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EVALUATION OF *PAECILOMYCES LILACINUS* FOR MANAGEMENT OF ROOT KNOT NEMATODE *MELOIDOGYNE INCOGNITA*, IN TOMATO

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ABSTRACT

The efficacy of *Paecilomyces lilacinus* for the management of *Meloidogyne incognita* along with FYM and carbofuran was evaluated to study the root knot index, population of *M. incognita* in root and in soil. By using (T_5) *P. lilacinus* (cfu 2x10^6) @ 50 gm/m^2 in nursery bed+ *P. lilacinus* @ 5 kg with 2.5 tons FYM/ha in main field found most effective and which improves plant growth and yield of tomato (228.3 Q/Ha) and lowest population of *M. incognita* in root, in soil (222.3 J_2 at 45 DAT and 309 J_2 at 60 DAT) and also lowest root knot index (2.67). Followed by chemical nematicide viz. carbofuran showed a significant effective in increase the growth parameters and suppression of *M. incognita* multiplication. *P. lilacinus* @ 50 gm/m^2 provided lowest gall formation (60.3-64.5%) followed by carbofuran (38-44%). All other treatments exhibited higher yield (12.8-32.7%) and reduction of soil population by 21.7-51.9% over untreated control.

INTRODUCTION

There is a great potential for genetic manipulation in tomato to enhance productivity through increasing pest and disease resistance, environmental stress tolerance and to study gene function and regulation (Pawar *et al*., 2012). The crop tomato is attacked by number of nematode pests. Globally, nematodes are common in almost all soils, with their distribution being determined by temperature, degree of moisture and soil particle size, along with the presence of acceptable food source (Bina *et al*., 2012). Among the plant parasitic nematodes, Root knot nematode (RKN), *Meloidogyne incognita* is a serious pathogen hampering the productivity in tomato crop significantly throughout the world (Mucksood and Tabreiz, 2010) and has been found to be very widely distributed with wide host range and cause very serious damage specially in vegetables. In India, annual yield loss due to *M. incognita* has been estimated to as 27.21% in tomato (Jain *et al*., 2007). Control of root knot nematodes has been primarily accomplished through chemical nematicides (Widmer and Abawi, 2000). Nematode control using chemical nematicides has become environmentally unsafe and economically unviable due to removal of efficient fumigant nematicides from world market on environmental grounds. There is a need to develop environmentally and economically sound alternatives to nematicides for sustainable nematode management in tomato ecosystem. Biological control of plant parasitic nematodes using fungi and bacteria has been found to be a feasible option. The antagonistic fungi play an important role as biocontrol agent for many plant parasitic nematodes (Jatala, 1986).

Paecilomyces lilacinus a saprophytic soil fungus has drawn many research attentions due to its promising effect in parasitizing and controlling population of phytonematodes (Jatala, 1986; Dube and Smart, 1987; Hewlett *et al*., 1988; Freitas *et al*., 1995; Nagesh *et al*., 1997; Khan *et al*., 2006; Kiewnick and Sikora, 2006; Brand *et al*., 2010). Of these the use of pathogenic fungus *Paecilomyces lilacinus* is one the most widely tested biological control agent for management of plant parasitic nematodes (Atkins *et al*., 2003). It has a high frequency of occurrence in the tropics and subtropic (Morgan *et al*., 1984; Chen *et al*., 1996) and can be found in most of agricultural soils (Brand *et al*., 2010). This study was undertaken to determine the appropriate application rate and time of biological agents for the control of root knot nematodes in tomato. Keeping in view the above aspects the present investigation was undertaken for ecofriendly management of root knot nematode on tomato by using biocontrol agent, *Paecilomyces lilacinus*.

MATERIALS AND METHODS

An investigation was conducted at Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, West Bengal, India during the year 2010-2011 for the management of root knot nematode *M. incognita* (Kofoid and White) Chitwood infesting tomato by application of biopesticide, *Paecilomyces lilacinus* and chemical pesticide, carbofuran. The soil was a typical alluvial soil (Entisol) with a sandy clay loam texture with good drainage, slightly acidic pH and moderate fertility.

The experimental design was a completely Randomised Block Design with nine treatments with four replications. The field was divided into plots, measuring 5m^2 (2 m x 2.5 m). In case of initial soil nematode population study, soil sample were collected from the experimental plot before ploughing while for final population study soil sample were collected from rhizosphere of the plant at 45 DAT and at harvest. For initial soil population study of *Meloidogyne incognita* 12 subsamples were collected and mixed thoroughly to prepare four composite samples, where as nematode population during 45 days after planting and at harvesting three subsamples from each plot were collected from rhizosphere of the plant and mixed thoroughly to prepare one composite sample weighing 500 g each, so 36 samples from cropped field at 45 days planting and harvesting time were collected and labeled properly. Nematodes were extracted from 200 cc composite soil samples by Cobb's decanting sieving technique followed by Baermann's funnel method (Christie and Perry, 1951). Roots of the plants for examination are to be separated from the plant, to be mixed together and then only 2g of roots are to be collected from the composite sample. Roots were then cleaned in tap water, cut into pieces of 2-3cm and stained by NaOCl-acid Fuchsine method (Byrd *et al*., 1983). Stained root samples were then checked under stereoscopic binocular microscope for taking observation on egg mass and nematode (juveniles+female) population. Vial counting fixed nematodes suspension was taken in a measuring cylinder to measure the total volume of nematodes suspension obtained from 200 cc soil thrice. Then 5 ml of thoroughly stirred suspension containing nematodes was taken for counting the nematode population to find out the average number of juveniles (J_2) per ml of suspension.

Shoot lengths were taken 10 plants/plot at the time of 45DAT and at the time of harvesting. Tomato fruits from each plot were harvested and weight of the fruit was measured at regular interval to get the total yield of plot. Number of gall/knot present on the tomato root was counted from three randomly selected plants/plot.

Nematode count has been done under Stereoscopic binocular microscope (Olympus SZ 11). The roots were washed free of soil for gall index in 1-5 scale (1= no galls or egg masses, 2= 1-10, 3=11-30, 4=31-100, and 5= >100 galls and /or egg masses per plant). Data were statistically analyzed and compared according to Duncan's Multiple Range Test at 5% level of probability.

RESULTS

Effect of treatments on the weight of seedlings

Application of carbofuran 3G @ 10/m2 in seed bed provided highest seedling weight (68.06g/ 25 seedlings) followed by 64.06g and 59.88g/ 25 seedlings in T_8 and T_1 respectively on par with each other. These two treatments are better than the other treatments like T_4 and T_5 provided 57.19g and 56.75g/ 25 seedlings respectively, however, significantly higher than untreated check (Table 1).

Effect of treatments on gall formation in tomato seedlings

P. lilacinus @ 50g/m2 provided lowest gall formation (60.3-64.5%) and significantly superior than all other treatments followed by carbofuran 3G @ 10g/m2 (38-44%). Other treatments used in this experiment also effective in reducing the gall formation in seedling of tomato (Table 1).

Effect of treatments on the egg masses, yield and population of *M. incognita* in root

At 45 DAT, lowest (81.5) population of *M. incognita* was observed in the T_5 treatment plots followed by 112.5 in T_4 an T_8 and significantly lower than that of all other treated plots. At harvest, the population of *M. incognita* in roots were slightly changed. It has found that lowest (97.75) nematode population was noticed in T_5 and statistically superior than all other treatments (Table 2).

Table 1: Effect of Treatments on growth on seedlings of tomato and shoot length

Treatment	Wt of 25 seedlings (g)	No. of galls /25 seedlings	Shoot length At 45 DAT	Harvest
T_1 = *Paecilomyces lilacinus* (cfu 2x 10^6) @ 50 g/m^2 in nursery bed	59.88 ^{b}	36.6 ^{d}	46.6^{ab}	123.9^c
T_2 = *P. lilacinus* @ 2.5 kg along with 2.5 tons of FYM / ha in main field	42.19^c	91.4^b	44.0^{bc}	123.6^c
T_3 = *P. lilacinus* @ 5 kg along with 2.5 tons of FYM / ha in main field	43.75^c	108.1^a	45.2^{bc}	136.8^{ab}
T_4 = T_1 + T_2	57.19^b	40.9^d	47.3^{ab}	133.2^b
T_5 = T_1 + T_3	56.75 ^{b}	37.2^d	48.8^a	142.9^a
T_6= Carbofuran3G @ 10g/m^2 (Seed bed)	68.06^a	57.7^c	44.6^{bc}	123.5^c
T_7 = 2.5 tons of FYM / ha in main field	44.38^c	101.8^{ab}	46.7^{ab}	126.0^c
T_8 =Carbofuran 3G @ 10g/m^2 (Seed bed) + carbofuran 3G @ 1kg a.i./ha in main field	64.06^{ab}	63.9^c	49.00^a	133.4^b
T_9 = Untreated control	45.50^c	103.0^{ab}	42.9^c	116.7^d
S. Em ±	2.35	5.3	1.1	2.2

*Data marked by common letter are not statistically significant according to DMRT at 5% level of probability

Table 2: Effect of treatments on root knot nematode population in root and yield of tomato

Treatment	Population in 2 g root					Yield Q/ha
	At 45 DAT		At harvest			
	Adult + Juvenile	Egg mass	Adult + Juvenile	Egg mass	RKI	
T_1 = *Paecilomyces lilacinus* (cfu 2x 10^6) @ 50 g/m^2 in nursery bed	130.3^{c}	13.0^{cde}	141.8^{c}	19.0^{d}	3.58^{bc}	208.9^{bc}
T_2 = P. *lilacinus* @ 2.5 kg along with 2.5 tons of FYM / ha in main field	147.5^{b}	19.5^{ab}	161.3^{b}	23.8^{b}	3.83^{bc}	201.3^{cd}
T_3 = P. *lilacinus* @ 5 kg along with 2.5 tons of FYM / ha in main field	118.5^{cd}	14.0^{cd}	136.8^{c}	20.0^{cd}	3.41^{c}	217.8^{ab}
T_4 = T_1 + T_2	112.5^{d}	12.3^{de}	131.0^{c}	16.8^{de}	2.92^{d}	220.3^{a}
T_5 = T_1 + T_3	81.5^{e}	7.8^{e}	97.75^{d}	11.5^{f}	2.67^{d}	228.3^{a}
T_6= Carbofuran3G @ 10g/m^2 (Seed bed)	153.3^{b}	17.7^{bc}	173.3^{b}	23.0^{bc}	4.00^{b}	194.0^{d}
T_7 = 2.5 tons of FYM / ha in main field	114.5^{d}	11.3^{de}	132.8^{c}	17.8^{de}	3.41^{c}	199.0^{cd}
T_8 =Carbofuran 10g/m^2 (Seed bed) + carbofuran 1kg a.i./ha in main field	112.5^{d}	10.3^{de}	129.0^{c}	14.8^{ef}	3.57^{bc}	221.9^{a}
T_9 = Untreated control	180.3^{a}	23.0^{a}	195.3^{a}	29.0^{a}	4.51^{a}	172.0^{e}
S. Em ±	5.1	1.6	4.2	1.2	0.14	3.5

*Data marked by common letter are not statistically significant according to DMRT at 5% level of probability

Table 3: Effect of treatments on the population of root knot nematode, *M. incognita* in soil

Treatment	Population of *M. incognita* J_2 / 200 cc of soil	
	45 DAT	Harvest
T_1 = *Paecilomyces lilacinus* (cfu 2x 10^6) @ 50 g/m^2 in nursery bed	361.3^{c}	476.8^{b}
T_2 = P. *lilacinus* @ 2.5 kg along with 2.5 tons of FYM / ha in main field	372.3^{bc}	473.6^{b}
T_3 = P. *lilacinus* @ 5 kg along with 2.5 tons of FYM / ha in main field	314.5^{d}	407.0^{c}
T_4 = T_1 + T_2	241.1^{e}	333.8^{de}
T_5 = T_1 + T_3	222.3^{e}	309.0^{e}
T_6= Carbofuran3G @ 10g/m^2 (Seed bed)	406.8^{b}	503.8^{b}
T_7 = 2.5 tons of FYM / ha in main field	387.5^{bc}	448.9^{bc}
T_8 =Carbofuran3G @ 10g/m^2 (Seed bed) + carbofuran3G @ 1kg a.i./ha in main field	305.5^{d}	389.0^{cd}
T_9 = Untreated control	455.8^{a}	643.3^{a}
S. Em ±	12.7	20.3

*Data marked by common letter are not statistically significant according to DMRT at 5% level of probability; **INP before transplanting = 315 J_2 / 200 cc of soil

At 45 DAT, lowest (7.8) egg masses were noticed in T_5 followed by 10.3 treated with carbofuran 10g/m2 + carbofuran 1kg a.i./ ha significantly superior than all other treated plots. At harvest, T_5, T_8 and T_4 provided 11.5, 14.8 and 16.8 egg masses respectively. Number of egg masses in all treated plots significantly lower than untreated control. The lowest root knot index (2.67) was found in T_5 which were significantly lower than all other treatment. All the treatments were significantly better than that of untreated control (Table 2).

The highest yield (228.3 Q/ha) of tomato was recorded in *P. lilacinus* @ 50g/m2 in nursery bed + *P. lilacinus* @ 5kg / ha+2.5 ton FYM/ha. All the treatments exhibited significantly higher yield of tomato in the time of 12.8-32.7% increase the yield over untreated control (Table 2).

Effect of treatments on the population of root knot nematode, *M. incognita* in soil

Initial nematode population before transplanting was 315 J_2 / 200 cc of soil. At 45 DAT, population reduction of *Meloidogyne incognita* juveniles was observed lowest (222.3 J_2/200 cc soil) in T_5 treated plots followed by 241.1, 314.5 and 361.3 J_2/200 cc soil in T4, T3 and T1. Other treatments also provided appreciable reduction in nematode population significantly lower than that of untreated control in the time of 10.8-52.2%. At harvest, the ranking of treatments were slightly changed with regard to nematode population in soil and T_5 provided lowest (309.0 J_2/200 cc soil) in T_5 treated plots. Significant reduction in soil population of J_2 of *M. incognita* in the time of 21.7-51.9% was noticed in all the treatments over untreated control (Table 3).

DISCUSSION

The antagonistic parasitic fungi, *P. lilacinus* and chemical pesticide, carbofuran 3G improved the plant growth and reduced the gall formation in tomato caused by *M. incognita* as compared to untreated control. Improvement of the plant growth and reduction of gall formation in *P. lilacinus* treated seedlings corroborates the previous finding recorded by the several workers (Lin *et al*., 1993; Khan and Saxena, 1997; Priya and Kumar, 2006; Kannan and Veeraval, 2008). They also reported that *P. lilacinus* reduces the gall formation and egg masses. Application of *Paecilomyces lilacinus* (2x10^6 cfu/ gm) @ 50g/m2 in nursery bed + *P. lilacinus* @ 5kg/ha along with 2.5 tons of FYM/ha reduces RKI and root knot nematode population both in roots and soil also. This finding collaborates with Barma *et al*. (2013) also reported that by using *P. lilacinus* with other materials reduces and took much better care for root knot nematodes. Kiewnick *et al*. (2011) and Ramakrishnan and Panduranga (2013) reported that applications of *P. lilacinus* significantly reduced RKI and number of egg masses.

The increased growth of the plant in *P. lilacinus* treated plots was also reported by Noe and Sessar (1995) and Pathan *et al*. (2005) which corroborates the present study. Priya and Kumar (2006) and Pramanik and Roy (2008) also observed that *P. lilacinus* increased the plant growth, including shoot and root length and seedling weight. At 45 DAT carbofuran 3G@10g/m2 + carbofuran 3G@1kg a.i./ha is found to be most effective treatment for increasing the growth of tomato crop. This finding is in confirmating with finding of Haq and Saxena (1986) who reported that carbofuran was the most effective for increasing the growth of plants. According to Khalil *et. al* (2012) the *Paecilomyces lilacinus* product was the best treatment in suppressing the root-knot populations in the soil also, *P. lilacinus* increased the shoot length and fresh weight of the root system. Several authors also reported that shoot length increased of tomato by using carbofuran (Rather *et al*. 2007; Ahmad & Siddiqui, 2009).

The treatments of fungal biopesticide, *P. lilacinus* and chemical pesticide carbofuran 3G were effective in reducing the invasion of nematode in root and root knot index (RKI). These findings are also supported by the earlier workers (Lin *et al*., 1993; Khan and Saxena, 1997). The reduction of nematode population (J_2) of *M. incognita* in soil by the application of *P. lilacinus* also corroborates with the findings of Cabanillas and Barker (1989) and Pramanik and Roy (2008). Rao *et al*. (2012) reported that application of *P. lilacinus* gives maximum improvement in fresh and dry weight of shoot and root over the nematode check and cause higher reduction in gall number and nematode population.

Considering the above all observation it may be concluded that application of *Paecilomyces lilacinus* ($2x10^6$ cfu/gm) @ 50g/m2 in nursery bed + *P. lilacinus* @ 5kg/ha along with 2.5 tons of FYM/ha performed better in suppressing the root knot nematode and providing higher yield of tomato. *P. lilacinus* is a good proposition as bio-control agent for the management of *M. incognita* in tomato.

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