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Research Article

BIOLOGICAL CONTROL OF LESION NEMATODES *Pratylenchus spp.* BY VARIOUS ANTAGONISTIC FUNGI

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Abstract: The lesion nematodes *Pratylenchus spp*. is described as one to the major limiting factor in crop production especially in chickpea. The approach of nematode management with bio-control agents will provide an environmental sustainable and suitable for plant and animal health. Fifteen soil inhibiting fungi were isolated from the *Pratylenchus* prone soils of different localities and isolated fungi were identified and attempts were made to find out their antagonistic behavior against *Pratylenchus thornei*. Out of 15 fungi, *P. pinophilum, T. harzianum, T. viride, Crisosparium sp. (T), Crisosporium sp. (R), F. moniliforme, Cladosporium sp. and A. alternata* were exhibited positive correlation against the existing population of P. thornei and their antagonistic behaviour was tested against P. thornei. Reduction in population of *P. thornei* was 90 percent in the presence of *T. viride*. However, *T. harzianum, Crisosporium sp. (T) and (R), Cladosporium sp.* were also drastically declined the nematode population. Nematode population was drastically declined in the presence of *T. viride* within 72 hours and further reached minimum after 168 hours, similar trend was shown by other test antagonistic fungi.

Keywords: *Lesion nematode, Pratylenchus sp,, P. pinophilum, T. harzianum, T. viride, Crisosparium sp. (T), Crisosporium sp. (R), F. moniliforme, Cladosporium sp. and A. alternata*

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Introduction

Chickpea (*Cicer arietinum* L.) is the most important Rabi pulse crop not only in Madhya Pradesh and ranked third among the important pulse crops of the world. It is mainly confined to Indian sub-continent where India alone contributed more than 70% of the global chickpea production. The important chickpea growing state of the India are Madhya Pradesh, Uttar Pradesh, Haryana, Panjab, Rajasthan and Maharashtra. Chickpea occupied in an area of 8.00 million hectare with the total production of 6.00 million tonnes. Madhya Pradesh alone contribute 2.68 million hectares of the total area and production of 2.48 million tonnes [1]. In India (9,10) reported the pathogenic effect of *Pratylenchus thornei on*. In recent years, there has been a surging interest in Phytoparasitic nematodes affecting crop plants leading to considerable losses. Soil samples collected during 1979 around the rhizoplane and rhizosphere of crops in Madhya Pradesh predominantly revealed the occurrence of *Pratylenchus* species [2].

Sharma, (1985) catalogued 22 species of plant parasitic nematodes associated with chickpea in different parts of the monopathogenic situation seldom if ever exists in nature, the root system of crop plants remains exposed to a multitude of taxonomically diverse organisms in causation of damage to the host crop in fields [3]. Since, several species of *Pratylenchus* ubiquitously distributed around chickpea plants, it is expedient to quality and their role is causation of the diseases in field so as to develop an understanding towards appropriate viable and feasible control measures needed for reducing the crop losses due to this obnoxious organism. During the last decade it has been observed that the yield of chickpea seems to be declined and beside fungi lesion nematodes *Pratylenchus thornei* may be one of the major constraints in the production of chickpea.

Materials and Method

Extraction of Lesion Nematode Population

The collected soil and root samples were brought to the laboratory. Two hundred cubic centimetre (cm^3) thoroughly mixed samples were soaked in water for 3 hours before extraction. Cobbs sieving and decanting method and modified Baermann Funnel Technique Christie and Perry, (1951) was followed for the extraction of nematode population [4]. Nematode suspension containing freshly washed and sieved, on 325 mesh sieves was, poured with the help of a jet of water. Assembly was later kept on a glass bowl holding approximately 50 ml of aqua guard distilled water. Extraction assembly was so placed, that the upper layer of water in glass bowls touches the stretched base of extraction dish to ensure a thin film leaving no air bubble. The extraction was carried out at room temperature (20-32^{0}C) and different stages of the nematodes were collected after 24 and 48 hours of washing. The nematode extraction was examined under stereo-binocular (80 x) and nematode population was identified as *Pratylenchus thornei* on the basis of characters of the *Pratylenchus thornei* (Filipjev 1936) Sher and Allen 1953.

Isolation of Soil Fungi

Serial dilution procedure

Goss, (1972) described the dilution plate method for isolation of soil micro organisms [5]. One gram sieved soil on dry weight basis was dispensed aseptically in 10 ml sterile water in a culture tube and shaken for 20 min. on a mechanical wrist action shaker. The suspension was serially diluted aseptically till the dilution of 1:1000 was obtained.

One ml as the desired end dilution from each replicate was transferred aseptically to each of the three sterile petri-dishes. Pre-cooled, Martin's Rose Bengal medium (15 ml) was poured into each petri-dish. The contents immediately mixed by rotating the dishes in a circular and side to side motion before the medium got solidified. The plates were incubated at 25±1^{0}C for seven days. Identification of the colonies which were developed in petri-dishes was made with the aid of research microscope, after isolation of individual fungus in pure culture on PDA, by observing the typical characteristics described in the text [6-8].

Isolation of Fungus from infected plants

The fungus which were associated with lesion nematodes within roots of the infected plants were isolated by time segment method and purified by hyphal tip method and maintained on potato dextrose agar slants [9].

Preparation of Antagonistic lot

Fungi isolated from eight different blocks of Rewa district were selected for antagonistic study on *Pratylenchus thornei*. Fungi were cultured in Potato Dextrose Broth (P.D.B.) for three weeks incubated at 25^{0}C. The fungal mycelial mat was removed by decanting and taking out the filtrate and mycelial mat separately.

Culturing of nematode population on Chickpea Plants'

The nematode population was extracted from chickpea infested roots. The roots were free from soil and washed gently in running tap water. The roots were later chopped into small, segments of 1 to 2 cm length and placed on extraction dish for recovery of the nematode population. The extracted nematode population were collected and utilized for investigation whenever required. The extraction dish was covered by enamel trays to minimize the evaporation.

Test of Pathogenicity of lesion nematode on Chickpea

To study the pathogenicity of lesion nematode on chickpea the experiment was conducted in 10 cm diameter earthen pots having steam sterilized soil and analysed in glass house. Chickpea surface disinfected seeds were sown in each pot. Freshly emerged lesion nematode inoculated in logarithmic series of 10, 100, 1000 and 10000 per plant, each treatment was replicated four times. The pots were examined 40 days after inoculation and observations on plant growth character were recorded [10-12].

Test of Pathogenicity of antagonistic fungi

To study the Pathogenicity of lesion nematode on chickpea, an experiment was conducted to determine soil environment for antagonistic fungi. Nature of association was determined by isolation of 15 fungi collected from different loci representing different agro-climatic situations. Isolated fungi were grown on growth substrate incorporated in the media. Isolated fungi were purified to the desired level. The identified fungi were catalogued as per the identification procedure. Inoculation of fungi was done on surface disinfected chickpea seeds raised in earthen pots filled with sterilized soil, containing 500/200 cm^3 freshly emerged *Pratylenchus thornei* population. The observations on different growth parameters of chickpea were recorded to ascertain pathogenic effect of the inoculated fungi.

Results

Effect of fungal antagonistic on the population of *Pratylenchus thornei* in soil *In vitro and in vivo* study was carried out to determine the effect of fungi on *P. thornei*. Replicated lab and pot experiment was undertaken during the course of investigation. Data summarised in [Table-1] revealed that *T. viride* reduced the nematode population in both lab and pot culture conditions to the tune of 1.79 and 2.93 respectively over check (control). *Cladosporium sp., Crisosporium sp.(T), Crisosporium sp (R), T. harzianum* and *A. alternata* was in next in reducing nematode were recorded both in lab. and pot condition which indicated that *P. thornei* behaviour was uniform in both the conditions. The reduction of nematode population ranged from 32.19 to 89.65 percent and 32.39 to 82.89 percent under lab and pot condition respectively.

Table-1 *Effect of fungal antagonists on the population of Pratylenchus thornei in soil**

Treatment	*In vitro*	*In vivo*
Aspergillus flavus (Sirmour)	128.75 (11.33)	127.50 (11.31)
Aspergilus niger (Teothar)	118.75 (10.90)	130.00 (11.41)
Penicillium pinophilum	43.75 (6.59)	43.75 (6.61)
Trichoderma harzanium (Mangawan)	18.75 (3.96)	13.75 (3.64)
Aspergillus flavus (Rewa)	118.75 (10.90)	115.00 (10.74)
*Crisosporium*sp. (Teothar)	12.50 (2.87)	18.75 (4.38)
Aspergillus flavus (Mangawan)	137.50 (11.73)	118.75 (10.88)
Fusarium oxysporium (Gangeo)	50.00 (6.98)	57.5 (7.55)
Fusarium moniliforme (Rewa)	37.50 (6.07)	40.00 (6.42)
Crisosporium sp.(Rewa)	12.50 (2.87)	10.00 (3.19)
Trichoderma viride (Rewa)	6.25 (1.79)	8.25 (2.93)
Aspergillus niger (Jawa)	118.75 (10.90)	133.75 (11.58)
Cladosporium sp. Sirmour	12.50 (2.87)	17.50 (4.18)
Alternaria alternata sp. (Rewa)	31.25 (5.56)	47.50 (6.84)
Fusarium moniliforme (Teothar)	43.75 (6.59)	48.75 (6.91)
Control	302.50 (17.30)	293.75 (17.13)
SEm±	0.81	0.41
CD 5%	2.31	1.18

**Mean of four replication*

Figure in parenthesis indicate logarithmic transformation value the nematode population to the extent of 2.87, 2.87, 2.87, 3.96, 5.56 in lab condition and 4.18, 4.18, 3.19, 4.38, 3.64 and 6.84 in pot respectively, and showed non-significant difference with *T. viride* but significant difference over control.

Application of research: The growth of chickpea was improved in the presence of all the antagonistic fungi, but application of *Trichoderma viride* increased the yield to the extent of two times as compared to control. Soil amended with antagonistic fungi drastically changed the population dynamics of *P. thornei* at different growth stages of the crop except *Alternaria alternata and Fusarium moniliforme. The T. viride and T. harzianum* found to be more antagonistic against *Pratylenchus thornei* as compared to *P. pinophillum, Crisosporium* (T) and (R) and Cladosporium spp. Nematode specific survey need to be undertakes to determine the trends of population dynamics and frequency of their distribution in other crops. More samples may be collected form soil exhibiting foliar symptoms beside the root rot and wilt. Botanical product, organic farming and resistant lines should be considered for the management of the nematodes.

Research Category: Plant Pathology

Abbreviations: T-Teothar, R-Rewa, S- Sirmour, P- Pratylenchus

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