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EFFECT OF DIFFERENT INOCULUM LEVELS OF *VERTICILLIUM CHLAMYDOSPORIUM* GODDARD ON *MELOIDOGYNE JAVANICA*, ROOT KNOT NEMATODE

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Abstract

Effect of *Verticillium chlamydosporium* at 1%, 2% and 3% (w/w) was studied against *Meloidogyne javanica* root knot nematode under greenhouse conditions. Maximum reduction in gall formation, egg mass production, root and soil densities of *M. javanica* was observed (at $p<0.001$) in treatments where *V. chlamydosporium* was applied @3% (w/w). Fungal application rates 1% & 2% (w/w) also provided disease suppression. Fresh shoot weight ($p<0.01$), plant height ($p<0.001$) and root length was increased in fungal treated plants compared to untreated controls. At the termination of experiment, maximum recovery of *V. chlamydosporium* was obtained in the treatments where both fungal and nematode inoculum was applied. It means, *V. chlamydosporium* increased in the presence of root knot nematode and could be used for biological control of root knot disease of tomato.

Introduction

Biological control agents of *Meloidogyne* spp. have been reported by many workers (Godoy *et al*, 1982; Janson *et al*, 1985; Jatala, 1986; Zaki, 1993; 1999; Amer-Zareen *et al*, 2000; Rodriguez-Kabana *et al*, 1984 and Stirling & Mankau, 1979), but some of them have been found to provide effective suppression of nematode densities under greenhouse and filed conditions upto some extant. The facultative parasitic fungus *Verticillium chlamydosporium* Goddard has demonstrated to be an effective natural enemy of plant parasitic nematodes with ovicidal properties (Williams, 1969; Kerry *et al*, 1980; Crump & Kerry, 1982; Zaki, 2000). Infestation of soil with the fungus in filed and greenhouse experiments have revealed its effectiveness against root knot disease and increased crop yield (Godoy *et al*., 1983; De Leij & Kerry, 1992; Zaki, 2000). *Verticillium chlamydosporium* is a rhizosphere colonizer and attacks root knot *Meloidogyne* spp. when fully developed females and egg masses appear on root surface This fungus can't provide effective control against initial damage by nematodes But subsequent generations of nematode after first generation will lead to less damage (De Leij, 1992). In the present study different inoculum rates of *V. chlamydosporium* were used against single population level of *Meloidogyne javanica* under greenhouse conditions to evaluate the effective inoculum level to suppress root knot nematodes on tomato plants.

Materials & methods

The fungus *Verticillium chlamydosporium* isolate Vc-10 was selected for this study, which was originally isolated from eggs of *Meloidogyne incognita* (De Leij, 1992). The fungus was maintained and multiplied on cornmeal agar (CMA, Oxoid), whereas chlamydospores were produced on milled barley and sand medium.

Chlamydospores were harvested through 10 µm sieve after 2-3 weeks growth. Chlamydospores were mixed with sand (1:10 w/w ratio) and used as fungal inoculum.

Sandy loam soil, pH 8.1 collected from experimental field of Botany Dept., University of Karachi, was transferred in 8 cm diam. plastic pots @ 300g/pot. Pots were inoculated with sand inoculum of chlamydospore @ 1%, 2% and 3% w/w by thoroughly mixing according to treatment plane. Pot receiving sand only served as control. One-month-old tomato seedlings were transplanted three plants/pot. After two week of seedling transplantation freshly hatched *M. javanica* juveniles were inoculated in the root zone @2500 J2/pot according to the treatment plane. Each treatment was replicated four times and pots were arranged in randomized complete block design. Pots were watered whenever needed. Experiment was terminated 50 days after nematode inoculation. Root was washed under running tap water, fresh weights and length of shoot and roots were recorded. Galls and egg masses were observed and counted under binocular microscope and RKI was determined using 0-5 scale of Taylor & Sasser (1978). The root invasion of nematode was assessed by staining roots in lactophenol acid fuchsin (Franklin, 1949), roots were destained, and homogenized and nematode population in roots was counted under binocular microscope. Nematode population was estimated g^{-1} of root. Nematode soil densities per 250 cc were estimated using Baerman modified funnel technique (Schindler, 1961). Soil colonization by *V. chlamydosporium* was determined g^{-1} soil sample by dilution method. Data obtained was statistically analyzed (Gomez & Gomez, 1984).

Results

Effect of *Verticillium chlamydosporium* on root knot *M. javanica* development: Root knot development was suppressed in all three fungal inoculum levels compared to control. Number of galls per root system were reduced significantly ($p<0.001$). Gall formation and egg mass production was suppressed by 47 % and 64%in treatments where *V. chlamydosporium* was applied @ 3% (w/w) (3600 chlamydospores g^{-1} soil) followed by @ 2% by 43 % and 45 % and 1 % inoculum 30 % & 28% respectively over control. Varying degree of suppression (at $p<0.001$) was observed in root and soil densities of *M. javanica* in all three fungal applications compared to control. There was a positive correlation in suppression of root & soil densities and fungal application rates. Maximum suppression in juveniles recovery from soil per 250 cc (55 %) and invasion in tomato roots (31%) compared to rest of treatments and untreated control, was observed with higher application rate (3 % w/w) of fungal inoculum. In set of posts where 2% fungal inoculum was applied 44% suppression in nematode soil density and 25% in root invasion over control followed by 1% chlamydospore inoculum with 30% and 15% reduction in soil and root densities of nematodes respectively over control was observed (Table 1).

Effect of *V. chlamydosporium* on growth of tomato plants: Data presented (Table 1) showed that fresh shoot weight and plant height was increased significantly in treatments where fungal inoculum was applied @ 1%, 2% & 3% (w/w) respectively both in nematode infested and nematode free soil. There was significant difference in plant height ($p<0.001$), fresh shoot weight ($p<0.001$) and root length ($p<0.001$) among the treatments. Increase in root weight ($p<0.001$) was

observed in nematode inoculated control compared to nematode free control and rest of treatments.

Fungal establishment in soil: Fungal colony forming units (cfu) g^{-1} for each treatment was assessed at zero day soon after fungal incorporation and at the termination of experiment (fig 1). At the termination of experiment it was observed that treatments where both fungal antagonist and nematodes were introduced recovery of fungal colony forming units (cfu) g-1 soil was greater compared to set of treatments having fungal inoculum only.

Discussion

This study indicates that *V. chlamydosporium* suppressed root knot disease severity at higher fungal application (3% w/w) with maximum reduction in gall formation 47 % and 64 % in egg mass production compared to untreated control. Similarly reduction in nematode soil and root densities were also affected by higher fungal inoculum application. In soil environment lower level of fungal antagonists are not able to suppress phytoparasitic nematodes successfully (de Leij, 1992). Recovery of *V. chlamydosporium* at the termination of experiment was greater in 3%>2% and 1% respectively in fungal inoculum and nematode infested soil compared where only fungal inoculum was applied. For the successful germination of fungal propagules in rhizosphere it depends upon its internal protein contents and some degree of externally available nutrients. The suppressive effect of *V. chlamydosporium* is host dependent therefore susceptibility of crop is considered most important in determining the survival and establishment of a biocontrol efficacy of microbial agent. Root knot female need more nutrients for egg production and therefore compete with the host for the pool of nutrients in the roots (Hussey, 1985). The increased metabolic activities of synsytia increased, stimulate mobilization of photosynthates from shoots to root s and in particular to the giant where they are removed and consume by the feeding nematodes (McClure, 1977). The fungus appeared to be a more prevalent on galled than on non-galled roots. This may be due to the leaching of more nutrients from nematode damaged plant tissues than undamaged tissues. *Paecilomyces lilacinus* is also reported more frequent on galled tissues than on undamaged (Stirling *et al.*, 1975; Hewlett., 1988). The direct relation between leakage of nutrients from the roots and the ability of the fungus to colonize the rhizoplane seems to be of great advantage for the development of *V. chlamydosporium* as a biological control agent because it would decrease dependency on other soil nutrition resources and therefore increase the reliability for the exploitation *V. chlamydosporium* against root knot disease of vegetables and other cultivated plants. In case of severe root knot infection it is assumed that in large galls, many egg masses embedded in galled plant tissues and are therefore chance increased for nematode to escape from fungal infection (de Leij, 1992). For the control of plant parasitic nematodes pre-sowing applications by *V. chlamydosporium* may provide better control or it should successful in fields which treated with nematicides where there is low nematode densities.

Table 1. Effect of different inoculum levels of *Verticillium chlamydosporium* on development of *Meloidogyne javanica* and growth of tomato plants.

Treatments	Plant height (cms)	Shoot weight (g)	Root length (cms)	Root weight (g)	Galls/root system	RKI (0-5)	Egg mass/ per root system	Population per g root	Population per 250cc soil
Untreated control	11.27	2.45	8.7	0.67	-	-	-	-	-
2500 eggs	9.33	1.87	5.5	3.19	84.94	4.77	65.61	165.99	946.60
Verticillium chlamydosporium @ 1%	14.44	3.04	11.2	0.83	-	-	-	-	-
V. chlamydosporium @ 2%	16.05	3.19	12.86	0.97	-	-	-	-	-
V. chlamydosporium @ 3%	16.89	3.34	13.02	1.99	-	-	-	-	-
V. chlamydosporium @ 1% + 2500 eggs	12.33	2.14	10.57	2.80	59.50	4.50	47.00	141.00	658.60
V. chlamydosporium @ 2% + 2500 eggs	15.88	2.07	11.44	1.03	48.10	3.44	36.33	124.00	533.20
V. chlamydosporium @ 3% + 2500 eggs	16.44	3.04	12.79	0.87	45.11	3.11	23.55	115.00	422.00
SED ($p<0.05$)	1.41	0.36	1.26	0.31	4.7	0.13	5.23	7.26	47.73
Significant level ($p<$)	***	**	***	***	***	***	***	***	***

**, $p<0.01$; ***, $p<0.001$

Population of *Verticillium chlamydosporium* in tomato rhizosphere

F1= *V. chlamydosporium* @ 1%, F2= *V.chlamydosporium* @ 2%

F3= *V. chlamydosporium* @ 3%, F1+N= *V. chlamydosporium* @1%+2500J2

F2+N= *V.chlamydosporium* @ 2%+ 2500 J2, F3+N= *V. chlamydosporium* @3%+ 2500 J2

Series 1= Initial fungal population (Zero day)

Series 2= Final fungal population (At harvest)

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