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BIO EFFICACY OF BIO-NEMATON (*PAECILOMYCES LILACINUS* 1.15%WP) AGAINST ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) IN CUCUMBER CROP

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Abstract

Cucumber (*Cucumis sativus* L.) is a vegetable crop produced at a huge scale However, its production is seriously threatened by root-knot nematodes *Meloidogyne* spp. Approximately 50% of grown vegetables are infected by *Meloidogyne* spp. In intensive cropping systems, root knot nematode has usually been managed with chemical nematicides for decades. Though, the potential harmful impact on the environment and humans has led to a total prohibit or restricted use of most chemical nematicides. With increased pressure on growers to reduce nematicide usage and without effective alternatives, there is great interest in biological control as a management tool for this destructive nematode. This study was done to evaluate the effect of the bio-nematon (*Paecilomyces lilacinus* 1.15%wp) against root-knot nematode (*Meloidogyne incognita*)in Cucumber crop. Experimental findings indicate that soil drenching with Bio- nematon *Paecilomyces lilacinus* 1.15% WP bio- nematicide @ 6.0 kg/ha first application at the time of sowing and second application: 30-60 days after sowing can be adopted to efficiently manage the root-knot nematode (*Meloidogyne incognita)* and increase the yield in cucumber. The test Bio- nematon is found very much safe to the in cucumber crop and a well to the environment as the treatments with *Paecilomyces lilacinus* 1.15% WP. Showed no phytotoxic symptoms and increased the beneficial nematodes population.

***Keywords*:** Cucumber, *Meloidogyne incognita* and *Paecilomyces lilacinus* 1.15% WP.

Introduction

Cucumber (*Cucumis sativus* L.) is a vegetable crop produced at a large scale. It is a tropical cucurbit originally from India. It grows best at high temperatures. However, its production is seriously threatened by root-knot nematodes *Meloidogyne* spp. (Sikora and Fernández, 2005). The cucumber is originally from South Asia, but now grows on most continents. Cucumber is an edible cucurbit popular throughout the world due to a good source of vitamins, minerals, fibber and roughages. Cucumber is truly a versatile vegetable because of wide range of uses from salads to pickles and digestive aids to beauty products. The caloric and nutritional value of cucumber is very low but it is a primary source of vitamins, mineral and fiber for human body (Keoprapari, 1997 and Filgueira, 2003). The annual production of cucumber in India is 698000 MT from 45000 ha area with productivity of 15.5 per ha only during 2012-13 (Anonymous, 2014). *M. incognita* infection can alter the levels of amino acids and organic acids and reduce chlorophyll contents (Saikia *et al*., 2013). Heavy infestations can cause stunting, reduced tillering, yellowing, and reductions in the biomasses and bioactive constituents of roots and leaves (Pandey *et al*., 2003). Soil fumigants have been used to control of *M. incognita* for over twenty years (Noling and Becker, 1994). In addition to their high cost, these chemical nematicides are not environmentally friendly and cause possible risks to non-target microorganisms. Therefore, the development of nonchemical and eco-friendly management strategies is importantly and immediately needed in controlling RKNs. With this aim, a study was conducted in bio efficacy of bio-nematon (*Paecilomyces lilacinus* 1.15%wp) against root-knot nematode (*Meloidogyne incognita*)in Cucumber crop under field condition

Materials and Methods

Multiplication of Root–Knot nematode

Root–Knot nematode (*Meloidogyne incognita*)

Three weeks old cucumber seedlings Hybrid cucumber Malini (Seminis were transplanted in five kg capacity earthen pots, filled with steam sterilized pot mixture. Single egg masses were collected in fresh dist. water and the larvae emerged from each individual egg mass in suspension was utilized for inoculation to start pure culturing. The suspension was inoculated in the soil near to basal part of cucumber plants at 15 days after transplanting. The pots maintained in the glass house were regularly watered with tap water. From this stock culture, well developed, egg mass were carefully teased out and transferred to Petri dish containing adequate amount of dist. water and incubated under laboratory condition.

Field studies

Field trial was conducted at makkanur (Dharmapuri district) in Tamil Nadu during January, 2018 - March, 2018 the well-known endemic area for the occurrence of Root – Knot nematode disease of cucumber. The Hybrid cucumber Malini (Seminis ma susceptible variety to root-knot nematode, was used for this study. All the agronomical practices were strictly adopted during the cropping period as per the crop production manual for horticultural crops published by the Department of Horticulture, Government of Tamil Nadu.

The initial nematode population is recorded before the treatment imposition and again, at 30 and 60 days after the application of the Bio-Nematon. The yield and beneficial nematode population were recorded at the time of final harvest. The in cucumber plants uprooted at random was assessed to record various parameters like number of galls and egg mass per root system. The root-knot nematode population per 200 g of soil and five g of roots were recorded at the time of final harvest.

The treatment details are given below

Tr. No	Treatment details	Methods of application
T_1	Bio-Nematon @ 4.0 kg / ha	1st Soil application at the time of transplanting, 2nd application: 30-60 days after transplanting
T_2	Bio-Nematon @ 5.0 kg/ha	1st Soil application at the time of transplanting 2nd application: 30-60 days after transplanting
T_3	Bio-Nematon @ 6.0 kg/ha	1st Soil application at the time of transplanting2nd application: 30-60 days after transplanting
T_4	Seed treatment@10gm/kg	Before sowing of seeds
T_5	Carbofuran 3G	15gm/plant
T_6	Control	

Estimation of root-knot nematode population in soil

Soil sample weighing 200 g was washed thoroughly and processed using combined "Cobb's sieving and Baerman funnel method" (Ayoub, 1977). Two hundred g of soil taken in a 1000 ml beaker was added with sufficient quantity of water to make a soil solution, stirred thoroughly and allowed to stand for the heavier particles to settle down. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh size, respectively. Residue from 325 and 400 mesh sieves were collected and poured over a tissue paper on a wire gauge placed on Baermann funnel. Level of water in the Baermann funnel was maintained to keep the tissue paper wet and left undisturbed for 48 h. After incubation for 48 h., the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting various plant parasite nematodes. Nematode population from this was finally calculated to 200 g of soil.

Estimation of root-knot nematode population in root samples

Five gm of root samples weighed and directly observed under stereo-binocular microscope for counting adult females of sedentary nematodes and the same was processed using root incubation method (Ayoub, 1977) for the extraction of active forms of sedentary nematodes along with migratory nematodes.

After incubation for 48 h the volume of suspension was made to 200 ml, out of which 10 ml was pipette out and used for counting various plant parasitic nematodes. Nematode count from this was finally estimated to five g of root sample. The number of egg masses of root-knot nematode per root system was counted by exposing the infected roots to 0.25 per cent tryptan blue for three min. as per the standard procedure suggested by Sharma and Kumar (1997).

Methodology for Root-Knot nematode assessment

Rated for Root-Knot Index (RKI) according to the following scale.

Gall Index	Number of Galls
1	01-05 galls
2	06-10 galls
3	11-15 galls
4	16-20 galls
5	20 galls

Fruit yield

The fruits were harvested at matured stage periodically and the fruit weight was recorded as Kg per plot.

Assessment of Phytotoxicity

Cucumber plants were observed for phytotoxic symptoms (If any) such as chlorosis, necrosis, scorching, epinasty and hyponasty on 7 days after treatment of bio-nematon (*Paecilomyces lilacinus* 1.15%WP) and grading was done as per CIB guidelines adopting 0-10 scale.

Leaf injury was graded based on visual rating on a 1-10 scale (CIB, 1989)

Grade	Crop injury (%)	Grade	Crop injury (%)
1	1 – 10% of leaf injury	6	51.1 - 60% of leaf injury
2	11.1 – 20% of leaf injury	7	61.1 - 70% of leaf injury
3	21.1 – 30% of leaf injury	8	71.1 – 80% of leaf injury
4	31.1 - 40% of leaf injury	9	81.1 - 90% of leaf injury
5	41.1 - 50% of leaf injury	10	91.1 - 100% of leaf injury

Statistical Analysis

The data collected were subjected to statistical analysis using computer aided IRRISTAT Version 92 software developed by the International Rice Research Institute, Philippines.

Experimental Results

The results of the present study (Table 1) clearly showed that application of Bio- Nematon (*Paecilomyces lilacinus* 1.15% WP) was found to be toxic to *M. incognita*. The minimum, nematode population and Root-Knot Index ware observed with T_3 viz.., Bio- Nematon 6 kg/ha) with 617.23 numbers before treatment, 182.71 numbers at 30^{th} day observation after treatment, 540.43 numbers at 60^{th} day after treatment and 26.09 Root-Knot Index respectively. This was followed by the treatment with Bio- Nematon as seed treatment with@ 10gm per kg and soil drenching @ 5 kg /ha at 30 and 60 DAP. Application of testing chemical carbofuran 3 G was also found to be highly toxic to the root not nematode and treatments with Bio- Nematon were on par with the carbofuran 3 G. The control plot recorded the maximum nematode population and Root-Knot Index. The data pertaining to the population of nematodes in root sample depicted in Table 2 revealed a similar trend as observed with the population of nematodes in soil samples treated with Bio- Nematon *Paecilomyces lilacinus* 1.15% WP.

Yield

The results indicated that all the treated plots had considerably increased yield and number of beneficial nematodes when compared to control. Among the treatments, the treatment T_3 recorded the maximum yield (18.73 t / ha) and number of beneficial nematodes (302.07 / 200g soil and 229.52/5g root). This was followed by treatments T4 and T2. The untreated control recorded minimum values of the above parameters (Table3).

Phytotoxicity

There was no phytotoxic symptoms *viz*., leaf tip injury, leaf surface injury, vein clearing, necrosis, epinasty and hyponasty in all the concentrations of Bio- Nematon *Paecilomyces lilacinus* 1.15% WP treated plots during the entire cropping period (Table 4).

Discussion

Cucumber is highly susceptible to *M. Incognita* as indicated by severity in root-knot development, nematode population densities plant growth suppression in the inoculated controls. Our results indicated that t soil drenching with Bio- nematon *Paecilomyces lilacinus* 1.15% WP bio- nematicide @ 6.0 kg/ha first application at the time of sowing and second application: 30-60 days after sowing can be adopted to efficiently manage the root-knot nematode (*Meloidogyne incognita)* and ,increase the yield in cucumber. Showed no phytotoxic symptoms and increased the beneficial nematodes population. in cucumber. Numerous biological agents have been used to control RKNs. To date, *P. lilacinus* has been one of the most effective fungi for the biological control of plant parasitic nematode populations under various conditions (Anastasiadis *et al*., 2008; Cannayane and Sivakumar, 2001; Kiewnick and Sikora, 2006). Similar findings have also been found on under field conditions, the inaculation of soil with *P. lilacinus* before planting has been noted to allow for the sporulation of vegetative colonies and estab- lish fungal population for longer time Culbreath *et al.*, 1986. Several action mechanisms of *P. lilacinus* have been suggested for the biological control of plant-parasitic nematodes. The main mechanism of action is direct infection during the egg stage. In addition, *P. lilacinus* was observed to produce leucinotoxins, chitinases, proteases and acetic acid to promote the infection (Khan *et al.*, 2004; Park *et al.*, 2004). A number of plant species, including cucumber, tomato and egg-plant, have been demonstrated to permit the extensive colonization of P. *lilacinus* and *S. racemosum* in their rhizospheres and are considered to be good hosts for these fungi (Boone *et al.*, 2005; Sun *et al.*, , 2008). The use of biocontrol agents in agriculture may potentially reduce the chemical inputs and decrease the cost of farmers in con- trolling various nematodes. However, the efficiency of bio control agents against RKNs depends on the interaction among the host plant, the nematode and the fungus (Kerry, 2000). In conclusion, soil drenching with Bio-nematon *Paecilomyces lilacinus* 1.15% WP bio- nematicide @ 6.0 kg/ha first application at the time of sowing and second application: 30-60 days after sowing can be adopted to efficiently manage the root-knot nematode (*Meloidogyne incognita)* and increase the yield in cucumber. The test Bio-nematon is found very much safe to the in cucumber crop and a well to the environment as the treatments with *Paecilomyces lilacinus* 1.15% WP. Showed no phytotoxic symptoms and increased the beneficial nematodes population.

Table 1: Effect of T -Stanes 'Bio-Nematon' (*Paecilomyces lilacinus* 1.15% WP $1X10^8$ gm) on the population reduction of *Meloidogyne incognita* in cucumber crop (soil)

Tr. No.	Dosage (kg/ha)	Nematode population level (200 g/ soil) PAT				% Reduction over 30 DAA after 2nd application	% Reduction over 60 DAA after 2nd application	RKI
		PBT	30 DAP	60 DAP	Mean			
T_1	Bio- Nematon 4.0 Kg	616.17	247.33	591.83	419.58	70.20	62.78	29.57
T_2	Bio-Nematon 5.0 Kg	611.34	201.21	580.19	390.07	75.76	63.51	27.02
T_3	Bio-Nematon 6.0Kg	617.23	182.71	540.43	361.57	77.99	66.01	26.09
T_4	Bio-Nematon Seed treatment 10gm/kg of seeds	603.17	231.73	671.43	451.58	72.08	57.77	29.21
T_5	Carbofuran 3G (1kg/ha)	619.71	220.41	741.13	480.77	73.44	53.39	25.83
T_6	**Control**	62938	830.13	1590.12	1198	-	-	65.43
	SEd CD (p=0.05)	0.04 0.05	0.04 0.07	0.05 0.12	--			0.03 0.05

Note: PBT: Population before treatment, PAT: Population after treatment, DAT: Days after transplanting, RKI: Root-Knot Index

Table 2: Effect of T-Stanes 'Bio-Nematon' (*Paecilomyces lilacinus* 1.15% WP $1X10^8$ gm) on the population reduction of *Meloidogyne incognita* in cucumber crop crop (Roots)

Tr. No.	Dosage (kg/ha)	Nematode population level (5 g/roots sample) PAT				% Reduction over 30 DAA after 2nd application	% Reduction over 60 DAA after 2nd application	RKI
		PBT	30 DAP	60 DAP	Mean			
T_1	Bio-Nematon 4.0 Kg	121.17	31.79	159.92	95.85	75.18	51.26	28
T_2	Bio-Nematon 5.0 Kg	122.41	27.73	156.71	92.22	78.35	52.24	26.92
T_3	Bio-Nematon 6.0 Kg	123.48	26.39	149.39	87.89	79.39	54.47	24.72
T_4	Bio-Nematon Seed treatment10gm/kg of seeds	122.91	25.79	195.42	110.60	79.86	40.44	29.72
T_5	Carbofuran 3G (1kg/ha)	121.62	24.79	191.24	108.01	80.64	41.71	29.02
T_6	**Control**	124.52	128.09	328.14	228,11	-	-	65.32
	SEd CD (p=0.05)	0.03 0.041	0.04 0.05	0.12 0.26				0.02 0.05

Note: PBT: Population before treatment, PAT: Population after treatment, DAT: Days after transplanting, RKI:Root-Knot Index

Table 3: Effect of T-Stanes Bio-Nematon (*Paecilomyces lilacinus*-1X10^8/gm) on cucumber yield and beneficial nematodes

Tr. No.	Dosage(kg/ha	Yield t/ha	% Yield increase over control	Number of beneficial nematodes	
				(5 g/roots sample)	(200 g/ soil)
T_1	Bio-Nematon 4.0 Kg	15.52	20.29	199.72	218.35
T_2	Bio-Nematon 5.0 Kg	16.43	24.71	201.52	257.41
T_3	Bio-Nematon 6.0Kg	18.73	33.95	229.52	302.07
T_4	Bio-Nematon Seed treatment10gm/kg of seeds	17.59	29.67	2117.41	298.43
T_5	Carbofuran 3G (1kg/Plant)	17.96	31.12	97.43	148.14
T_6	**Control**	12.37	-	69.82	120.34
	SEd CD (p=0.05)	0.14 0.26	--	0.89 1.97	1.23 2.64

Table 4: Phytotoxicity evaluation of T-Stanes Bio-Nematon (*Paecilomyces lilacinus* 1.15%WP) in cucumber

Tr. No	Dosage(kg/ha)	Leaf injury on tips/ surface*	Wilting	Vein clearing	Necrosis	Epinasty	Hyponasty
T_1	Bio-Nematon 4.0 Kg	1	Nil	Nil	Nil	Nil	Nil
T_2	Bio-Nematon 5.0 Kg	1	Nil	Nil	Nil	Nil	Nil
T_3	Bio-Nematon 6.0Kg	1	Nil	Nil	Nil	Nil	Nil
T_4	Bio-Nematon Seed treatment10gm/kg of seeds	1	Nil	Nil	Nil	Nil	Nil
T_5	Carbofuran 3G (1kg/ha)	1	Nil	Nil	Nil	Nil	Nil
T_6	**Control**	1	Nil	Nil	Nil	Nil	Nil

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