Plant Archives Vol. 15 No. 2, 2015 pp. 667-670 *ISSN 0972-5210*

PERFORMANCE OF A HIGHLY POTENT STRAIN OF *TRICHODERMA HARZIANUM* POSSESSING EGG PARASITIC CHARACTER AGAINST ROOT KNOT NEMATODE IN ADDITION TO MYCOPARASITISM ISOLATED FROM VEGETABLE GROWING AREAS OF NORTHERN INDIA

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Abstract

In the present paper, seven isolates of *Trichoderma* particularly *T. harzianum* collected from vegetable growing areas of Punjab, Haryana and three districts of Western U.P. tested separately against the pathogenic wilt causing fungus, *Fusarium oxysporium* f. sp. *lycopersici* through *in vitro* dual culture test. In this test, the isolates of fungal bioagent collected from rhizosphere and rhizoplane of solanaceous crops separately exhibited distinct variation in their potentiality against the pathogenic fungus. Out of all the seven isolates, the one collected from district Gautam Buddha Nagar of Western U.P. (India) exhibited highly potential properties in inhibiting the pathogenic wilt fungus followed by the strain from Ludhiana, all other strains under investigation also showed remarkable performance. The response of the same isolate tested against eggs of *Meloidogyne incognita* also expressed high egg parasitization. Thus, both myco and egg parasitization properties have been confirmed on the present locally isolated strain from through *in vitro* studies. The strain was thereafter selected and maintained on PDA and starch rich sorghum grains for both, field application alone and also as core component of the evolved and evolving indigenous IPM packages.

Key words : *Trichoderma harzianum*, *Fusarium oxysporium* f. sp. *lycopersici*, *Meloidogyne incognita,* egg parasitization, indigenous IPM package.

Introduction

During a series of surveys of the vegetable growers' fields in and around villages of Punjab, Haryana and Western U.P. during 2012- 2014, a remarkably wide number of solanaceous vegetables were recorded to be heavily infested with root knot nematodes and soil borne wilt causing fungus as well, the former having been reported to predispose the hosts for the fungal attack causing disease-complexes with synergistic effect leading to much more damage to the common hosts 35%-80% (Powell, 1971 and Bhagwati *et al*., 2000).

It was found that in efforts to get increasingly higher yields, the ignorant farmers of surveyed area of Northern India were indiscriminately overusing very high doses of toxic and expensive chemical pesticides and fertilizers to combat soil borne diseases including root knot nematode leading to environmental health hazards ruining also the soil fertility, natural biodiversity and health of the consumers including the farmers themselves by wrecking their immune system.

Although chemical pesticides *viz*. DD, DBCP, MBr as fumigants have been widely used around many developed European countries including US during 60s and 70s. All these chemicals in addition to few more in recent years *viz*. like, Endosulphan, Phorate Chloropicrin etc have been banned onwards 1982 worldwide, mainly causing environmental health hazards and for their carcinogenic properties. In recent years as an alternative therefore ecofriendly methods for the management of pests, diseases and root knot nematode are attempted by Plant Protectionists through the use of sustainable/non-chemical components in the form of package out of which fungal bioagents are in forefront.

Among the beneficial mycoflora, the role of *Trichoderma* spp. have been considered globally the most outstanding member to combat the soil borne fungi including a number of genera like *Fusarium*, *Rhizoctonia*,

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Pythium etc. and also against soil borne root knot nematodes infecting a wide range of field crops (Lych, 1987; Goswami and Singh, 2002; Goswami *et al*., 2005).

It is estimated that 90% of all anatagonistic fungi used in Plant Protection belong to the genus *Trichoderma* (Benitez *et al*., 2004). The multifarious activity of *T.harzianum* provides a potential for their extensive applications in different branches of economy this species are frequently used in the degradation of organic pollutants as bioremediation it detoxifies phenols, cyanides and nitrates(Lynch and Moffat, 2005) Regarding the potentiality of two species of *Trichoderma*, in recent years the potentiality of *T. harzianum* is reported to be substantially higher then *T. viride*, which also grow faster both on PDA and also on starch rich sorghum grains (Goswami and Neetu Singh, 2013). In the present study, an attempt has been made to find out most potent isolate which is considered as the core component for improvising the performance of evolving packages along with other sustainable components *viz*. vermicompost, oilseed cakes, botanical antagonist and AM fungus a phosphate solubilizing biofertilizer.

Materials and Methods

Extensive surveys of vegetable growing areas particularly tomato, brinjal and okra around some districts of Northern India during 2012-2014 concentrating on collection of *Trichoderma* spp particularly *T. harzianum* were carried out through collection of soil and root samples from the rhizosphere and rhizoplane of major soil borne diseases including root knot nematode affected crops. Soil samples out of above collection were subjected to soil dilution method (Martin, 1950) through which mycoflora appeared on Potato dextrose agar medium on incubating at 22±2^{0}C in BOD Incubator.

Among the mycoflora appearing from the above colonies the consistently occurring ones *viz. Aspergillus niger*, *A. terrus*, *Penicillium oxalicum*, *Neocosmospora vasinfectum*, *Trichoderma harzianum*, *Gleocladium virens*, *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani* were isolated, subcultured and identified (Barnett and Hunter, 1987). All the species were separately maintained on PDA and PD broth (Lilly, 1965) for *in vitro* studies.

Mycoparasitizm of *T. harzianum* : The seven strains of *T. harzianum* were evaluated separately against *Fusarium oxysporum* f. sp. *lycopersici* by the dual culture technique (Dennis and Webster, 1971) by using measured amount of PDA medium (25ml) in each plate maintaining three replicates. The petriplates were allowed to keep under incubator at 22±2^{0}C, which was observed after a week and inhibition percentage was calculated by the formula described by Vinset and Budget (1990).

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

Where, I = Per cent inhibition, C = Colony diameter of radial growth in control and T = Colony diameter of radial growth in the treatment.

Egg parasitization

All the seven isolates of *T. harzianum* were grown separately on PDA on 90 mm diameter Petri dishes aseptically and incubated for five days alongwith two egg masses each then the egg masses transfer to 1% Agar and incubated at 26^0 C to allow the egg parasitic to grow the egg masses with fungal growth were isolated, teased and stained with cotton blue lectophenol followed by observation under stereo binocular. The number of eggs parasitized in each egg mass was counted and percentage of parasitized eggs was calculated.

Results and Discussion

Heavy infestations of major soil borne maladies *viz*. root knot nematodes and wilt causing fungus both known as 'vascular feeders' invading alone and concomitantly on the common host resulting in severe damage in crop productivity ranging from 30%-80% to the field crops (Powell, 1971; Bhatti and Jain, 1977; Goswami and Singh, 2010). Consequently being a matter of great concerns, the farmers, out of ignorance and also in a notion to get instant results, promptly started using indiscriminately over dose of toxic chemical pesticides which, in turn, caused heavy damage to the soil, plant and finally to human health by wrecking their immune system. Instances were also gathered about the farmers being compelled to commit suicides due to falling into debt mainly because of high cost farming.

With the mission to combat the above maladies and also for improving the soil, plant and human health, the authors initiated attempts in evolving economic and farmers' friendly management package comprising of sustainable components like neem oil seed cake and/or botanicals antagonist as soil health enricher alongwith application of the most vital partner of the package *i.e. T. harzianum* designated as 'core component' to combat the soil borne maladies.

The present investigation of searching for a potent isolate of the core component, *T. harzianum* the role of which is of paramount importance in reducing the

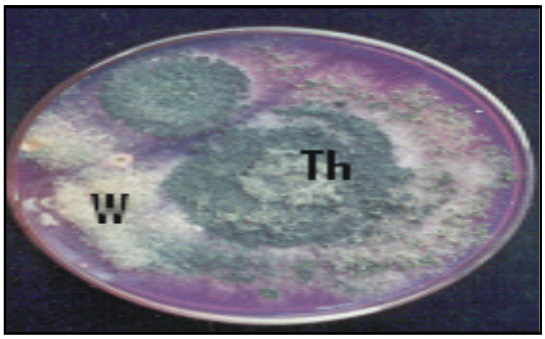

Fig. 1 : *T. harzianum* (Th5) masking the wilt fungus (W) in dual test (90.66%).

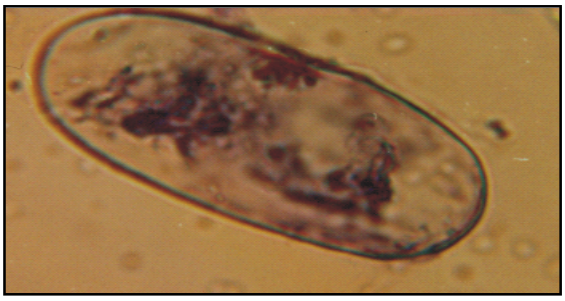

Fig. 2 : Egg of RKN parasitized by Th-5.

Table 2 : Evaluation of different isolates of *T. harzianum* on eggs of *M. incognita*.

S. no.	Fungal isolates	% Parasitized eggs
1	*T. harzianum* (Jalandhar)	53.50
2	*T. harzianum* (Ludhiana)	51.27
3	*T. harzianum* (Hisar)	46.75
4	*T. harzianum* (Karnal)	47.31
5	*T. harzianum* (Gautam Buddha Nagar)	69.11
6	*T. harzianum* (Bulandshahar)	59.83
7	*T. harzianum* (Ghaziabad)	61.33
8	Control	0.0
	CD @ 5%	2.37

approach is essential for the development of sustainable methods of biological control thus the key factors that influence interaction between the nematodes, fungi and plants are soil environment, microbial flora and host plants, Goswami *et al.* (2005) in support of the above observations initiated the investigation with a number of

Table 1 : Interaction between different isolates of *T. harzianum* against *F. oxysporum* f.sp. *lycopersici* in dual culture test (after a week).

S. no.	Test fungus	Colony diameter of *F. oxysporum* f.sp. *lycopersici* (mm)	Inhibition % (I) = C-T/ C × 100
1	*T.harzianum* (Jalandhar)	15.22	83.08
2	*T.harzianum* (Ludhiana)	14.81	83.54
3	*T.harzianum* (Hisar)	18.43	79.52
4	*T.harzianum* (Karnal)	16.99	81.12
5	*T.harzianum* (Gautam Buddha Nagar)	8.4	90.66
6	*T.harzianum* (Bulandshahar)	13.11	85.43
7	*T.harzianum* (Ghaziabad)	12.32	86.31
8	Control	90.00	0.0
	CD @ 5%	1.71	-

development of fungal diseases (Bell *et al.*, 1982; Papavizas, 1985; Lych, 1987; Tran, 1998; Benitez, 2004) and for its additional characters like stimulate plant growth, enhance their stress resistance and accelerate composting (Chet, 1987 and Harman, 2000).

In addition to growth hormonal and multifolded activities of fungi from *T. harzianum* providing a potential for their extensive applications in degradation of organic pollutants and bioremediation which detoxifies phenols, cyanides and nitrates (Lynch and Moffat, 2005).

Deacon (1991) considered that an ecological

isolates of *T. harzianum* collected from different agro climatic regions of the country and observed low, mild and highly potential explaining further the distinct variability on the potentiality of the strains belonging to the same species against both wilt causing fungus and root knot nematode showing mycoparasitism and/or egg parasitization respectively in addition to the toxic nature.

In the present investigation, out of seven isolates tested, the one collected from Gautam Buddha Nagar of Western U.P. exhibited most significantly outstanding in comparison to all other strains against wilt causing fungus

F. oxysporum f. sp. *lycopersici* through dual culture test (fig. 1) followed by isolate from Ghaziabad while the least was observed in the isolate collected from Hisar (table 1).

Another *in vitro* test of egg parasitization capacity by isolates of *T. harzianum* showed maximum invasion in isolate collected from GautamBuddha Nagar (69.11%) (fig. 2) followed by Ghaziabad (61.33%), Bulandshahar (59.83%), Jalandhar (53.50%), Ludhiana (51.27%) and the least egg parasitization was found in Hisar (46.75%) after 12 days (table 2).

In the present paper therefore the selection of the most potent isolate possessing both myco and egg parasitic nature collected from Gautam Buddha Nagar is expected to further improve the quality of the package which in turn shall prove to be ideal for a better quality and productivity of crops.

Acknowledgement

We acknowledge the constant encouragement and moral support of our Founder President Hon'ble Dr. Ashok Kumar Chauhan, Amity University, Sector – 125, Noida (U.P.), India. We also thank the Department of Science and Technology for financial support.

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