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RESEARCH ARTICLE

EFFECT OF BIOPESTICIDES ON PENETRATION OF *MELOIDOGYNE INCOGNITA* JUVENILES ON KING CHILLI ROOTS

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ABSTRACT

The potential of three biopesticides (Biofor-PF-2, Biozine-PT and Biogreen-5) of Assam Agricultural University to provide protection against root penetration of infective juvenile of *Meloidogyne incognita* race 2 on King chilli was assessed in pots in net house condition. All the biopesticides were found to reduce the nematode penetration to various levels. Soil application with Biogreen-5 was the most effective of all the tested products in preventing juvenile penetration by up to 69.7 per cent, 6 days after inoculation with nematode compared to that in untreated check.

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* race 2 is a serious pest in vegetable growing areas of Assam. King chilli (*Capsicum chinense*) is an important commercial crop in North-East India. It is known to be parasitized by many pests including root-knot nematode. Root-knot nematode with their stylet pierces the hard tissues by using a biochemical process resulting in formation of galls in the root system. The crops infested with this nematode appear to be stunted in growth, low flowering and low yield (Gitanjali *et al.*, 2016). Avoidable yield loss of 53.36% due to infestation of this nematode have been recorded in green chillies (*Capsicum annum*), Jaipur local (Yadav and Mathur, 1999). Integrated pest management approach, including biological approach and nutrient enrichment along with resistant varieties will provide a long term solution instead of chemical approach alone. Biological control and other eco-friendly disease control measures have recently gained increasing interest. Several strains of *Pseudomonas*, *Bacillus*, *Beauveria bassiana*, *Metarhizium anisopliae* are able to suppress a variety of plant diseases caused by soil-borne plant pathogens as well as insect pests and hence are of considerable agricultural value (Kloepper, 1993). The experiment was performed to determine whether soil treatment with the commercial biocontrol agents interfere nematode penetration to the root.

MATERIALS AND METHODS

An experiment was conducted to study the influence of biopesticides on root penetration of *M. incognita* in King chilli (local variety) under net house conditions in the Department of Nematology, Assam Agricultural University, Jorhat, Assam.

Preparation and sterilization of soil mixture: Sandy loam soil collected from a field of AAU, Jorhat was passed through 10 mesh sieve. The soil, river sand and organic manure mixed in the ratio of 3:1:1 and transferred to an autoclave for sterilization at 20 lb pressure. These sterilized soil were allowed to cool down at room temperature before use and kept in a sterilized tray. Seeds of King chilli (local variety) were surface sterilized with 0.01% HgCl₂ for 2 min and washed twice with distilled water. Seeds were sown in the tray. Watering was done whenever necessary.

Preparation of nematode inoculums: Galled roots of tomato infested by *Meloidogyne incognita* race 2 were carefully washed using gentle flow of water to remove the adhering soil particles. Egg masses were collected by the aid of a needle. The obtained culture was reared on tomato seedlings planted in pots filled with sterilized sand: clay media (1:1). Pots were kept under net house conditions for 45-60 days to maintain the nematode inoculum for further studies. A stock culture of the second-stage juveniles was obtained from the collected mature egg-masses after immersion in sterilized water for 7-10 days.

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Table 1. Effect of biopesticides on penetration of *Meloidogyne incognita* juveniles on King chilli roots. (Average of 5 replications)

Treatment (w/w)	Number of juveniles penetrated/5 g rootDays after inoculation											
	2	% (-)ve over control	4	% (-)ve over control	6	% (-)ve over control	8	% (-)ve over control	10	% (-)ve over control	12	% (-)ve over control
Biofor-PF-2	14.2 (3.82) ^c	60.5	26.8 (5.22) ^c	56.7	35.2 (5.97) ^c	54.8	42.4 (6.54) ^c	51.2	46.2 (6.83) ^c	51.8	50.8 (7.16) ^b	47.0
Biozin - PTB	20.0 (4.50) ^b	44.0	36.2 (6.05) ^b	41.6	45.0 (6.74) ^b	42.3	52 (7.24) ^b	40.2	55.0 (7.44) ^b	42.7	58.2 (7.66) ^b	37.8
Biogreen-5	10.2 (3.22) ^d	71.6	18.8 (4.39) ^d	69.6	23.6 (4.90) ^d	69.7	30.2 (5.53) ^d	65.2	35.2 (5.97) ^d	63.3	38.8 (6.26) ^b	59.5
Carbofuran 3G	6.2 (2.57) ^e	82.0	10.4 (3.29) ^e	83.0	12.4 (3.58) ^e	84.1	12.2 (3.55) ^e	85.9	9.5 (3.17) ^e	90.0	10.2 (3.26) ^b	89.3
Control	36.0 (6.03) ^a		62.0 (7.90) ^a		78.0 (8.85) ^a		87.0 (9.35) ^a		96.0 (9.82) ^a		96.0 (14.11) ^a	
CD(P=0.05)	(0.55)		(0.20)		(0.24)		(0.22)		(0.16)		(5.7)	

Figures in parentheses indicate $(X+0.5)^{1/2}$ transformed values. Figures indicating common letters do not differ significantly at P=0.05.

Propagation of the bioagent: The talc based biopesticides viz., Biofor-PF-2 (contains beneficial microbes: *Pseudomonas fluorescence*, *Trichoderma harzianum*), Biozin-PTB (contains beneficial microbes: *Pseudomonas fluorescence*, *Trichoderma viride*, *Bacillus brevis*) and Biogreen-5 (contains beneficial microbes: *Trichoderma viride*, plant growth promoting rhizobacteria, *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae*) were procured from Department of Plant Pathology, AAU, Jorhat. One kg of each biopesticides was mixed with 10 kg of dry cowdung for 15 days. After 15 days of incubation the mixer was ready for application. The tested concentration of fungi (2×10^9 spores /g) and bacteria (1×10^9 CFU/g) was determined by the aid of haemocytometer.

Soil amendment with biocontrol agent: One month old seedlings of King chilli (local variety), were transplanted singly in 10 cm diam. earthen pots containing 500 g of sterilized soil-sand mixture. Ten days after transplantation, the roots of each plant were inoculated with $1 J_2$ /g soil. For inoculation of *M. incognita*, soil around the roots was carefully removed so that the roots were not damaged. The talc formulations of biocontrol agents viz., Biofor-PF-2, Biozin-PTB and Biogreen-5 were applied at 10% (w/w) immediately after inoculation of nematodes. Treatment with Carbofuran 3G 0.1% (w/w) was included for comparison. Check treatment without biocontrol agents or nematicide was included. There were 5 replications for each treatment. The experiment was conducted in a completely randomized design. Observations were recorded on penetration of juveniles at 2, 4, 6, 8, 10, 12 days after inoculation of nematodes. The top part of the plants was cut down at the base and the pots were then, inverted and tapped for intact root system. It was then, carefully washed under a stream of water. After removing the soil debris, the root system was gently pressed between blotting papers followed by chopping the roots into 5g pieces. The chopped roots were then stained as per technique of Byrd *et al.*, (1983). Total number of nematodes was calculated from the figures available per 5 g roots. The data were subjected to square root transformation for statistical analysis and critical differences were determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The nematode penetration in King chilli roots was observed up to 12 days after inoculation (DAI). The results of the *in vitro* studies indicated that significant ($P < 0.05$) reduction in penetration of *M. incognita* in all the biopesticide treated King chilli plants. The results revealed that Biogreen-5 and Biofor-PF-2 had profound effect of root penetration of *M. incognita* to

King chilli. Data in the Table 1 showed that level of nematode penetration was reduced by 71.6, 69.6, 69.7, 65.2, 63.3 and 59.5 per cent in Biogreen-5 treated seedlings over control at 2, 4, 6, 8, 10 and 12 DAI respectively. It was followed by Biofor-PF-2 (60.5, 56.7, 54.8, 51.2, 51.8 and 47.0) and Biozin-PTB (44.0, 41.6, 42.3, 40.2, 42.7, 37.8) and differed significantly ($P=0.05$) over control. Oostendrop and Sikora (1990) reported that the sugar beet cyst nematode, *Heterodera schachtii* penetration was decreased due to *P. fluorescens* treatment. The mechanism responsible for the reduction in nematode penetration was attributed to the ability of the bacterium to envelop or bind to root surface lectins, thereby interfering with normal host recognition by the nematode. Further, disruption in the movement of infective juveniles due to fatty acids (Djian *et al.*, 1991) and reduced attractiveness of root tips due to ammonia excreted by these bacteria (Castro *et al.*, 1990) and in addition the ability of rhizobacteria to colonize root surface (Dobbelaere *et al.*, 2003) which masked the probable penetration sites available on the root probably delayed and reduced root invasion and subsequent egg deposition, resulting in reduced nematode population per root system thus affecting the total reproduction in comparison to untreated check. Fluorescent pseudomonads were reported to produce iron chelating siderophores (Kloepper *et al.*, 1980), antibiotics and hydrogen cyanide (Ahl *et al.*, 1986) and these compounds were implicated in lower penetration of infective juvenile, reduction of pathogenic rhizosphere microorganisms, creating an environment favourable for root growth (Leong, 1986). Reduction in nematode population in roots might be due to premature egg hatching and reduction in viability and mobility of juveniles induced by secondary metabolites such as 2, 4-diacetylphloroglucinol (PHL) and lytic enzymes produced by *P. fluorescens* (Elsharif and Grossmann, 1996; Dunne *et al.*, 1998). Delayed nematode egg hatch and reduced motility of J_2 of *Meloidogyne* spp. due to culture supernatants of *Pseudomonas* sp. (Sharma *et al.*, 1998) and *Bacillus* sp. (Padgham *et al.*, 2005; Jonathan and Umamaheswari, 2006) are also known. The mechanisms which mediate these effects include the production of metabolites (Siddiqui and Mahmood, 1995; Kalaiarasan, 2000; Siddiqui and Shaukat, 2003) which reduce hatch and attraction and/or degradation of specific root exudates which control nematode behavior. Siddiqui and Shaukat (2002) reported that two rhizobacteria, *Pseudomonas aeruginosa* strain IE-6S+ and *P. fluorescens* strain CHA0, used as a bare root-dip treatment or as a soil drench, substantially reduced *M. javanica* juvenile penetration into tomato roots under glasshouse conditions. Reduced nematode penetration in the biocontrol inoculated seedling is probably related to the nematotoxic metabolites produced by these bioagents, which reduced egg hatch and

mobility of juveniles. Thus fewer J₂ were available to invade host plant root. Dababat (2007) and Dababat and Sikoro (2007) reported that the endophytes inoculated plants exudates reduced the total number of nematodes attracted to the inoculation area by up to 80 per cent over uninoculated control. Similar alleviating impact on nematode disease intensity by reducing the penetration rate has been exhibited by a number of rhizobacteria. *Pseudomonas fluorescens* and *Bacillus megaterium* were found to reduce J₂ penetration by *M.graminicola* in rice seedlings (Anita and Rajendran, 2005; Padgham *et al.*, 2005). *In vitro* studies demonstrated the activity of metabolites derived from *Trichoderma* treated soil extracts and direct parasitic interactions between the fungus and the nematode (Sharon *et al.*, 2001). Several metabolites of microbial origin have been reported to possess antibiosis effects against nematode activities. Volatile fatty acids suppress egg hatching by impairing embryogenesis of *M.incognita* (Bansal and Bajaj, 2003). It can be inferred that *Pseudomonas* and *Bacillus* sp. are having high activity against *M.incognita*. Rhizobacteria especially plant growth promoting bacteria are considered most ideal candidates for nematode control due to the nematotoxic metabolites produced and accumulated to the toxic level during their growth (Bansal and Bajaj, 2003) and also due to their high rate of multiplication, spread and colonization in rhizosphere and on plant root surface (Gutierrez Manero *et al.*, 2001; Dobbelaere *et al.*, 2003). Developing and further characterizing more beneficial microorganisms will in future provide a comprehensive and sustainable crop protection.

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