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INVITRO AGGRESSIVENESS OF *TRICHODERMA* SPP AGAINST *FUSARIUM OXYSPORUM* INCITING ROOT ROT OF SOYBEAN.

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ABSTRACT

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

KEYWORDS: Soybean (*Glycine max* L.), *Fusarium oxysporum, Trichoderma* species dual culture.

INTRODUCTION

The main cause of reduction of the crop yield are the diseases. Plant diseases are infections which are caused by variety of pathogens namely bacteria, fungi, viruses, nematodes, insects etc. According to the American Phytopathological Society (APS) fungi are the No. 1 cause of crop yield loss from 10 to 100 % worldwide. They causes the severe diseases like root rot, late blight, downy mildew, wilt, pulse seed-borne diseases, powdery mildews, rusts and smuts which having a significant impact on yield and quality, hence managing them becomes the first part of crop production (Chiranjeevi *et al*., 2002). Soybean [Glycine max (L.) Merrill.] is a native of Northern China. It is the most important legume crop in the world. Soybean is also called ‘Golden bean’, ‘Miracle bean’ and ‘Crop of planet’. Soybean is capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with

Rhizobium bacterium, so the crop improves soil fertility and economizes crop production not only for themselves but also for the next crop grown in rotation especially, cereal crops (Cook, 1967).

Soybean has great nutritional value. It is easily digestible and is considered as one of the richest and cheapest source of proteins, hence called as 'Poor man's meat'. It is one of the world's most important source of oil and protein. Soybean contains 30% oil of dry seed weight and it is an important source of protein, which reaches 50% of dry seed weight along with calcium, iron, zinc, carotene, thiamine and ascorbic acid (Chet *et al*., 1981; Kumar and Mukerji, 1996). It has great medicinal importance as soya based food helps to control diabetics and melts all kinds of stones in the urinary bladder. Seeds were primarily used as pulses by the local population and the green and dried vegetative part were used as foliage for cattle.

In addition, 100g soybean contains 250 mg calcium, 590 mg phosphorous, 12.50 mg iron, 462 calories, 10.50 g fats and 466 mg vitamins A, B and D. The major soybean growing countries in the world are USA, Brazil, Argentina, China, India and European community (Mukhopadhyay, 2005).

Soybean serve as one of the valuable crop in India with innumerable possibilities of not only improving agriculture but also supporting industries, its cultivation has increased tremendously. In India, it is grown on an area of 11.25 million hectare with a production of 14.22 million metric tonnes and productivity of 1268 kg/ha in the year 2017 (Mukhopadhyay, 2005). Amongst the soybean producing nation, India occupies dismal fifth position in production though we are third after USA and China in area.

Among the different states in India, Maharashtra is having total area of 39.24 lakh hectare and production of 51.37 lakh metric tonnes with productivity of 1309 kg/ha. (Chet *et al*., 1981; Kumar and Mukerji, 1996).

Root rot of soybean

Nene (1985) reported more than 68 pathogens so far from different soybean growing countries. According to Nene (1985) major fungal diseases of soybean are root rot (*Fusarium oxysporum* Schlecht.), leaf spot (*Alternaria alternata*), Charcoal rot (*Macrophomina phaseolina*), Damping-off (*Rhizoctonia solani*) but among these root rot of soybean is very

serious disease in India which cause 80 to 90% yield loss. According to Tewari and Mukhopadhyay (2003) root rot of soybean caused by *Fusarium oxysporum* is one of the most serious disease which shows both the symptoms of wilt and root rot.

Nene (1985) observed that the disease can appear at any stage of the crop, symptoms in a highly susceptible cultivar can develop any time between 20 days after sowing till as late as podding stage. Root rot of soybean spread through soil as mycelium grows on roots and enters through soil water, farm equipments and wounds (Agrios, 1984).

The main symptoms are spreading of roots vertically and shows dark brownish to blackish rotten patches on the internal tissues, diseased plant are get stunted, yellowing and drying of leaves and browning of vascular bundle. Drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally death of plants (Chet *et al*., 1981; Kumar and Mukerji, 1996) Mycelium enters in to xylem vessels and acquire whole vascular system of host results in to blockage of water and mineral transport results in to wilting and yellowing of plant. According to Kumar and Mukerji, (1996) in the absence of host *Fusarium oxysporum* can survive in the soil up to five years. The disease has assumed great importance in Maharashtra state during the past few years due to severe yield loss.

Therefore, biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi (Chet *et al*., 1981; Kumar and Mukerji, 1996). During the last decade, species of *Trichoderma* have emerged as the most powerful bioprotectants for the management of a wide variety of plant diseases by virtue of their broad spectrum action against a number of plant diseases caused by fungi, bacteria, viruses and even nematodes (Mukhopadhyay, 2005). 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 Soybean growing areas of different districts of Maharashtra state. It suffers severely by root rot disease incited *Fusarium oxysporum. In vitro* screening with our arbitrary system of bio-antagonists effective against soil borne pathogens is a simplistic

approach to understand a small sector of biological system in disease control. Root rot infected materials were collected and cut into small pieces (2mm) by 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 potato dextrose agar (PDA) medium & produced an *Fusarium oxysporum* species (Simmons, 2007; Subramaniam, 1971; Ellis, 1971).

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 potato dextrose agar (PDA) and *Trichoderma* selective medium (TSM) by dilution plate technique (Johnson, 1957). The isolated species were identified by reculturing on another petriplates 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 (Kubicek and Harman, 2002). All *Trichoderma* spp. were purified by 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 *Trichoderma atroviride Trichoderma viride*, *T. harzianum*, *T. virens*, T. *koningii* 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 was inoculated at 7 cm 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. 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 X C - 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 Mungikar (1997).

RESULTS AND DISCUSSION

Isolation and identification of test pathogen

Diseased root rots were found as a dark blackish coloured spots on roots. Plant also shows wilting symptoms, defoliation and loss of chlorophyll. Such symptoms were collected from different locations of Maharashtra and fifteen isolates of *Fusarium oxysporum* were isolated. The culture was deposited at Plant pathology laboratory department of botany Shivaji University Kolhapur.

Isolation of *Trichoderma* spp

Isolates of six species of *Trichoderma atroviride*, T. *viride, T. harzianum, T. virens, T. koningii., T. pseudokoningii*. were isolated from irrigated and non-irrigated rhizosphere soil.

Dual culture

Results indicated that all *Trichoderma* species showed antagonistic activity. *T.atroviride, T.koningii* and *T. harzianum* showed 90% and 80 % antagonistic activity than others in case of sensitive isolate of test fungus. Resistant isolate of pathogen was restricting the antagonism in some extent. Overall, all *Trichoderma* species were found more than 60% antagonistic nature (Table1) (Plate 1).

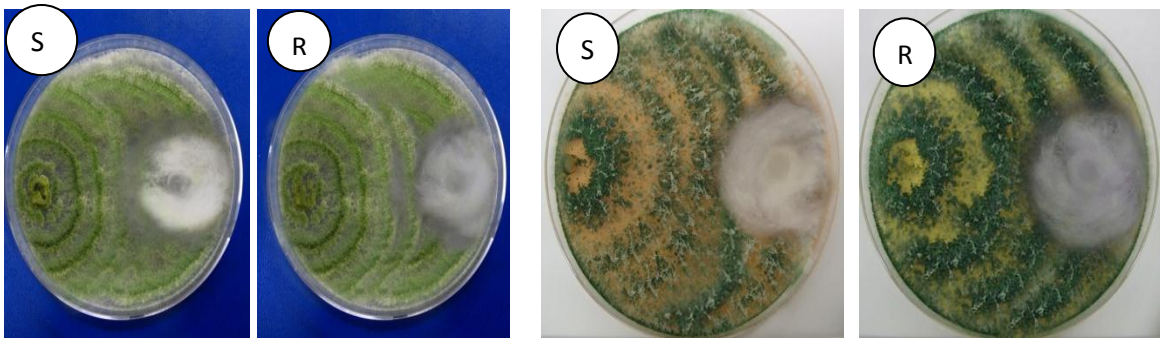
Several workers have been reported that the use of *Trichoderma* species against number of plant pathogenic fungi (Brisa *et al.*, 2007; El-Mougy *et al.*, 2007; Harman, 2006).Akbari and Parakhi (2007) reported *T.viride*-I and *T.hamatum*-IV&V isolates showed strong antagonism against *Alternaria alternata* causing blight of sesame. 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 *Fusarium oxysporum f sp psidii* (61-69%) & *F.solani* (58-68%) (Gupta and Mishra, 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. Among the *Trichoderma* species *T. viride* showed the best performance *in vitro* biological control of *Fusarium oxysporum* followed by others(Irfan 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 *Fusarium roseum* (Bouziane *et al*., 2011). Recently, Waghmare and Kurundkar (2011) reported 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).

Table 1: Influence of *Trichoderma* species against *Fusarium oxysporum*.

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

S-Sensitive R- Resistant



T. atroviride ***T. harzianum***

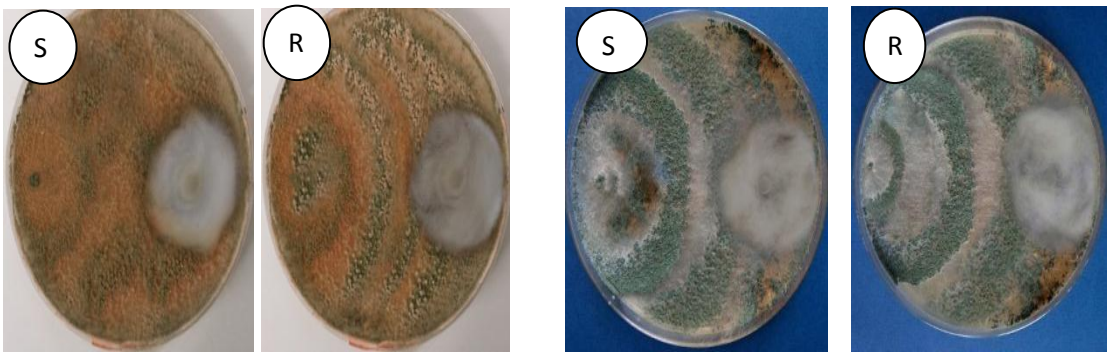


Fig. 1. *In vitro* inhibition of growth of *Fusarium oxysporum* by *Trichoderma* spp. in dual culture method.

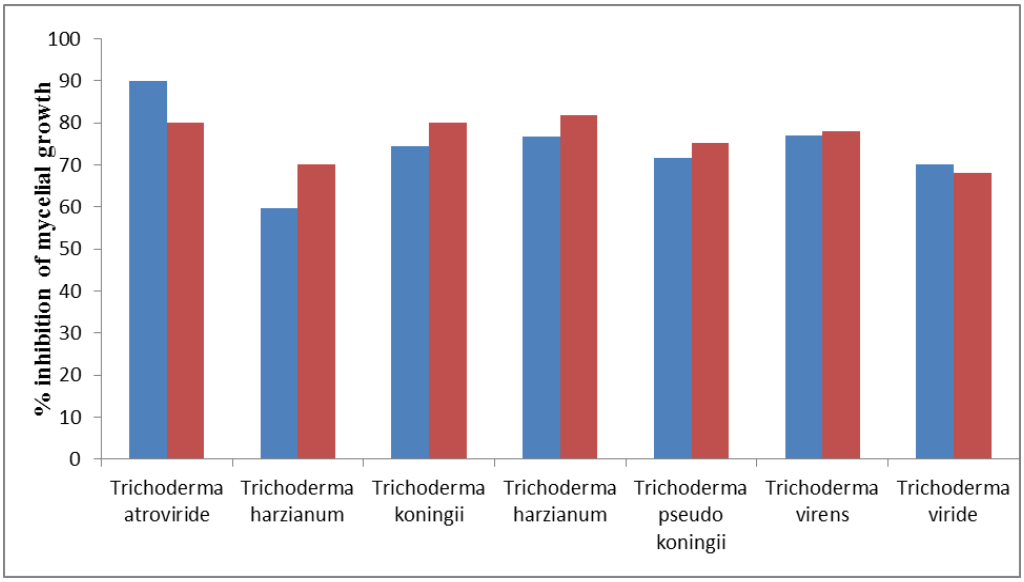


Fig 2: *In vitro* inhibition of growth of *Fusarium solani* by *Trichoderma* spp. in dual culture method.

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 oxysporum*.

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