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Evaluation of Bio-Nematon (*Purpureocillium lilacinum* 1.15%WP) against root-knot nematode (*Meloidogyne incognita*) in tomato

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Abstract
A field experiment was conducted to evaluate the bio-efficacy of Bio-Nematon (*Purpureocillium lilacinum* 1.15%WP) in comparison to carbofuran 3%G against root-knot nematode (*Meloidogyne incognita*) in tomato cv. Patharkuchi (local susceptible) at the C-Block Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal during the main and late *rabi* season of 2016-17. Five treatments comprising rhizospheric soil drenching with Bio-Nematon 1.15%WP @ 46g, 57.5g and 69g *a.i./* ha, root zone application of carbofuran 3G @ 1000 g *a.i.*/ha and an untreated control were replicated four times. The study revealed that Bio-Nematon @ 57.5-69 g *a.i.*/ha was effective and economic for the management of root knot nematode infestation along with simultaneous crop health improvement and fruit yield enhancement. Increase in fruit yield by 11.6% and 11.4% over untreated control was recorded with Bio-Nematon @ 69 g *a.i.*/ha in the 1st and 2nd season, respectively. It implies that the fungus, *P. lilacinum* is a potential biological control agent of *M. incognita*. There was no detrimental effect of Bio-Nematon @ 46-69 g *a.i.*/ha on the beneficial nematode population in the tomato field. The incremental cost benefit ratio (ICBR) revealed the superiority of all the doses of Bio-Nematon over the treated check and untreated control. The maximum ICBR was recorded in soil drenching with Bio-Nematon @ 69 g *a.i.*/ha being, 1:9.33 and 1:12.89 in the main and late *rabi* crop, respectively. The study emphasizes that Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha can safely be recommended for the management of menacing infestation of root-knot nematodes in tomato crop.

Keywords: Bio-Nematon, beneficial nematodes, *Meloidogyne incognita*, *Purpureocillium lilacinum*, tomato

1. Introduction
Tomato (*Solanum lycopersicum* L.) belonging to the family Solanaceae, is one of the most important commercially grown vegetable crop of tropics and sub-tropics. It is grown for its edible fruits, which can be consumed either fresh or in processed form and provide a tremendous amount of vitamin A, vitamin K, vitamins B_1, B_3, B_5, B_6, B_7 and vitamin C and minerals. India is the second largest producer of tomato after China. As per the estimates of the Horticulture Statistics Division of the Department of Agriculture, Cooperation and Farmers Welfare under Ministry of Agriculture and Farmers Welfare, Govt. of India the annual production of tomato in the country was 19.7 million tons from an area of 0.81 million ha during 2017 [1]. Madhya Pradesh, Karnataka, Andhra Pradesh, Telangana, Gujarat, Odisha and West Bengal are the major tomato growing states in India. West Bengal among all the states of India in respect of acreage 0.57 million ha and production 1.23 million tons of tomato in 2016-2017 [1]. Quite a large number of pests infest the tomato crop in its different pheno-phases with substantial reduction of fruit yield both in terms of quality and quantity. It suffers from several biotic stresses like infestation of insects, mites, nematodes, fungi, bacteria, viruses, phytoplasma etc. Among plant feeding nematodes, root gall inducing nematode species, *Meloidogyne incognita* (Kofoid and White) Chitwood is the most dominant and widely prevalent species inflicting serious loss of tomato fruit yield across the globe. An avoidable yield loss of 30.57 - 46.92% and 77.5% had been reported in tomato due to infestation of *M. incognita* and *M. javanica*, respectively [2]. Plants suffered from infestation of *M. incognita* reveal stunting, yellowing and a general unthrifty appearance along with characteristic root galls which ultimately result in reduction of growth and yield. Wide host range, intensive cultivation of susceptible crops over the period, continuous mono-cropping, high biotic potential of the nematode, non-availability of the resistant crop cultivars and registered

nematicides make the situation more complicated to restrain the nematode infestation.

To secure the yield and profits, there is an urgent need to manage this unseen, soil borne plant-parasite. Tomato growers of the country are in deep grief for a solution to tackle the menacing effect of this dreaded nematode. However, we cannot overrule the adverse impact of commercially available chemical pesticides with nematistatic action on non targets. Human health and the environment is evidently a major concern in today's world. There is an urgent need and noble reason to rely upon biological agent to check the yield reduction due to root-knot nematode infestation. Biological control is being considered as a component of integrated nematode management programme. *Purpureocillium lilacinum* (Thom) Luangsa-ard, Hou- braken, Hywel-Jones & Samson is an efficient nematophagous fungus and ovoparasite. It has shown promising results as a biocontrol agent against the destructive root-knot nematode species [3-7]. It is being widely used as a biocontrol agent against *M. incognita* [8, 9]. *P. lilacinum* enters the females of *M. incognita* through the anal or vulval opening [10]. They colonize the gelatinous egg matrix of root-knot nematode and penetrate the nematode's eggs through formation of appressoria [11]. In understanding of economic importance of root-knot nematode on tomato crop, the present work was planned to evaluate the efficacy of commercial formulation of *P. lilacinum* 1.15% WP (Bio-Nematon) against *M. incognita* on tomato.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted in a sick micro-plot having high population of *M. incognita* race 2 at the C-Block Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. The experimental site was located at 22°59'13.20''N latitude and 88°27'16.05"E longitude with an elevation of 14.32 m above mean sea level. The soil of the experimental field was *Gangetic* alluvial (Entisol) having sandy loam texture with good drainage facility, pH 6.7 and moderate in fertility. Weather condition during the period of experimentation revealed 33.5 ^{0}C minimum and 10.9 ^{0}C maximum mean temperatures,

58.2- 96.8% mean RH and 167 mm total rainfall during September to December, 2016 to and 32.4^{0}C minimum and 8.5 ^{0}C maximum mean temperatures, 42.6- 96.3% mean RH and 18.3 mm total rainfall during January to March, 2017.

2.2 Experimental details

Thirty days old seedlings of tomato cultivar Patharkuchi (local susceptible) at 4-5 leaves stage was transplanted during main and late *rabi* season of 2016-17. Recommended package of practices for raising tomato was adopted. All the plots received N:P_2O_5:K_2O::100:80:80 kg/ha. One third of the recommended dose of N and full dose of P_2O_5 and K_2O were given as basal application. Remaining amount of nitrogen was top dressed in two equal halves once at 20 days after transplanting (DAT) and again at 40 DAT.

The experiment was laid out in Randomized Complete Block Design. Each plot was measuring 9 sq. m. Four replicates of five treatments *viz*., T_1: Bio-Nematon (*P. lilacinum* 1.15% WP) @ 46 g *a.i.*/ha; T_2: Bio-Nematon (*P. lilacinum* 1.15% WP) @ 57.5 g *a.i.*/ha;

T_3: Bio-Nematon (*P. lilacinum* 1.15% WP) @ 69 g *a.i.*/ha; T_4: Carbofuran 3G @ 1000g *a.i.*/ha; and T_5: Untreated check were used in the study. Treatments were delivered once at the time of transplanting and again at 30 days after transplanting. Bio-Nematon was used exclusively for drenching of rhizosphere after dissolving the required amount into water @ 500ml/ha. Required amount of carbofuran 3G was applied in a ring around the plant individually and watered subsequently.

2.3 Observations

Observations were recorded on soil nematode population before transplanting, at 30 DAT and 60 DAT, root knot index at 60 DAT and yield. The nematode population was recorded from the soil sample (200 cc) and root sample (5g) collected from each plot. About 3 sub-samples of 200 cm^3 each was collected from each treatment and pooled. Combined Baermann and Cobb's Sieving Technique was adopted to extract the nematodes from the soil [12]. The root samples were washed free of soil and dirt particles clung to the roots under gentle flow of tap water and cut into pieces of about 1cm length. Thereafter, roots were split longitudinally and placed on double layer facial tissue paper resting on aluminum wire-gauze and follow the modified Baermann Technique. At 60 DAT, five grams of root samples were collected and after washing they were rated for root-knot index (RKI) in 0-5 scale (0 = no gall, 1= 1-5 galls, 2 = 6-10 galls, 3 = 11-15 galls, 4 = 16-20 galls, 5 = > 20 galls). Effect of treatments on the non-plant parasitic nematodes and phytotoxicity on the tomato crop in 0-10 scale (0 = 0, 1-10 = 1, 11-20 = 2, 21-30= 3, 31-40 = 4, 41-50 =5, 51-60 = 6, 61-70 = 7, 71-80 = 8, 81-90 = 9 and 91-100 = 10) was also assessed.

2.4 Statistical analysis

The average nematode population in each treatment was recorded and computed for statistical analysis. Number of galls per root system was recorded for the calculation of root-knot index (RKI). Data on the yield at each harvest was collected and used for computation of yield per ha. Based on yield, the incremental benefit cost ratio was worked out. Data were subjected to statistical analysis after making suitable transformation [13].

3. Results

3.1 Effect of Bio-Nematon 1.15%WP on reduction of *M. incognita* population in tomato

Soil population of *M. incognita* before treatment initiation did not vary significantly between plots of tomato crops in both the main and late *rabi* seasons of 2016-17. Reduction of *M. incognita* population over the mean nematode population after 2nd application of Bio-Nematon was observed maximum in T_3 i.e. Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha being, 47.9% and 55.7% in the 1st and 2nd crop season, respectively (Table 1 & 2). Reduction of *M. incognita* population at 30 days after 2nd application of Bio-Nematon was observed maximum with T_2 i.e. Bio-Nematon 1.15%WP @ 57.5 g *a.i.*/ha being, 36.6% during 1st crop season and with Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha being, 40.8% during 2nd crop season (Table 1 & 2). Application of Bio-Nematon 1.15%WP @ 57.5 g *a.i.*/ha was statistically at par with the standard treated check (carbofuran 3%G @ 1000 g *a.i.*/ha) to keep the nematode population low during 1st crop season (Table 1) but was statistically superior over it to keep the nematode population low during 2nd crop season (Table 2).

Drenching of rhizospheric soil of tomato twice (before transplanting and at 30 DAT) with Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha revealed significantly minimum root gall index at

60 DAT being, 2.3 and 1.3 during main and late *rabi* season crop, respectively (Table 1 & 2). Root-knot indices of tomato in drenching of Bio-Nematon (*P. lilacinum*1.15%WP) @ 46 and 57.5 g *a.i.*/ha once immediately after transplanting and again at 30 days after transplanting of tomato were statistically at par with the soil application of carbofuran 3% G @ 1000 g *a.i.*/ha in the main *rabi* crop (Table 1). However, root-knot indices of tomato in two rounds of soil drenching with Bio-Nematon 1.15%WP @ 46 and 57.5 g *a.i.*/ha at transplanting and at 30 days after transplanting were significantly lower than the treated check (carbofuran 3% G @ 1000 g *a.i.*/ha) in the late *rabi* season crop (Table 2).

3.2 Effect of Bio-Nematon 1.15% WP on tomato yield

Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha achieved maximum fruit yield of tomato being, 222.8 and 312.5 q/ha during the main and late *rabi* season, respectively. Increase in fruit yield by 11.6% and 11.4% over untreated control was recorded with Bio-Nematon @ 69 g *a.i.*/ha for the 1st and 2nd season, respectively.

3.3 Incremental cost benefit ratio (ICBR)

Incremental cost benefit ratio revealed the superiority of all the doses (46-69 g *a.i.*/ha) of Bio-Nematon over the treated check carbofuran @ 1000 g *a.i.*/ha and untreated control. The maximum ICBR was recorded in soil drenching with Bio-Nematon @ 69 g *a.i.*/ha being, 1:9.33 and 1:12.89 in the main and late *rabi* crop, respectively (Table 3 & 4).

3.4 Effect of Bio-Nematon 1.15% WP on the phytotoxicity of tomato crop

None of the doses of Bio-Nematon reveal phytotoxic effect on tomato plant as observed 7 days after their application in both seasons crops (Table 3 & 4).

3.5 Effect of Bio-Nematon 1.15% WP on beneficial nematodes

Beneficial nematode fauna comprising of rhabditids, dorylaimids and mononchids were observed maximum in untreated control and was followed by Bio-Nematon @ 46 g *a.i.*/ha. Beneficial nematode fauna in Bio-Nematon @ 57.5-69 g *a.i.*/ha was at par with the treated check carbofuran 3G @ 1000 g *a.i.*/ha in the 1st season crop (Table 3). However, Bio-Nematon @ 46-69 g *a.i.*/ha recorded significantly more number of beneficial nematodes than the treated check carbofuran 3G @ 1000 g *a.i.*/ha in the 2nd season crop (Table 4).

4. Discussion

In present study, soil drenching with the commercial formulation of *P. lilacinum* 1.15% WP i.e. Bio-Nematon @ 69 g *a.i.*/ha achieved 47.9% and 55.7% reduction of *M. incognita* population over the mean nematode population after its 2nd application in the 1st and 2nd crop season, respectively. According to Goswami and Mittal [14] *P. lilacinum* showed greatest egg parasitizing efficiency (80%) compared to other fungi. Fungal propagules colonized on egg masses and eggs inside the egg masses were parasitized by fungus, due to which egg content was reduced and some eggs were found deformed. Similar results on root-knot nematode management of okra were also noted by earlier researchers [15].

In the present experimentation, drenching of rhizospheric soil of tomato twice (before transplanting and at 30 DAT) with Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha revealed significantly minimum root gall index at 60 DAT being, 2.3 and 1.3 during main and late *rabi* season crop, respectively. Root galling**,** number of egg masses and nematode population was reduced by 66, 74 and 71%, respectively following pre-plant soil treatment of *P. lilacinum* in tomato as compared to untreated check [16, 17]. The reduction in nematode population in tomato roots and reduction in gall formation were observed by about 67-77% and 30%, respectively applying *P. lilacinum* [18]. When compared with popular nematicides such as azadirachtin, oxamyl, *Pseudomonas fluorescens*, *Bacillus subtilis* and abamectin against the *M. incognita*, *P. lilacinum* (Bio-Nematon) was most effective in reducing both egg and gall masses by 76.9 and 88.2% respectively [19]. Ethyl acetate extract of *P. lilacinum* resulted in 100% mortality of *M. javanica* larvae after 24 h of exposure as compared to *Pseudomonas aeruginosa* extract which resulted in 64% mortality of the same [20]. Increase of plant height, root length, shoot dry weight and root dry weight by 51.28%, 87.0%, 55.53%, 67.62%, respectively over untreated check was observed following seed treatment with *P. lilacinum* @ 5 ml/kg along with soil application of vermicompost @ 2.5 t/ha enriched with *P. lilacinum* @ 10 ml/kg [21]. In the same study, final nematode population in soil and in the roots of okra was reduced to 171.0 J2/200cc soil and 41.25/ 5g root, respectively with the lowest root knot index of 2.0. Simultaneously, highest fruit yield @ 7.19 t/ha (36.6% higher than control) was recorded in the same study. Here, authors observed increase in fruit yield of tomato by 11.6% and 11.4% over untreated control in two rounds of soil drenching with Bio-Nematon @ 69 g *a.i.*/ha in the 1st and 2nd season, respectively.

The present study unveiled the superiority of all the doses of Bio-Nematon over the treated check and untreated control with regard to the economics of the treatment imposition. The maximum ICBR was recorded in soil drenching with Bio-Nematon @ 69 g *a.i.*/ha in the main and late *rabi* crops. *P. lilacinum* was observed as the most economic biological agent for managing *M. incognita* population in tomato besides chemical nematicides [21]. The results obtained from the present study are in conformity with those of previous researchers.

Paucity of information pertaining to the phytotoxicity of commercial formulation of *P. lilacinum* made difficult to compare the present finding with earlier information.

The fungal bioagent, *P. lilacinum* prefer to colonize on egg masses of root-knot nematodes and parasitize them. They can parasitize juveniles and females of root-knot nematodes too. In contrast, beneficial nematodes which are more agile in nature than that of root-knot nematodes and have tendency to lay eggs scatteredly in the soil may possibly escape parasitization from the fungal inoculants. This may be reason of having presence of good number of population of beneficial nematodes in Bio-Nematon treated plot.

Table 1: Effect of Bio-Nematon on reduction of *M. incognita* population in tomato during main *rabi* season of 2016-17

Treatments	Nematode population / 200 cc soil + 5g root PAT				% Reduction over mean after 2nd application	% Reduction over 30 days after 2nd application	RKI (0-5 Scale)
	#PBT	30 DAT	60 DAT	Mean			
T_1: Bio-Nematon 1.15WP @ 46 g *a.i.*/ha	211.8	158.5	135.0	168.4	25.3* (19.3)	21.8 (14.2)	3.8
T_2: Bio-Nematon 1.15WP @ 57.5 g *a.i.*/ha	254.0	168.9	106.0	176.3	39.3 (39.6)	37.5 (36.6)	3.5
T_3: Bio-Nematon 1.15WP @ 69 g *a.i.*/ha	248.8	122.0	78.5	149.7	44.1 (47.9)	37.0 (36.0)	2.3
T_4: Carbofuran 3G @ 1000 g *a.i.*/ha (treated check)	225.8	176.3	127.3	176.4	31.9 (27.7)	31.1 (27.1)	4.3
T_5: Untreated Control	222.5	268.5	427.4	306.1	4.1 (0.0)	4.1 (0.0)	5.0
SEm (±)	13.22	7.48	9.18	6.76	2.81	3.46	0.37
LSD (0.05)	NS	23.05	28.29	20.83	8.66	10.66	1.14

Note: PBT: Population before treatment, PAT: Population after treatment, DAT: Days after transplanting, Data shown are (x+0.5) angular transformed values, Figures in parentheses indicate original values, # soil population only

Table 2: Effect of Bio-Nematon on reduction of *M. incognita* population in tomato during late *rabi* season of 2016-17

Treatments	Nematode population / 200 cc soil + 5g root PAT				% Reduction over mean after 2nd application	% Reduction over 30 days after 2nd application	RKI (0-5 Scale)
	#PBT	30 DAT	60 DAT	Mean			
T_1: Bio-Nematon 1.15WP @ 46 g *a.i.*/ha	203.5	173.3	137.8	171.5	26.6* (19.7)	27.1 (20.4)	1.8
T_2: Bio-Nematon 1.15WP @ 57.5 g *a.i.*/ha	200.8	120.0	73.0	131.3	42.0 (44.3)	39.2 (39.5)	1.5
T_3: Bio-Nematon 1.15WP @ 69 g *a.i.*/ha	229.3	95.1	56.3	126.9	48.6 (55.7)	40.0 (40.8)	1.3
T_4: Carbofuran 3G @ 1000 g *a.i.*/ha (treated check)	199.5	144.0	94.5	146.0	36.7 (35.2)	36.2 (34.4)	3.0
T_5: Untreated Control	179.8	200.0	262.3	214.0	4.1 (0.0)	4.1 (0.0)	4.5
SEm (±)	11.33	6.29	5.27	5.88	1.58	1.16	0.33
LSD (0.05)	NS	19.38	16.24	18.12	4.87	3.57	1.02

Note: PBT: Population before treatment, PAT: Population after treatment, DAT: Days after transplanting, Data shown are (x+0.5) angular transformed values, Figures in parentheses indicate original values, # soil population only

Table 3: Effect of Bio-Nematon on tomato yield, ICBR, beneficial nematode population and phytotoxicity during main *rabi* season of 2016-17

Treatments	Yield (q/ha)	% Yield increase over control	ICBR	Number of beneficial nematodes /200 cc soil	Phytotoxicity (0-10 Scale)
T_1: Bio-Nematon 1.15WP @ 46 g *a.i.*/ha	207.4	3.9	1:4.00	184.9	0
T_2: Bio-Nematon 1.15WP @ 57.5 g *a.i.*/ha	212.0	6.2	1:5.59	155.6	0
T_3: Bio-Nematon 1.15WP @ 69 g *a.i.*/ha	222.8	11.6	1:9.33	145.5	0
T_4: Carbofuran 3G @ 1000 g *a.i.*/ha (treated check)	203.8	2.1	1:1.26	141.5	0
T_5: Untreated Control	199.7	--	--	263.0	0
SEm (±)	4.84	--	--	12.96	--
LSD (0.05)	14.91	--	--	39.93	--

Note: ICBR= incremental cost benefit ratio, labour wages – Rs 247/man-day, price of Bio-Nematon - Rs.413/kg, price of carbofuran 3G - Rs.110/kg

Table 4: Effect of Bio-Nematon on tomato yield, ICBR, beneficial nematode population and phytotoxicity during late *rabi* season of 2016-17

Treatments	Yield (q/ha)	% Yield increase over control	ICBR	Number of beneficial nematodes /200 cc soil	Phytotoxicity (0-10 Scale)
T_1: Bio-Nematon 1.15WP @ 46 g *a.i.*/ha	299.4	6.7	1:9.77	238.5	0
T_2: Bio-Nematon 1.15WP @ 57.5 g *a.i.*/ha	307.6	9.6	1:12.27	235.3	0
T_3: Bio-Nematon 1.15WP @ 69 g *a.i.*/ha	312.5	11.4	1:12.89	180.8	0
T_4: Carbofuran 3G @ 1000 g *a.i.*/ha (treated check)	288.5	2.8	1:2.44	129.8	0
T_5: Untreated Control	280.6	--	--	489.9	0
SEm (±)	5.40	--	--	10.17	--
LSD (0.05)	16.64	--	--	31.34	--

Note: ICBR= incremental cost benefit ratio, labour wages – Rs 247/man-day, price of Bio-Nematon - Rs.413/kg, price of carbofuran 3G - Rs.110/kg

5. Conclusions

The present investigation on the bio-efficacy and phyto-toxicity of Bio-Nematon (*P. lilacinum* 1.15%WP) on tomato crop conducted during two consecutive seasons revealed that Bio-Nematon @ 57.5-69 g *a.i.*/ha was effective and economic for the management of root knot nematode (*M. incognita*) infestation along with improved crop vigour and enhanced fruit yield. It implies that the fungus, *P. lilacinum* is a potential biological control agent of root-knot nematode. There was no detrimental effect of Bio-Nematon @ 46-69 g *a.i.*/ha on beneficial soil dwelling nematode population in tomato field. The ICBR revealed the superiority of all the doses of Bio-Nematon over the treated check and untreated control. The maximum ICBR was recorded in soil drenching with Bio-Nematon @ 69 g *a.i.*/ha. The study emphasizes that Bio-Nematon 1.15%WP @ 69 g *a.i.*/ha can safely be recommended to the tomato growers for the management of menacing infestation of root-knot nematodes.

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