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Invitro mycoparasitism activity of *Trichoderma* spp against *Fusarium solani* inciting root rot of chickpea (*Cicer arietinum* L.)

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ABSTRACT

Root rot of chickpea (*Cicer arietinum* L.) is caused by *Fusarium solani*. This paper describes the efficacy of *Trichoderma* spp. against sensitive and resistant isolates of *F, solani* by dual culture method under *invitro* conditions. *Trichoderma virens, T. atroviride, T. viride, T. harzianum, T. koningiopsis, T. stilbohypoxyli,and T. pseudokoningii* species were used for the antagonistic study. Results indicate that all *Trichoderma* species showed great antagonistic activity. But among them, *T. virens, T. atroviride, T. viride* showed 90% and 80 % antagonistic activity than others in case of a sensitive isolate of test fungus. The resistant isolate of the pathogen was restricting the antagonism to some extent.

Keywords: Chickpea (*Cicer arietinum* L.), *Fusarium solani, Trichoderma* species dual culture.

INTRODUCTION: The main cause of reduction of the crop yield is the diseases. Plant diseases are infections that are caused by a variety of pathogens namely bacteria, fungi, viruses, nematodes, insects. According to the American Phytopathological Society (APS) fungi are the No. 1 cause of crop yield loss from 10-100% worldwide. They cause severe diseases like root rot, late blight, downy mildew, wilt, pulse seed-borne diseases, powdery mildew, rusts, and smuts which having a significant impact on yield and quality, hence managing them becomes is first part of crop production (Chiranjeevi *et al.*, 2002).

Pulses are an important part of the daily diet for most Indians as they contain 2 to 3 times more protein than cereals. Chickpea (*Cicer arietinum* L.) is the most important pulse food crop among major rabi pulses of India and belongs to the family Leguminosae. Chickpea is not only important human food but also used in traditional farming systems. According to Chiranjeevi *et al.* (2002). the dry land, fixes atmospheric nitrogen in the soil and increases soil fertility. It has very great nutritional value. According to Cooke (1908) after dehulling chickpea is valued for its nutritive seeds with a protein content of 25.3 to 28.9%. Raw chickpea seeds contains per 100g: 357 calories, 140-440 mg Ca, 2-4.8g ash, 190-382mg P, 4.5-15.69% moisture 0.8 to 6.4% fat, 9mg Fe, 1.3-2.9mg niacin, 0.12-0.33 mg riboflavin and 0-225µg β carotene (Chet *et al.*, 1981). According to Chiranjeevi *et al.* (2002), chickpea is the very richest source of fiber, fat, and proteins. Chickpea is used as medicine for cholera, diarrhea, snakebite, warts and blood purification. Chickpea is the most hypocholesterolemic legume among all food legumes. Seeds are antibilious. Chickpea seeds are eaten fresh, roasted, and boiled. Seed flour can be used to make soup, tasty food stuffs, bread and served as a side dish. The major regions where chickpea is cultivated in India are Maharashtra, Uttarpradesh, Karnatak, Haryana, West Bengal, Gujrat, Bihar and Chhattisgarh. At present, chickpea production has either remained static or decreased is mainly due to the diseases and poor management practices. Nene *et al.* (1984) reported that is about 67 fungi, 3 bacteria, 22 viruses, and 80 nematodes. The major fungal diseases are ascochyta blight, black root rot,Rhizoctonia dry root rot, verticillium wilt, rust, etc. Among these black root rot of chickpea caused by *Fusarium solani* (Mart.) Sacc. is a very serious disease in India. Tewari and Mukhopadhyay (2001) reported that black root rot is caused by *Fusarium solani* (Mart.) Sacc. is one of the most serious diseases, which causes severe yield loss of up to 60 to 80%. The disease shows both the symptoms of wilt and root rot. Nene *et al.* (1984) observed that the disease can appear at any stage of the crop, symptoms in a highly susceptible cultivar can develop any time between 25 days after sowing till as late as the podding stage. Chickpea black root rot spread through the soil as mycelium grows on roots and enters through soil water, farm types of equipment and wounds. Initially, infected crop does not show external rotting, but their roots spread vertically and show blackish rotten patches on the internal tissues. The main symptoms of the diseased plant are get stunted growth, yellowing and drying of leaves, and browning of vascular bundle. Mycelium enters into xylem vessels and acquires the whole vascular system of the host results in to wilting and yellowing of the plant. According to Cooke (1908), in the absence of a host *Fusarium solani* can survive in the soil for up to six years. The disease has assumed great importance in Maharashtra state during the past few years due to severe yield loss.

OBJECTIVES: Thus the present study was aimed to evaluate the antagonistic activity of *Trichoderma* spp. in laboratory conditions.

MATERIALS AND METHODS

Isolation and identification of test pathogen: Surveys were conducted in chickpea growing areas of different districts of Maharashtra state. It suffers severely by root rot disease incited *F. solani. In vitro* screening with our arbitrary system of bio-antagonists effective against soil-borne pathogens is a simplistic approach to understanding a small sector of the biological system in disease control. Root rot infected materials were collected and cut into small pieces (2mm) by the sterilized

blade. The pieces were then washed with sterilized distilled water thrice and dried by sterilized blotting paper. In each case, surface disinfested tissue plated on Czapeckdox agar (CDA) medium & produced a *F. solani* (Simmons, 2007).

Isolation of *Trichoderma* spp: Rhizosphere soils of irrigated and non irrigated plants were collected from different regions of Maharashtra. From the rhizosphere soil samples, desired *Trichoderma* species were isolated by using Czapeckdox agar (CDA) and *Trichoderma* selective medium (TSM) by dilution plate technique (Johnson, 1957). The isolated species were identified by reculturing on other Petri plates containing sterilized TSM. The isolated species were identified up to species level based on colony characters, growth of fungus, and structure of mycelium, conidiophores, and conidia (Harman and Kubicek, 2002). All *Trichoderma* spp. were purified by the hyphal tip technique. The isolated strains of *Trichoderma* spp were maintained throughout the study by periodical transfers on PDA and TSM slants under aseptic conditions to keep the culture fresh and viable.

Dual culture experiment: The Antagonistic efficacy of different species of *T. virens, T. atroviride, T. viride, T. harzianum, T. koningiopsis, T. stilbohypoxyli,* and *T. pseudokoningii* were tested against the isolated sensitive and resistant pathogenic fungus by dual culture experiment (Morton and Stroube, 1955). *Trichoderma* spp. and test fungus were inoculated at 7cm apart. Three replicates were maintained for each treatment and incubated at 28 ± 2° C for 7 days. Monoculture plates of both served as control. Seven days after incubation radial growth of test fungus and *Trichoderma* isolates were measured. The colony diameter of test fungus in dual culture plate was observed and compared with control. The growth inhibition was calculated by using the formula: 100 XC - T/C, Where C = growth in control and T = growth in treatment (Vincent, 1947).

Statistical analysis: Statistical analyses of the experiments were performed by using the book of (Jolicoeur, 2012).

RESULTS AND DISCUSSION

Isolation and identification of test pathogen: Diseased root rots were found as dark blackish coloured spots on roots. The plant also shows wilting symptoms, defoliation, and loss of chlorophyll. Such symptoms were collected from different locations of Maharashtra and twenty-five isolates of *F. solani* were isolated. The culture was deposited at the Plant Pathology Laboratory Department of Botany Shivaji University Kolhapur.

Isolation of *Trichoderma* spp: Isolates of seven species of *T. virens, T. atroviride, T. viride, T. harzianum, T. koningiopsis, T. stilbohypoxyli, T. pseudokoningii*. were isolated from irrigated and non-irrigated rhizosphere soil.

Dual culture: Results indicated that all *Trichoderma* species showed antagonistic activity. *T. virens, T. atroviride, T. viride* showed 90% and 80% antagonistic activity than others in case of a sensitive isolate of test fungus. A resistant isolate of the pathogen was restricting the antagonism to some extent. Overall, all *Trichoderma* species were found more than 60% antagonistic nature (table 1, figure 1&2). Several workers have been reported that the use of *Trichoderma* species against the number of plant pathogenic fungi (Harman, 2006; El-Mougy *et al.*, 2007; Ramos *et al.*, 2007). Akbari and Parakhia (2007) reported *T.viride*-I and *T.hamatum*-IV&V isolates showed strong antagonism against *Alternaria alternata* causing blight of sesame. The high inhibitory effect of volatile toxic substances emitted by *Trichoderma* spp. on the radial growth of *Fusarium* spp. has also been reported by Dubey *et al.* (2007). The inhibition was high with the direct use of *Trichoderma* spp. in dual culture against *F. oxysporum f sp psidii* (61-69%) & *F. solani* (58-68%) (Gupta and Misra, 2009). Kumar *et al.* (2007) tested three species of *Trichoderma* i.e. *T.virens, T. viride* & *T. harzianum* against *F.moniliforum var subglutinas,* and found them effective.

CONCLUSION: *Trichoderma* species play an important role in controlling fungal plant pathogens. The use of *Trichoderma*-based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost-effective, easy to produce, and easy to apply formulations. Our results concluded that the tested *Trichoderma* spp reduced the growth of *Fusarium solani*.

Among the *Trichoderma* species, *T. viride* showed the best performance *in vitro* biological control of *F. solani* followed by others (Sahi and Khalid, 2007). *Trichoderma viride* reached the confluence of the Petri dish four days after sowing so that different fungal isolates occupy a surface of 29% to *F. roseum* (Bouziane *et al.*, 2011). Waghmare and Kurundkar (2011) reported the efficacy of *Trichoderma* species against *Fusarium oxysporum* f. sp. *carthami* causing wilt of safflower and isolates no. 29 and 33 were found to minimum growth of the pathogen as compared to others. The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda *et al.*, 2011).

Trichoderma species	Isolates	Radial growth of *F.solani*	Radial growth of *Trichoderma* species	% Inhibition
Trichoderma virens	S	10	80	90.00
	R	18	70	77.77
Trichoderma atroviride	S	18	72	80.00
	R	21	69	78.00
Trichoderma viride	S	30	60	66.66
	R	21	69	76.66
Trichoderma harzianum	S	22	68	76.00
	R	25	65	72.22
Trichoderma koningiopsis	S	20	72	79.00
	R	18	70	77.00
Trichoderma stilbohypoxyli	S	22	68	76.00
	R	25	65	72.22
Trichoderma pseudokoningii	S	22	68	76.00
	R	25	65	72.22
CD (p=0.06)				

Table 1: Influence of *Trichoderma* species against *F. solani*.

REFERENCES: Akbari, L. and A. Parakhia, 2007. Eco-friendly approaches to manage blight of sesame. Journal of mycology and plant pathology, 37(3): 389-400.

Bouziane, Z., L. Dehimat, W. Aziz, M. Benabdelkader and N. K. Chaouche, 2011. The antagonism between *Trichoderma viride* and other pathogenic fungal strains in *Zea mays*. Agriculture biology journal of North America, 2(4): 584-590.

Chet, I., G. Harman and R. Baker, 1981. Trichoderma hamatum: Its hyphal interactions with *rhizoctonia solani* and *pythium*

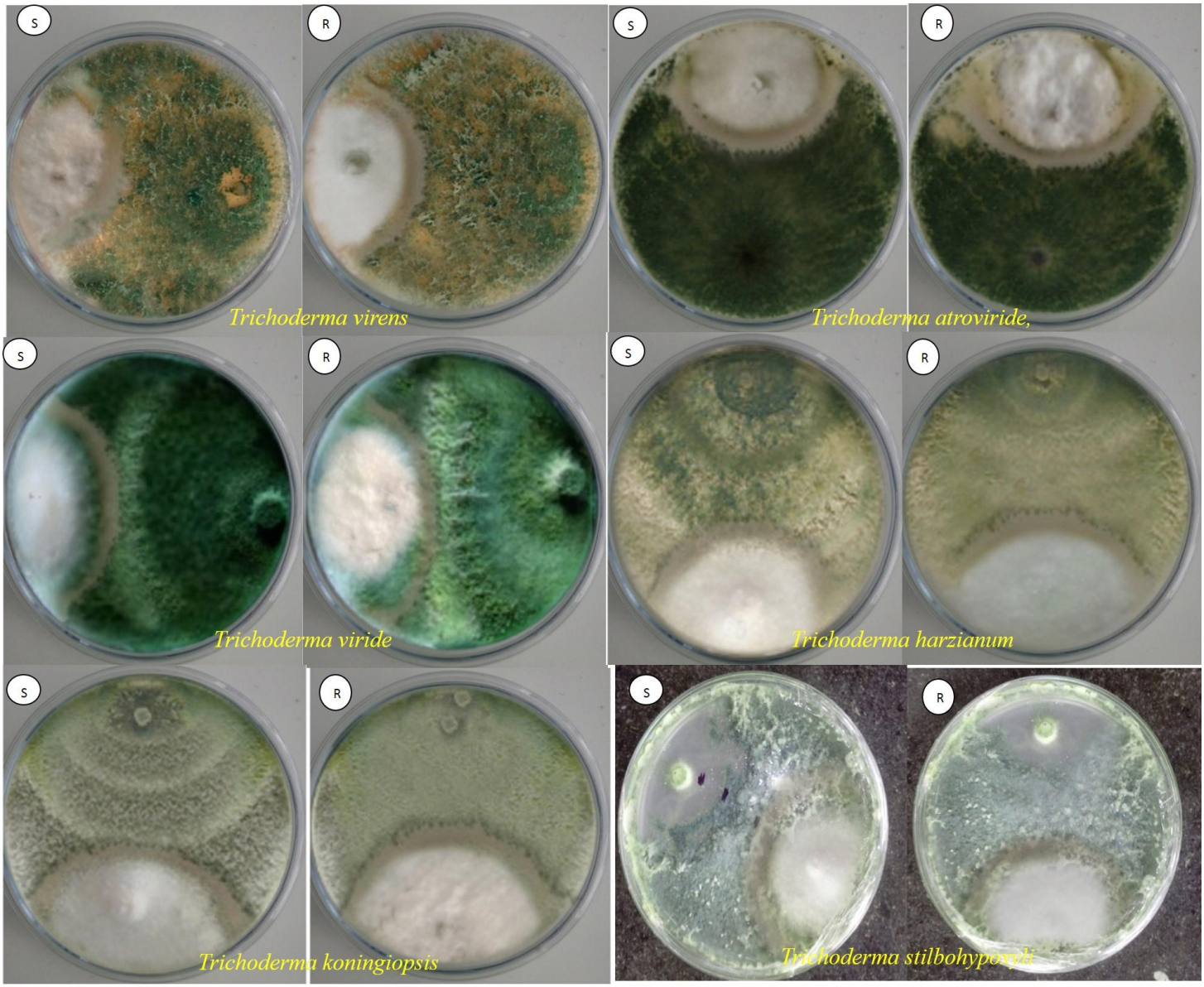

Figure 1: *In vitro* inhibition of growth of *F. solani* by *Trichoderma* spp. in dual culture method.

Figure 2: *In vitro* inhibition of growth of *Fusarium solani* by *Trichoderma* spp. in dual culture method.

spp. Microbial ecology, 7(1): 29-38.
Chiranjeevi, C., I. P. Reddy, M. Naryanamma and Neerja, 2002. Effect of shoot clipping and insecticides on the incidence of fruit borer in brinjal. In: International conferece on vegetables. Bangalore, India.
Cooke, T., 1908. The flora of the presidency of bombay. Bishen Singh Mahendra Pal Singh.
Dubey, S. C., M. Suresh and B. Singh, 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. Sp. Ciceris for integrated management of chickpea wilt. Biological control, 40(1): 118-127.
El-Mougy, N. S., N. G. El-Gamal and M. M. Abdel-Kader, 2007. Control of wilt and root rot incidence in *Phaseolus vulgaris* L. By some plant volatile compounds. Journal of plant protection research, 47(3): 255-265.
Gupta, V. and A. Misra, 2009. Efficacy of bioagents against fusarium wilt of guava. Journal of mycology plant pathology, 39(1): 101.
Harman, G. E., 2006. Overview of mechanisms and uses of *Trichoderma spp*. Phytopathology, 96(2): 190-194.
Harman, G. E. and C. P. Kubicek, 2002. Trichoderma and Gliocladium: Basic biology, taxonomy and genetics. CRC Press.
Johnson, L., 1957. Effect of antibiotics on the numbers of bacteria and fungi isolated from soil by the dilution-plate method. Amer Phytopathological Soc 3340 Pilot Knob Road, St Paul, Mn 55121, Phytopathology: pp: 630-631.
Jolicoeur, P., 2012. Introduction to biometry. Springer Science & Business Media.
Kumar, P., A. Misra and B. Pandey, 2007. In vitro evaluation of *trichoderma spp.* Against vegetative mango malformation pathogen *Fusarium moniliforme* var. Subg/utinans. Journal of ecofriendly agriculture, 2: 187-189.
Morton, D. and N. Stroube, 1955. Antagonistic and stimulatory effects of microorganism upon *Sclerotium rolfsii*. Phytopathology, 45(8): 419-420.
Nene, Y., M. Sexena and K. Singh, 1984. A review of ascochyta blight of chickpea. Martinus Nijhoff/Dr. W. Junk Publishers. The Haque, Netherland, 1(1): 17-36.
Rajkonda, J., V. Sawant, M. Ambuse and U. Bhale, 2011. Inimical potential of *Trichoderma* species against pathogenic fungi. Plant sciences feed, 1(1): 10-13.
Ramos, B., F. M. Alves-Santos, M. A. García-Sánchez, N. Martín-Rodrigues, A. P. Eslava and J. M. Díaz-Mínguez, 2007. The gene coding for a new transcription factor (ftf1) of *Fusarium oxysporum* is only expressed during infection of common bean. Fungal genetics biology, 44(9): 864-876.
Sahi, I. Y. and A. Khalid, 2007. In vit o biological control of *Fusarium oxysporum-r* causing wilt in *Capsicum annuum*. Mycopathology, 5(2): 85-88.
Simmons, E., 2007. Alternaria. An identification manual. Utrecht.
Tewari, A. and A. Mukhopadhyay, 2001. Testing of different formulations of *Gliocladium virens* against chickpea wilt-complex. Indian phytopathology, 54(1): 67-71.
Vincent, J., 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159(4051): 850-850.
Waghmare, S. and B. Kurundkar, 2011. Efficacy of local isolates of *Trichoderma spp.* Against *Fusarium oxysporum F. Sp. Carthami* causing wilt of safflower. Advances in plant sciences, 24(1): 37-38.