JBiopest 13(2):187-194(2020)

On field management of root-knot infestation in tomato and brinjal through bioformulations of *Paecilomyces lilacinus* and *Pochonia chlamydosporia*

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ABSTRACT

Bioformulations like wettable powder, aqueous liquid and granules of *Paecilomyces lilacinus* (Nematofree) and *Pochonia chlamydosporia* (Nematofree+) were evaluated against root-knot infestation in tomato and brinjal at two farmer's field. The application of the bio-nematicides were carried out at transplanting stage of the crops as root dipping (@ 10gm or ml/ 1L of water) and soil treatment (@5Kg or L/acre incorporated with FYM).The root-knot infestation data was recorded by observing the foliage symptoms of root-knot infestation i.e., stunting, premature wilting, and leaf chlorosis. All the six treatments i.e., T1: Nematofree (WP), T2: Nematofree (Liquid), T3: Nematofree (Granule), T4: Nematofree+ (WP), T5: Nematofree+ (Liquid), T6: Nematofree+ (Granule) showed significant reduction in the root-knot nematode infestation in both tomato crop (90 to 96%) and brinjal (75 to 86%). Among all formulation types, the wettable powder formulation of both bio-nematicides provided promising result in reduction of root-knot infestation symptoms. Nematofree+ was found to be more effective than Nematofree in controlling the infestation. Application of both the bio-nematicides also increased the final yield in case of both tomato and brinjal.

Keywords: Root-knot nematode, bio-nematicide, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, tomato, brinjal, field study.

MS History: 02.04.2020 (Received)-18.10.2020 (Revised)- 10.11.2020 (Accepted)

Citation: Swati Deep, Mohammad Muslim, Nitul Tyagi, Ajay Kumar and Vimala Prakash. 2020. On field management of root-knot infestation in tomato and brinjal through bioformulations of *Paecilomyces lilacinus* and *Pochonia chlamydosporia.* *Journal of Biopesticides*, 13(2):187-194.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) and Brinjal (*Solanum melongena* L.) of the family Solanaceae, are two major vegetable crops grown in India. One of the major constraints in production is biotic stress due to plant parasitic nematodes which is considered as one among the major pests in the vegetable cultivation (Kumar *et al.*, 2020). In India, about 21.3% annual loss amounting to Rs. 102,039.79 million (1.58 billion USD) has been estimated due to plant parasitic nematodes. Among plant parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) stand out as one of most destructive and frequently observed nematodes in agriculture, causing mean percent losses of 23.03% in horticultural crops (fruits 25.5%, vegetables 19.6%, spices 29.5%), 18.23% in field crops (cereals 18.8%, pulses 23%, oilseeds 11.8%, fiber crops 19.75%). Out of total 19.6% loss in vegetables, 23% and 21% loss was reported in tomato and brinjal respectively (Kumar *et al.*, 2020). Similarly, economic loss of 11-35% in tomato and 10-42% in brinjal was reported earlier (Gowda *et al.*, 2017). Nematodes generally attack plants by forming root galls below ground which can be detected with symptoms of chlorosis, yellowing and wilting with stunted growth. Plants die before

attaining maturity as the root galls slows down the root functions for nutrient and water uptake and translocation. In tomato and brinjal, the root galls are generally large in size in comparison to other solanaceae crop like chilli where very small size galls are developed with poor root growth (Bernard *et al.,* 2017;Gowda *et al.,* 2017). Biocontrol technology is developing as most promising and effective measures for control of plant parasitic nematodes as it is non-toxic to the mammals and beneficial soil micro fauna/flora, non-polluting to groundwater and have no residual effect on farm produce. The fungus, *Paecilomyces lilacinus* (Thom) Samson and *Pochonia chlamydosporia* Zare, (syn. *Verticillium chlamydosporium)* have been reported as most potential biocontrol agents against root-knot nematodes and other plant parasitic nematodes ; namely *Meloidogyne* spp., *Globodera rostochiensis, G. pallida, Tylenchulus semipenetrans, Rotylenchulus reniformis* etc. in wide range of crops (Silva *et al.,* 2017; Abd-Elgawad and Askary 2018; Moosavi and Zare, 2020). Both of them successfully control root-knot nematode by parasitizing the eggs and females of root-knot nematodes (Wabere, 2016). Also, it is reported to reduce the number of plant parasitic nematodes up to 57-58% (Tahseen *et al.,* 2005). Evaluation of different formulation and method of application of the above two nematofagous fungi i.e., *P. lilacinus* and *P. chlamydosporia* in controlling plant nematodes has been researched separately globally (Kiewnick and Sikora, 2006; Cardona et al, 2014;Ciancio *et al.,* 2016 a, b; Abd-Elgawad and Askary 2018; Anusha *et al.,* 2018).

The current research paper aims at comparison of effect of liquid, wettable powder and granular formulation of *P. lilacinus* and *P. chlamydosporia* on field management of root-knot infestation in solanaceae vegetable crops tomato and brinjal in India.

MATERIALS AND METHODS

Product details

Three different formulations such as wettable powder, liquid and granule of Bionematicides Nematofree (*P. lilacinus*) and Nematofree+ (*P. chlamydosporia*) (Technology Innovation Centre, IPL Biologicals Limited, Gurugram, India) are used. As per product specifications the wettable powder contains fungal spores/mycelia (microscopically checked and confirmed) and fermentation solids – 1-2%, carrier powder – 81 –84% and moisture – Q.S %. The liquid formulation contains fungal spores/mycelia and fermentation solids – 2-5 % suspended in aqueous solution whereas, the granular formulation contains fungal spores/mycelia and fermentation solids – 1-2%, Bentonite granules – 85 –88% and moisture - Q.S %. The colony forming unit (CFU) of both the bionematicide product of *Paecilomyces lilacinus* and *Pochonia chlamydosporia* is maintained at 2 x 10^8 per gm or ml each and the pH of all the formulations is between 5 to 7.

Experimental design

Field trial was conducted to understand the effects of different types of formulations (i.e., wettable powder, liquid and granules) of both Nematofree and Nematofree+ on root-knot infestation in vegetables (Tomato and Brinjal). The efficacy of these bio products were compared with untreated control. The trial was conducted at two different farmer's field in Rajasthan, India where infestation of nematode (*Meloidogyne* spp.) was observed up to 70-80%. The trial site included a tomato field at village- Benada, tehsil-Bassi, district-Jaipur. The tomato field was divided into 28x5 m plots. Soil pH was observed as 8.46. Another trial site was a brinjal field at village-Jhoraka bud, tehsil- Malakhera, district-Alwar. The plot size was 16x16m having soil pH of 7.20. Regular agricultural practices was followed by the farmers i.e., recommended dose of Urea, DAP, MOP was applied during land preparation stage at both sites. Mode of

application of the bionematicidal formulation was root dipping (@ 10gm or ml/ 1L of water) and soil treatment (@5Kg or L/acre).The soil in the trial field was incorporated with bio-nematicides enriched FYM. The treatments were T1: Nematofree (WP), T2: Nematofree (Liquid), T3: Nematofree (Granule), T4: Nematofree+ (WP), T5: Nematofree+ (Liquid), T6: Nematofree+ (Granule) and T7: untreated control. Three- four week old tomato seedlings (variety: Viswanath) and brinjal seedling (variety: P.P.L) was transplanted during the month of July, 2018. The treatments were done following randomized block design (RBD) pattern with four replications each. At an interval of 15 to 20 days after each treatments, the total no. of infested plants were counted per plot per replication per treatment by visually observing the symptoms of infestation i.e., stunting, premature wilting, leaf chlorosis and the infestation percentage was calculated. Tomato and brinjal fruits were plucked three to four times in the cropping season and the final yield was recorded.

Data analysis

Data was subjected to one way ANOVA by using SPSS 16.00 Software. Least significant differences (LSD) were calculated at $p<0.05$ to test for significant differences between different treatment means.

RESULTS

The experiment was carried out at two different farmer's field with the solanaceae crops; tomato and brinjal. The data was recorded by observing the foliage symptoms of root-knot nematode infestation i.e., stunting, premature wilting, and leaf chlorosis. All the treatments were carried out at transplanting stage of the crops. The root-knot infestation symptoms started appearing in tomato crop at 30-45 days after transplanting (DAT) and final observation were recorded at 60-75 DAT. All the WP, liquid and granular formulations of both bio-nematicides; Nematofree and Nematofree+ were found significantly effective in reducing the root knot infestation in tomato crop as compared to the control (Table 1).

About 90 to 96% reduction in root-knot infestation was recorded. A significant difference was detected among the three types of formulations for each bio-nematicide, whereas, the infestation reduction data of same formulation type for both the bio-nematicides was found non-significantly different. The maximum reduction in nematode infestation was found with the wettable powder formulations of both Nematofree and Nematofree+ followed by their respective liquid and granules on 30-45 DAT ($F=56.18$; $df=6.27$; $P=0.012$) and 60-75 DAT ($F=62.89$; $df=6.27$; $P=0.003$). A significant increase of 26 to 35% in yield was recorded in tomato crop as compared to the control ($F=102.38$; $df=6.27$, $P=0.001$).

For brinjal crop, the symptoms of root-knot infestation started appearing on 45-60 DAT and final observation were recorded on 75-90 DAT. A significant reduction in the root-knot infestation in brinjal crop was found in all the six treatments having different formulations (WP, liquid and granules) of Nematofree and Nematofree + as compared to untreated control (Table 2). Alike tomato crop result, in brinjal crop also the WP formulations of Nematofree and Nematofree + showed minimum nematode infestation followed by liquid and granules on both 45-60 DAT ($F=54.19$; $df=6.27$, $P=0.001$) and 75-90 DAT ($F=92.71$; $df=6.27$; $P=0.001$). The final yield of brinjal was also found to be significantly increased by the WP formulations as compared to the control. Among the two bionematicides, Nematofree+ was found to be more effective than Nematofree in reducing the root-knot nematode infestation symptom and increasing the yield in both tomato and brinjal crop.

Table 1. Effect of bio-nematicides on tomato crop.

Bio-nematicides	Treatments	Total number of plant	Infested plant		Infestation (%)		% Reduction in the infestation over control		Yield (quintal/acre)	Increase in the yield over control (%)
			30-45 DAT	60-75 DAT	30-45 DAT	60-75 DAT	30-45 DAT	60-75 DAT		
Nematofree (WP)	T1	84.25±3.49^{b}	0.47±0.50^{c}	2.70±0.43^{c}	0.56±0.55^{c}	3.20±0.44^{d}	96.55	96.54	24.20±0.06^{a}	26.34
Nematofree (Liquid)	T2	85.75±3.26^{b}	0.50±0.50^{c}	3.50±0.50^{c}	0.59±0.59^{c}	4.07±0.48^{c}	96.88	95.50	23.88±0.34^{a}	24.65
Nematofree (Granule)	T3	80.50±4.03^{c}	1.00±0.81^{b}	7.18±1.63^{b}	1.20±0.82^{b}	8.86±1.69^{b}	92.91	90.68	22.20±0.06^{b}	15.90
Nematofree+ (WP)	T4	89.00±4.30^{a}	0.49±0.50^{c}	2.55±0.50^{c}	0.54±0.54^{c}	2.85±0.41^{d}	96.42	96.73	25.85±0.34^{a}	34.94
Nematofree+(Liquid)	T5	86.50±2.95^{b}	0.50±0.50^{c}	3.07±0.82^{c}	0.59±0.58^{c}	3.53±0.86^{c}	96.97	96.05	24.27±0.09^{a}	26.67
Nematofree+(Granule)	T6	87.25±4.20^{a}	1.02±0.81^{b}	6.99±1.08^{b}	1.20±0.82^{b}	8.08±1.59^{b}	92.84	90.97	22.89±0.22^{b}	19.47
Untreated Control	T7	82.75±3.96^{c}	17.74±4.71^{a}	78.25±4.26^{a}	21.23±4.64^{a}	94.53±0.78^{a}	0.00	0.00	19.17±0.38^{c}	0.00
LSD (P<0.05)		2.27	0.13	1.32	0.20	0.78			2.04	
df		6.27	6.27	6.27	6.27	6.27			6.27	
F		119.02	72.51	84.12	56.18	62.89			102.38	

Note: All the data are mean value of four replications ± Standard deviation value.
Means followed by same letter in column are not significantly different (P<0.05) by Tukey's HSD.

Table 2. Effect of bionematicides on brinjal crop.

Bio-nematicides	Treatments	Total number of plant	Infested plant		Infestation (%)		% Reduction in the infestation over control		Yield (quintal /acre)	Increase in the yield over control (%)
			45-60 DAT	75-90 DAT	45-60 DAT	75-90 DAT	45-60 DAT	75-90 DAT		
Nematofree (WP)	T1	90.00±4.29^{b}	2.75±0.41^{d}	9.25±1.35^{d}	3.02±0.31^{d}	10.27±1.56^{c}	91.71	85.19	37.79±0.13^{b}	15.34
Nematofree (Liquid)	T2	85.50±3.22^{c}	4.50±0.92^{c}	13.10±0.74^{c}	5.23±0.43^{c}	15.34±0.58^{b}	86.42	79.03	36.50±0.21^{b}	11.39
Nematofree (Granule)	T3	96.00±4.13^{a}	6.50±1.05^{b}	15.42±1.45^{b}	6.75±0.36^{b}	16.06±0.78^{b}	80.14	75.29	34.66±0.35^{c}	5.77
Nematofree+ (WP)	T4	91.25±3.16^{b}	3.25±0.48^{e}	8.75±0.56^{d}	3.52±0.17^{d}	9.57±0.26^{c}	90.19	85.99	39.37±0.09^{a}	20.17
Nematofree+(Liquid)	T5	88.25±4.03^{b}	4.54±0.87^{c}	13.46±0.34^{c}	5.16±0.26^{c}	15.25±0.53^{b}	86.28	78.45	36.91±0.32^{b}	12.64
Nematofree+(Granule)	T6	89.25±4.19^{b}	6.01±0.98^{b}	13.79±0.28^{c}	6.71±0.29^{b}	15.44±0.18^{b}	81.63	77.85	34.69±0.07^{c}	5.86
Untreated Control	T7	82.75±3.46^{c}	33.25±3.26^{a}	62.50±4.17^{a}	40.10±3.24^{a}	75.61±2.98^{a}	0.00	0.00	32.77±0.17^{d}	0.00
LSD (P<0.05)		2.89	0.36	1.04	0.28	1.79		1.84		
df		6.27	6.27	6.27	6.27	6.27		6.27		
F		112.16	65.23	89.27	54.19	92.71		88.93		

Note: All the data are mean value of four replications ± Standard deviation value.
Means followed by same letter in column are not significantly different (P<0.05) by Tukey's HSD.

DISCUSSION

Paecilomyces lilacinus and *P. chlamydosporia* are the most potential fungal antagonists which successfully control root-knot nematodes by parasitizing eggs and females. These promising opportunistic fungi have been extensively studied and utilized for the management of root-knot nematode in various vegetable crops such as okra, cucurbits, tomato, brinjal and chilli. The formulations of these bio-nematicides can be applied as seed treatment, nursery bed treatment, field application and on standing crop under open field as well as protected cultivation (Gowda *et al.,* 2017). In the present study different formulations such as 1% wettable powder, 2% aqueous suspension liquid and granular formulations of *P. lilacinus* and *P. chlamydosporia* are evaluated on field conditions against root-knot infestation in tomato and brinjal crop. Seedling treatment by root dipping (@ 10gm or ml/ 1L of water) and soil treatment (@5Kg or L/acre) incorporated with FYM were applied at transplanting stage of both tomato and brinjal crop. All the six treatments having different formulations of two bio-nematicides; Nematofree and Nematofree+ showed significant reduction in the root-knot nematode infestation in both tomato crop (90 to 96%) and brinjal (75 to 86%). Among all formulation types, the wettable powder formulation of both bio-nematicides has provided promising result in reduction of root-knot infestation symptoms. The bio-formulation of *P. chamydosporia* (Nematofree+) was found to be more effective than the bio-formulations of *P. lilacinus* (Nematofree) in controlling the infestation. Application of both the bio-nematicides also increased the final yield significantly for both tomato and brinjal crop.

Similar results were also reported by different workers like Abd-Elgawad and Askary (2018) who compiled use of different bio-nematicides for integrated management. *P. chlamydosporia* based bio-formulations were found effective in open-field conditions with soil/leaf treatments followed by irrigation for root knot nematodes (RKNs) control on potato, carrot and other crops (Manzanilla-López *et al,* 2013; Ciancio *et al.,* 2016 a, b; Sellitto *et al.,* 2016; Bontempo *et al.,* 2017). Darnetty and Liswarni (2018) found 55-70% suppression of root-knot nematode by *P. lilacinus* isolates in Tomato. Application of 5 tons of FYM enriched with 2.5 kg of each *P. lilacinus* (2×10^6cfu/g) + *Pseudomonas fluorescens* (2×10^8cfu/g) have been found highly effective for reducing the root-knot nematode disease by increasing in marketable yield in okra. Similarly combined application of talc based formulations such as *Trichoderma viride* (30g/10 m^2), *P. chlamydosporia* (20g/10 m^2) and neem cake (0.15 kg/10 m^2) showed greater plant growth with a significant reduction of root-knot nematode (*M. incognita*) in chilli (Gowda *et al.,* 2017). Hano and Khan (2016) found that *Paecilomyces lilacinus* formulations both in suspension concentrates (25% SC) and wettable powder (25% WP) are effective in reducing nematode population in soil, improving plant growth parameters and enhancing tomato yield. The severity of root galling and egg mass production was found more significantly ($P< 0.05$) suppressed with the application of *P. lilacinus* in Tomato (Udo *et al.,* 2014). Bio-efficacy of bio-nematicide formulation of *P. lilacinus* (1% WP) was evaluated against root-knot nematode and it was found that seed treatment @ 20g/kg, nursery bed treatment @50g *P. lilacinus*/m^2 and application of farm yard manure (5 tons) enriched with 5kg of *P. lilacinus*/ha was significantly effective in the management of *M. incognita* and these treatments increased the yield of tomato significantly (Rao *et al.,* 2012). Sharma *et al.* (2007);Khalil *et al.* (2012) found reduction in number of galls in tomato and soil nematode population by application of *P. lilacinus*. The talc-based formulation (at 8×10^6spores/g) of *P. lilacinus*

when applied as seed treatment, seedling-dip and soil application was found to be highly effective against *M. incognita* on tomato (Priya and Kumar, 2006).

Thus it can be concluded that formulations of both *P. chamydosporia*(Nematofree+) and *P. lilacinus* (Nematofree) prepared by IPL Biologicals Ltd., India, can be an effective measure for control of root-knot infestation in vegetable crops when applied as seedling treatment (@ 10gm or ml/ 1L of water) and soil application (@5Kg or L/acre) incorporated with FYM.

Acknowledgement

The authors thank the Technology Innovation centre of IPL Biologicals Limited (Previously known as International Panaacea Limited), Gurugram for providing the bio-nematicide formulations and the farmers of Rajasthan for carrying out this investigation at their field.

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