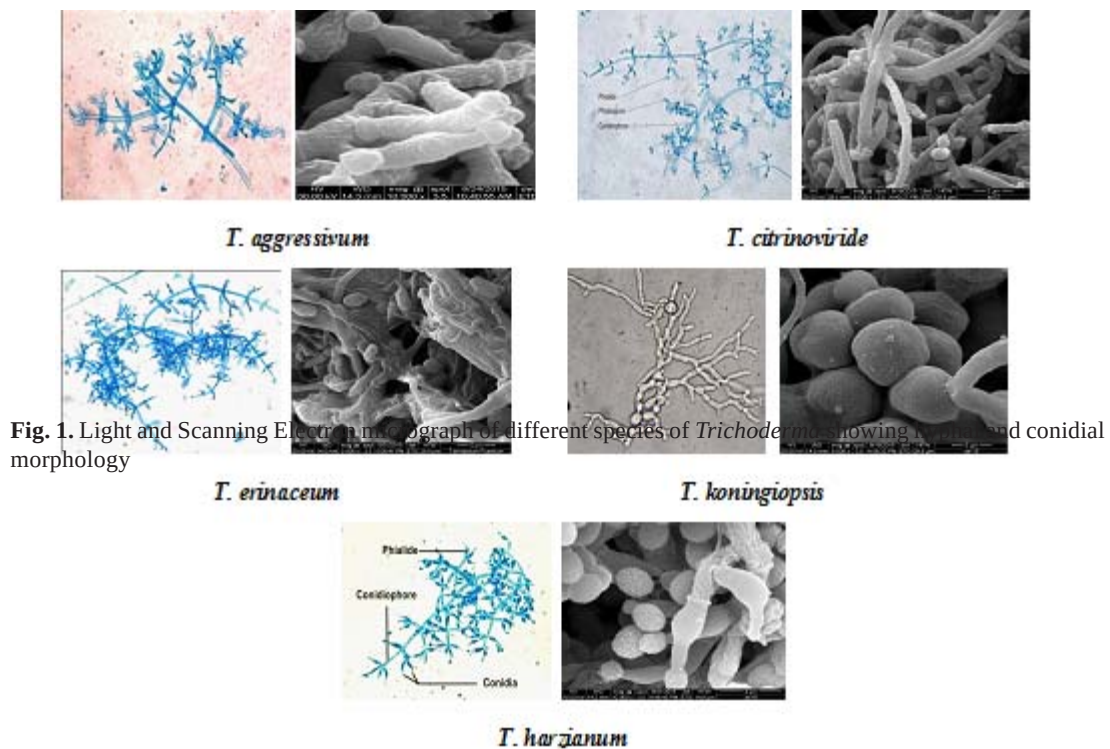
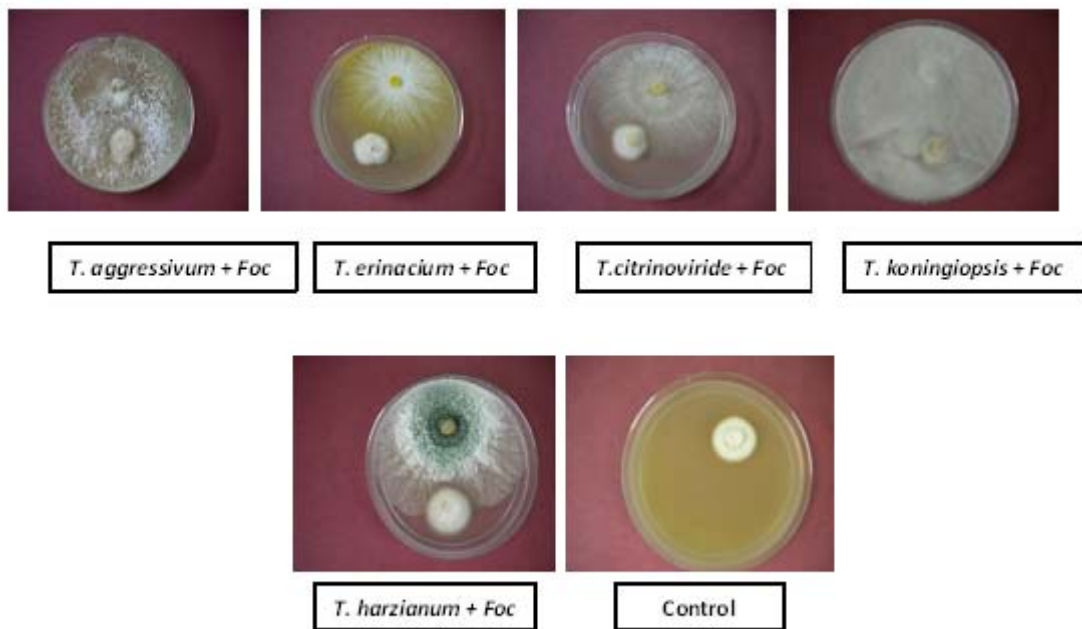


Fig. 1. Light and Scanning Electron micrograph of different species of *Trichoderma* showing hyphal and conidial morphology

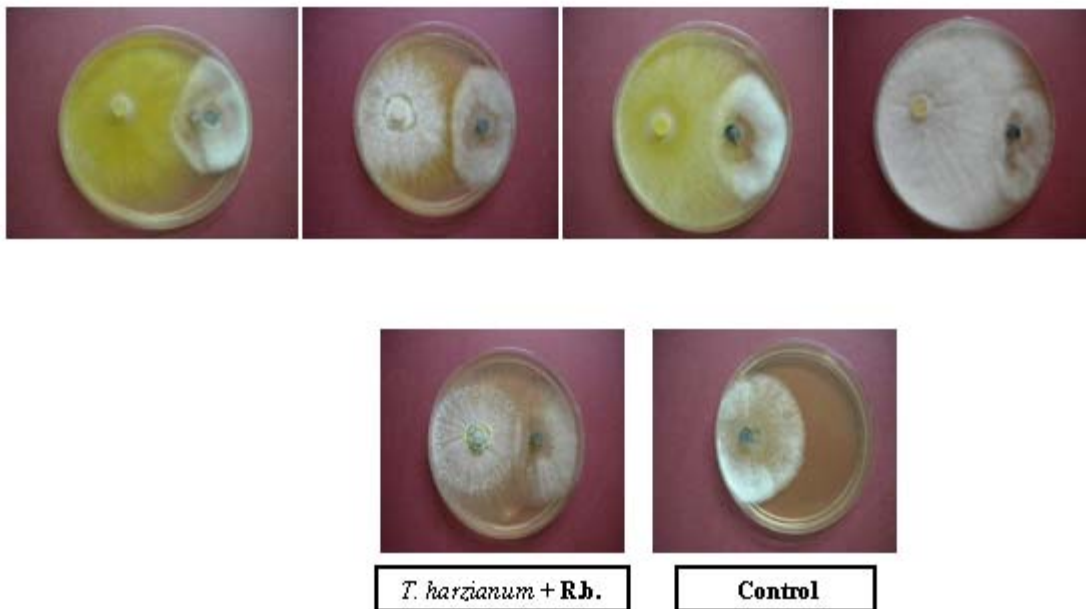
Table 2. In vitro antagonistic potential of *Trichoderma* isolates against *R. bataticola* through dual culture

<i>Trichoderma</i> spp.	Growth of <i>Foc</i> after 72h (mm)		Growth of <i>R.b.</i> after 72h (mm)	
	Mycelial growth	% inhibition in mycelial growth	Mycelial growth	% inhibition in mycelial growth
<i>T. aggressivum</i>	14.2	52.6	40.0	27.2
<i>T. citrinoviride</i>	18.0	40.0	40.2	26.9
<i>T. erinaceum</i>	20.0	33.3	42.5	22.7
<i>T. koningiopsis</i>	13.5	55.0	35.0	36.3
<i>T. harzianum</i>	15.0	50.0	40.2	26.9
Control	30.0	-	55.0	-
CD@5%	4.2		3.3	



**Foc* = *Fusarium oxysporum* f.sp. *ciceri*

Fig. 2 (a). Antagonistic potential of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *ciceri*.



* *R.b.* = *Rhizoctonia bataticola*

Fig. 2 (b). Antagonistic potential of *Trichoderma* spp. against *Rhizoctonia bataticola*

ciceri and *R. bataticola* included only a few isolates of a particular species so it is difficult to draw a conclusion on the species specificity. In

the present investigations antagonistic effects of five *Trichoderma* species revealed that there is a significant variability in their ability to parasitize,

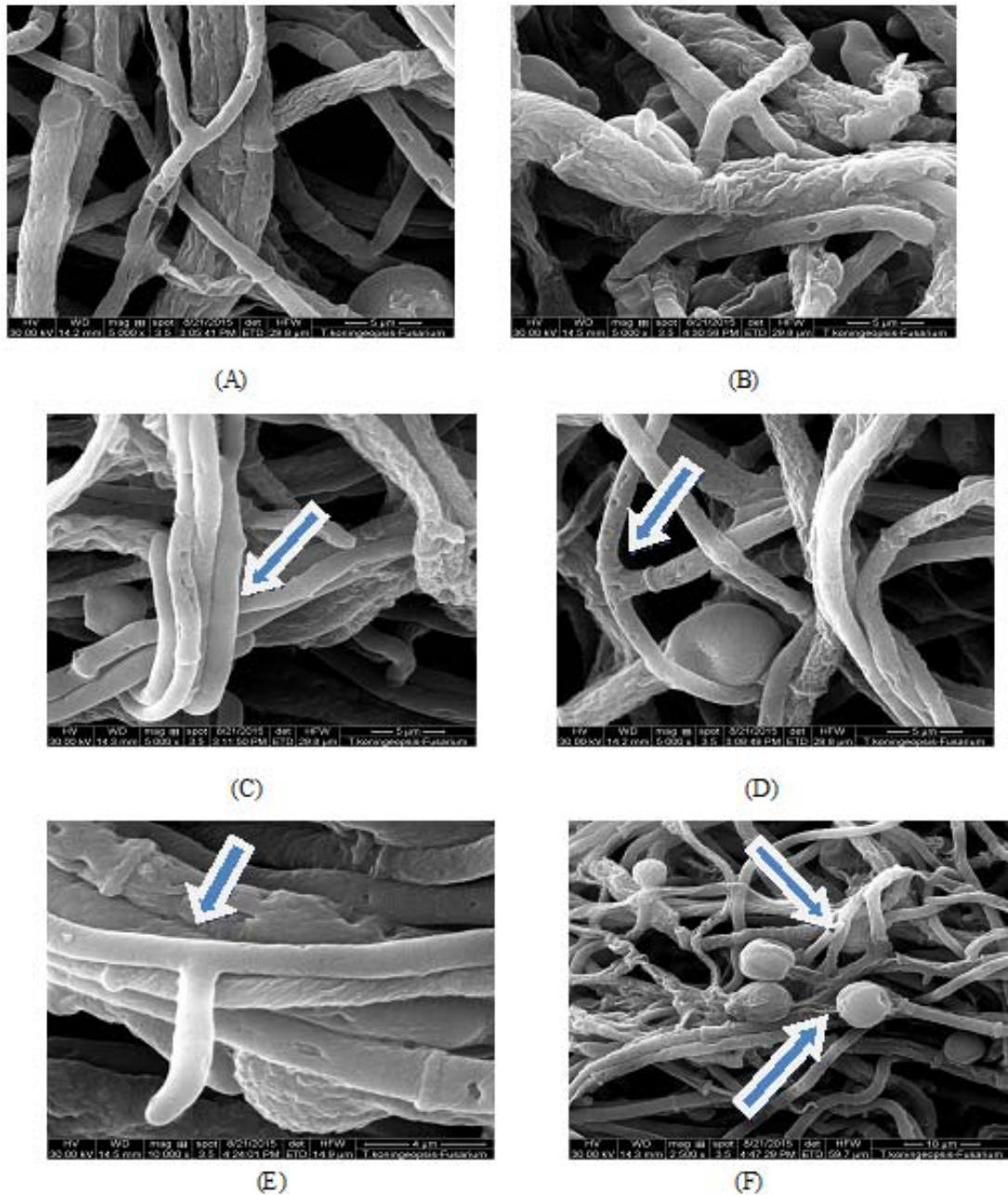


Fig. 3. (A-F) (A & B) Scanning electron micrograph on mycoparasitism of the *F. oxysporum f.sp. ciceri* hyphae by the hyphae of *T. koningiopsis* with pincer shaped structure moving longitudinally and parallel to the hyphae of the pathogen (C & D) Coiling of hyphae and hyphal tip of *T. koningiopsis* attached to and penetrating the hyphae of *F. oxysporum ciceri* (E & F) *T. koningiopsis* hyphal tip, hooks and chlamydospores adhere to the hyphae of *F. oxysporum ciceri* causing hyphal depression.

macerate and kill the mycelial, conidial and sclerotial inoculum of the test pathogens. Conidia and Sclerotia are first colonized by the antagonists followed by penetration and finally killing. *Trichoderma koningiopsis* is found best in

mycoparasitism of *Fusarium oxysporum*, f.sp. *ciceri* and *R. bataticola* as compared to other antagonists studied. These findings supported by findings of earlier workers (Elad *et al.*, 1983; Kohl and Schlosser, 1989; Sreenivasaprasad and

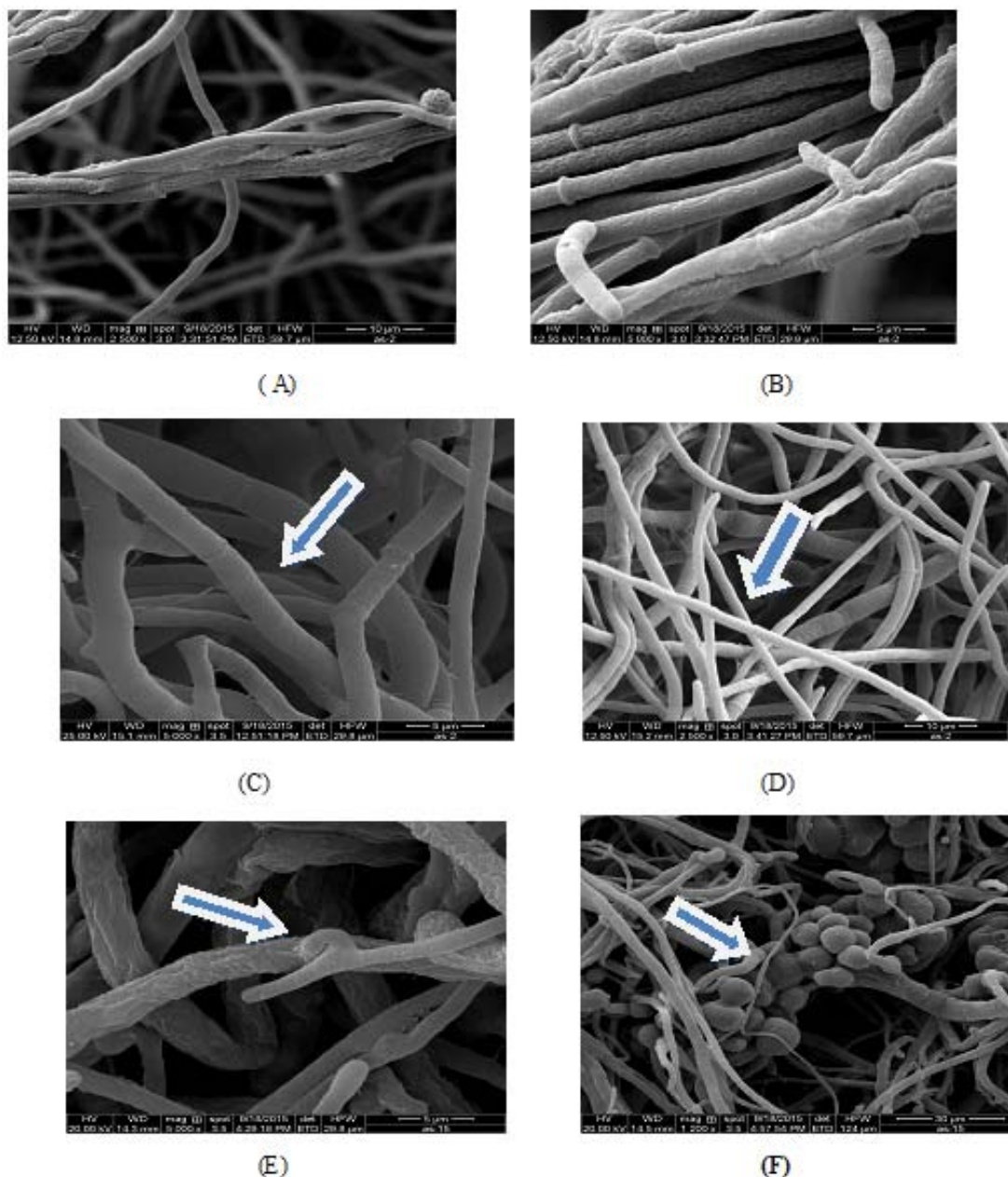


Fig. 4. (A-F) (A & B) Scanning electron micrograph on parasitic action of *T. koningiopsis* against *R. bataticola*, moving longitudinally and parallel to the hyphae of the pathogen (C & D) Hyphal tip of *T. koningiopsis* attached and penetrating the hyphae of *R. bataticola* (E & F) *T. koningiopsis* hyphal tip and conidia adhere to the hyphae of *R. bataticola* causing hyphal swelling and hyphal growth depression.

Manibhushanarao, 1993; Amrutha *et al.*, 2014).

DISCUSSION

Cultural characteristics comprising growth rate, colony colour and colony appearance were regarded as taxonomically useful characteristics for *Trichoderma* (Samuels *et al.*, 2002a). Studies revealed that all five *Trichoderma* spp. did not much differ in cultural characteristics with most isolates exhibiting rapid growth, effuse conidiation and/or loosely arranged conidia in pustules. The same findings like rapid growth at 25°C to 30°C were recorded by Samuels *et al.* (2002a). Gams and Bissett (2002), Lin and Heitman (2005) and Samuels *et al.* (2002a) also confirmed the presence of terminal and/or intercalary chlamydospores in cultures. Morphological characterization was conventionally used in the identification of *Trichoderma* species, and it remains as a potential method to identify *Trichoderma* species (Anees *et al.*, 2010; Gams and Bissett 2002; Samuels *et al.*, 2002a).

Trichoderma is a well known biocontrol agent with multiple modes of action such as competition (Howell, 2003), induced resistance (Harman, 2006), solubilization of inorganic plant nutrients (Altomare *et al.*, 1999), inactivation of the pathogen's enzymes involved in the infection process (de Meyer *et al.*, 1998) and mycoparasitism (Barnett and Binder, 1973). Various workers stated that *Trichoderma* spp. produces cell wall degrading enzymes (CWDEs) including chitinases, α -1,3-glucanases, proteases and α -1,4-glucanases, antibiotics and antibiotic peptides, such as peptaibols to combat with the pathogen (Flores *et al.*, 1997; Elad and Kapat, 1999; Dennis and Webster, 1971; Fujiwara *et al.*, 1982; Vinale *et al.*, 2006; Iida *et al.*, 1994).

In case of antibiosis in dual culture it was observed that *Trichoderma koningiopsis* was found best in controlling growth of the test pathogens among all *Trichoderma* spp. Varying modes of hyphal interactions and degree of inhibition in growth and development of *Foc* and *Rb* were studied to investigate mechanism of control. Understanding the mechanism of action involved in the biocontrol process is of primary importance in establishing these characteristics.

This can provide much insight about where and when the interaction occurs and how the pathogen will be affected. In order to survive and mycoparasitize *Trichoderma* spp. produces a wide variety of toxic and antibiotic metabolites such as trichodermol, trichodermin, harzianolide, terpenes, polypeptides (Lorito *et al.*, 1994; Dickinson *et al.*, 1995; Sivasithamparam and Ghisalberti, 1998; Vinale *et al.*, 2006; Vinale *et al.*, 2008; Andrabi *et al.*, 2011) and extracellular hydrolytic enzymes (Thrane *et al.*, 2000; Eziashi *et al.*, 2006) which were involved in the inhibition, competition, and mycoparasitism of phytopathogenic fungi. In this regard our results support these findings by showing that *Trichoderma koningiopsis* produces strong antibiosis and competitive growth against pathogens in agar plates (Fig. 2a & 2b).

Knowledge on the mechanism of antagonism is must and would prove very useful for the effective disease control. Scanning Electron Microscopy (SEM) of hyphal interaction between *Trichoderma* spp. and *Fusarium oxysporum-Rhizoctonia bataticola* (wilt complex pathogens) indicated that biocontrol agents parasitized the mycelium first. They penetrate and finally resulting into lysis or collapse of hyphae of the pathogens. Among the *Trichoderma* spp. *T. koningiopsis* showed more mycoparasitic ability making contact with host hyphae, running parallel to it, production of hook like structure and emptied the cells. This research was carried out to screen five *Trichoderma* spp. against wilt & dry root rot pathogens of chickpea under in vitro. Electron microscopic observations revealed that all *Trichoderma* spp. interacted with the pathogens. *T. koningiopsis* grew toward the pathogen and coiled around the host cells, penetrating and destroying the hyphae. Penetration into host cells was apparently accomplished by mechanical activity.

Elad *et al.*, (1983) demonstrated hyphal interaction between *T. harzianum* and *T. hamatum* with *Sclerotium rolfsii* and *Rhizoctonia solani* by Scanning Electron Microscopy (SEM). *Trichoderma* spp. adhere the host surface by coiling, hooks or appressoria. Lysed sites and penetration holes were found in hyphae of the plant pathogenic fungi, following removal of parasitic hyphae.

Based on the antagonistic potential and hyphal

morphologies observed at SEM we would suggest *T. koningiopsis* as a strong antagonist. These findings are new as SEM investigations on *Trichoderma* spp. with wilt complex creating fungi in chickpea are not reported earlier from India. *T. koningiopsis* may play an important role in the biological control of soil borne diseases of chickpea in U.P. (India).

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REFERENCES

- Morton DT, Stroube NH. Antagonist and stimulatory effect of microorganisms upon *Sclerotium rolfsii*. *Phytopath.* 1955; **45**: 419-420
- Singh RS, Singh N. Effect of oil cake amendment of soil on population of some wilt causing species of *Fusarium*. *Phytopath. Zei. Schrift*, 1970; **26**: 160-167.
- Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma* II Production of volatile antibiotics. *Trans. Brit. Mycol. Soc.* 1971; **5**: 41-48.
- Barnett HL and Binder FL. The fungal host parasite relationship. *Annu. Rev. Phytopathol.* 1973; **11**: 273-292.
- Elad Y, I Chet, Henis Y. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparas* 1981; **9**:1 59-67.
- Elad Y, I Chet, Henis Y. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Can. J. Microbiol.* 1982; **28**: 719-725.
- Fujiwara A, Okuda T, Masuda S, Shiomi Y, Miyamoto C, Sekine Y, Tazoe M, Fujiwara M. Fermentation, isolation and characterization of isonitrile antibiotics. *Agric. Biol. Chem.* 1982; **46**: 1803-1809. Gilbert J, Tekauz A. 2000. Review: recent developments.
- Cook RJ, Baker KE. The nature and practice of biocontrol of plant pathogens. *Ame. Phyto. Soc. St. Paul. Minn.* 1983; **539**.
- Elad Y, I Chet, Boyle P, Henis Y. Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy and fluorescence microscopy. *Phytopathol.* 1983; **73**: 85-88.
- Kohl J, Schlosser E. Decay of sclerotia of *Botrytis cinerea* by *Trichoderma* spp. at low temperatures. *J. Phytopathol.*, 1989; **125**: 320-326.
- Sreenivasaprasad S, Manibhushanarao K. Efficacy of *Gliocladium virens* and *Trichoderma longibrachiatum* as biocontrol agents of groundnut root and stem rot diseases. *Int. J. Pest. Mngmt* 1993; **39**: 167-171.
- Iida A, Sanekata M, Fujita T, Tanaka H, Enoki A, Fuse G, Kanai M, Rudewicz PJ, Tachikawa. Fungal metabolites XVI. Structures of new peptaibols, trichokindins I-VII, from the fungus *Trichoderma harzianum* Chem. Pharm. Bull. 1994; **42**: 1070-1075.
- Lorito M, Peterbauer C, Hayes CK, Harman GE. Synergistic interaction between fungal cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore germination. *Microbiol.* 1994; **140** (3):623-629.
- Mukhopadhyay AN. Biocontrol of soil borne fungal plant pathogens current status and future prospects and potential limitations. *Ind. Phytopath.*, 1994; **47**:119-126.
- Dickinson JM, Hanson JR, Truneh A. Metabolites of some biological control agents. *Pestic. Sci.*, 1995; **44**(4):389-393.
- Samuels GJ. *Trichoderma*: A review of biology and systematics of the genus. *Myc. Res.* 1996; **100**: 923-935.
- Flores A, Chet I, Harrera-Estrella A. Improved biocontrol activity of *Trichoderma harzianum* by over expression of the proteinase-encoding gene prb1. *Curr. Genet.*, 1997; **31**: 30-37 doi:10.1007/s002940050173.
- Gams, W. and Bissett, J. (1998). Morphology and Identification of *Trichoderma*. In: *Trichoderma and Gliocladium, Basic Biology, Taxonomy and Genetics*, Harman, G.E. and C.P. Kubicek (Eds.). **1**: Taylor and Francis, London, UK., pp: 3-34.
- de Meyer G, Bigirimana J, Elad Y, Hofte M. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *Eur. J. Plant Pathol.* 1998; **104**: 279-286, doi:10.1023/A:1008628806616.
- Sivasithamparan K, Ghisalberti EL. Secondary metabolites in *Trichoderma* and *Gliocladium*. p 139-191. In *Trichoderma and Gliocladium* Vol I (CP Kubicek, GE Harman, eds.) Taylor and Francis Ltd London UK 300 pp; 1998.
- Altomare C, Norvell WA, Bjorkman T, Harman GE. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum*

- Rifai 1292–22. *Appl. Environ. Microbio*, 1999; **165**: 2926–2933.
22. Desai S, Schlosser E. Parasitism of *Sclerotium rolfsii* by *Trichoderma*. *Ind. Phytopath*, 1999; **52**(1): 47-50.
23. Elad Y and Kapat A. The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. *Europ. J. Plant Pathol.*, 1999; **105** :177-189.
24. Sumeet, Mukherjee KG. Exploitation of protoplast fusion technology in improving biocontrol potential. In *Biocontrol Potential and its Exploitation in Sustainable Agriculture Crop Diseases Weeds and Nematodes*. 39-48.
25. Thrane C, Jensen DF, Tronsmo A. Substrate colonization strain competition, enzyme production in vitro and bio-control of *Pythium ultimum* by *Trichoderma* spp. isolates P1 and T3. *Eur. J. Plant Pathol*, 2000; **106** (3): 215-225.
26. Pisi A, Roberti R, Zakrisson E, Filippini G, Mantovani W, Cesari A. SEM investigation about hyphal relationships between some antagonistic fungi against *Fusarium* spp. foot rot pathogen of wheat. *Phytopathol. Mediterr.*, 2001; **40**:37-44.
27. Golve VM, Kurundkar BP. Biological control of pigeonpea wilt with *Pseudomonas fluorescens*. *J. Mycol. Pl. Pathol*. 2002; **44**: 573-578.
28. Gams, W. and Bissett, J. Morphology and identification of *Trichoderma*. In: Kubicek, C.P. and Harman, G.E. (eds.). *Trichoderma and Gliocladium: Basic biology, taxonomy and genetics*. Taylor & Francis Ltd, 2002; pp. 3-31.
29. Samuels, G.J., Chaverri, P., Farr, D.F. and McCray, E.B. *Trichoderma* Online. Systematic Mycology and Microbiology Laboratory, ARS, USDA; Retrieved 24, August 2014 from <http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm>
30. Howell CR, Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: history and evolution of current concepts. *Plant Dis.*, 2003; **89**:(11)1195-1200.
31. Eziashi EI, Uma NU, Adekunle AA, Airede CE. Effect of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium. *Afri. J. Biotechnol*. 2006; **5**(9):703-706.
32. Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathol*. 2006; **96**:(2)190-194.
33. Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M Sivasithamparam K. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. *Lett. Appl. Microbiol*. 2006; **43**(2):143-148.
34. Dubey S, Suresh M, Singh B. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. *Biol. Control*. 2007; **40**: 118–127.
35. Shaigan S, Seraji A, Moghaddam SAM. Identification and investigation on antagonistic effect of *Trichoderma* spp. on tea seedlings white foot and root rot (*Sclerotium rolfsii* Sacc.) in vitro condition. *Pak. J. Bio. Sci* 2008; **19**:2346–2349.
36. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. *Trichoderma*-plant-pathogen interactions. *Soil. Biol. Biochem*, 2008; **40**(1):1-10.
37. Jagathambigai V, Wijeratnam RSW, Wijesundera RLC. Control of *Fusarium oxysporum* wilt diseases of *Crossandra infundibuliformis* var. *danica* by *Trichoderma viride* and *Trichoderma harzianum*. *Asian. J. Pl. Pathol*. 2009; **3**:50-60.
38. Andrabi M, Vaid A, Razdan VK. Evaluation of different measures to control wilt causing pathogens in chickpea. *J. Plant Prot. Res*. 2011; **51**(1):55-59.
39. Rahman, A., Begum, M.F., Rahman, M., Bari, M.A., Ilias, G.N.M. and Alam, M.F. Isolation and identification of *Trichoderma* species from different habitats and their use for bioconversion of solid waste. *Turk. J. Biol.*, 2011; **35**: 183-194.
40. Mukherjee M, Mukherjee PK, Hoewitz BA, Zachov C, Berg G, Zeilinger S. *Trichoderma*-plant-pathogen interactions: Advances in genetics of biological control. *Indian J. Microbiol*. 2012; **54**: 522–529.
41. Hassan-El SA, Gowen SR, Pembroke B. Use of *Trichoderma hamatum* for biocontrol of lentil vascular wilt disease: efficacy, mechanisms of interaction and future prospects. *J. Plant Prot. Res* 2013; **53**:1.
42. Singh A, Mo S, Srivastava M, Kumar V, Bansal A. Antagonistic activity of *Trichoderma viride* against different pathogens of *Fusarium oxysporum* isolated from legume crops of UP. *Progr. Res*. 2013; **8**(1): 47-50.
43. Qualhato TH, Lopes FAC, Steindorff AS, Brandaõ RS, Santos R, Jesuino A, Jose CU. Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production. *Biotechnol. Lett*. 2013; **35**:1461–1468.
44. Amrutha VG, Reddy NPE. Mycoparasitism of *Trichoderma* spp. on *Rhizoctonia bataticola* the causal agent of dry root rot of chickpea. *Int. J. App. boil. & Pharma. Tech* 2014; **5**: 1 95.

45. Singh A, Srivastava M, Kumar V, Sharma A, Pandey S, Mo. S. Exploration and Interaction of *Trichoderma* species and their metabolites by Confrontation assay against *Pythium aphanidermatum*. *Int. J. Sci. and Res.* 2014; **3**:7.