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CONTENTS

Biointensive management of *Meloidogyne enterolobii* in tomato under glasshouse conditions

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ABSTRACT: *Meloidogyne enterolobii* is a tropical or subtropical nematode and has a broad host range, including cultivated plants and weeds. For decades, the control of sedentary nematodes has relied heavily on chemical nematicides. The present investigations were carried out to study bio-intensive management of *Meloidogyne enterolobii* causing root-knot disease in tomato. The experiments were laid out using different treatments of biocontrol agents (*Trichoderma asperellum* (Ta-14), *Pseudomonas flourescens* (Psf-173), PBAT 3 (consortium of Ta14 & Psf173) and *Paecilomyces lilacinus* (commercial) with the combination of Biofumigation (*Brassica juncea* & *Raphanus sativus*). Nematicide carbofuran was used as chemical check. All the treatments showed positive effect on the growth parameters of tomato plants. Combination of PBAT3+Biofumigation(M) resulted in maximum fresh root (7.38g) and shoot weight (36.07g) with minimum number of root galls (18.33galls per root system) as compared to the combination of other treatments.

Key words: Bioagent, biofumigation, nematode, tomato

Tomato (*Solanum lycopersicum* L.) is one of the most ubiquitous and remunerative vegetable crop in India. It is one of the member of family Solanaceae. Tomato have great nutritional importance which are rich in vitamins A, C, thiamine, riboflavin, and ni-acin as well as some minerals like potassium and sodium (Janes, 2004). Globally, India second in ranks with regard to production (184 million tons/ annum) and consumption (144 million tons/annum, Anonymous 2017). The crop is attacked by several destructive pests and diseases that cause severe damage. One of the important pests of tomatoes is the plant-parasitic nematodes, especially root-knot nematodes. Root-knot nematode *Meloidogyne* spp., is the most important limiting factor of plant productivity (Sasser and Carter, 1985; Sikora and Greco, 1993). These pests very difficult to manage, being obligate sedentary endoparasites with an inherent capacity to cause complex diseases by interacting with other fungal and bacterial pathogens, (Fourie and McDonald, 2000). Considerably, in vegetables crop this polyphagous pest is reported to cause an average 10% yield losses. In highly susceptible crops, such as tomato, eggplant and melons it is reported to cause 30% of yield loss (Gowda *et al.,* 2017). *Meloidogyne enterolobii* (Yang and Eisenback, 1983) is a tropical or subtropical nematode and has a broad host range, including cultivated plants and weeds. It is now considered to be one of the most important root-knot nematode species as it displays wide virulence in economically important crops and has a higher pathogenicity and reproductive potential than either *M. incognita* or *M. arenaria* (Subbotin, 2019). This nematode is polyphagus and can infect vegetable crops like tomato, okra, capsicum, sweet potato, ornamentals and fruit crops like guava, melon, fig etc. From decades, chemical nematicides heavily relied method for the control of sedentary nematodes. Although crop rotation and resistant crop cultivars have also been used as complementary methods (Li *et al*., 2015). Since many of the most commonly used nematicides are expensive or are being withdrawn from the market *viz.*, (Dibromochloropropane (DBCP) dated 25th July 1989), Aldicarb (dated 17th July 2001), Carbofuron 50% SP (dated 17th July 2001), Dazomet (31st Dec, 2008), Phorate (to be completely banned until 31st December, 2020) (Anonymous, 2019) due to their persistence in the soil, harmful effect on humans or contamination of ground water. To mitigate these challenges, investigators are concentrating their efforts on integrating biological control strategies for nematode management (Jatala, 1986).

In a particular rhizosphere commonly more than one microorganism occurs with plant parasitic or saprozoic nematodes. Constant association results in a biological balance that may manifest itself in the form of direct parasitism by attachment and penetration by one or more pathogenic microorganisms in the nematode eggs, juveniles and even adult nematodes, causing death and possibly allowing subsequent invasion by many saprophytic microorganisms. Egg masses, sedentary females may be directly invaded by pathogenic or some opportunistic organisms that draw their nutrients from the mucilaginous compounds present in the invaded body or indirectly invaded by the action of toxic, diffusible metabolites produced by one or more organisms on various developmental stages of nematodes. These toxins often render nematodes (particularly eggs and juveniles) more vulnerable to infection or to the activities of organisms that are either nonvirulent, slightly pathogenic, or basically saprophytic in nature (Jatala, 1986).

Biofumigation in recent years has emerged for the management of nematode pests as an effective nonchemical alternative. As originally defined, the term "biofumigation" demonstrates the suppressive effects of Brassicaceae family plant on noxious soilborne pathogens and is specifically attributed to the release of biocidal isothiocyanates (ITCs) due to the hydrolysis of glucosinolates (GSLs, thioglucosides) present in crop residues, catalysed by myrosinase (MYR, β-thioglucoside glucohydrolase) isoenzymes The secondary metabolite GSL (located in cell vacuole) is sequestered within the plant's tissues along with the hydrolysing enzyme MYR (stored in cell wall or cytoplasm) (Matthiessen and Kirkegaard, 2006; Motisi *et al*., 2010).

Considering the emergence of soil health problems and depletion of environment due to the spurious use of nematicides to control soil borne nematodes, there is an urgent need to find novel, environmentally friendly and effective management strategies for their management, thereby conserving natural resources. Keeping in view the importance of Biological control and Biofumigation as ecofriendly management techniques, the present investigations were carried out to study bio-intensive management of *Meloidogyne enterolobii* causing root-knot in tomato plant roots.

MATERIALS AND METHODS

The studies were carried out during 2017-2018 and 2018-2019 in the glass house and laboratory of Bio Control, Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand

Source of crop seeds

Seeds of tomato crop var-Pant T-3, raddish crop var-Japanese White were obtained from the Vegetable Research Centre (VRC) and mustard var- Kranti from the Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand).

Nematode Culture preparation

The stock culture of root knot nematode to be used in the experiment were derived from single egg mass of *M. enterolobii* originated from infected feeder roots and soil around the vicinity of guava plant, collected from Horticulture Research Center, Pantnagar.UK. The stock culture was established by placing single egg mass of the nematode beneath the root system of tomato seedlings.

Extraction of second stage juveniles of the *Meloidogyne enterolobii*

Isolated egg masses teased carefully in the sterilize distilled water with the help of needle, which were kept in incubator at 27±1^{0}C for up to 7 days for complete hatching of eggs. This hatched juvenile suspension was managed in such a way that 1 ml suspension contained 100 J2 individuals. This concentrated freshly hatched *M. enterolobii* juvenile suspension was used for further studies. Freshly hatched juveniles of *M. enterolobii* were counted in counting dish under stereo zoom microscope at various magnifications.

Identification of root-knot nematode species

The roots infested with root-knot nematode were

washed in tap water. The females dissected out from well-developed galls of the root under stereo-binocular microscope and transferred to Petri plate containing water. The posterior portion of the female is cut with the help of sharp blade (Taylor and Netscher, 1974) and the body contents are cleaned by peacock feather pick. Cleaned posterior portion of the female is further trimmed and transferred to a clean microscopic slide containing a drop of glycerine. A coverslip is placed on it, sealed with nail polish and observed under stereo-binocular microscope. The species confirmation is done through the perineal pattern as described by (Chitwood,1949).

Pathogenicity test

Soil mixture was prepared by mixing soil, sand and vermicompost (2:1:1). This was sterilized in the autoclave and filled in surface sterilized pots. Three-week-old tomato seedlings raised in the sterilized soil were transplanted in the pots. These pots were placed in glasshouse having regulated temperature and moisture. After the establishment of the plant, each pot was inoculated with 2000 second stage juveniles of *M. enterolobii* at root zone after removing surface soil. After 45 days of inoculation, the tomato plants were uprooted. There was extensive formation of galls on the roots. Roots showing formation of galls was washed carefully and galls were removed and cut open with the help of dissecting needles. Thereafter, perineal pattern of females was prepared and examined under the microscope for the confirmation of test nematode (Mateille *et al*., 1995).

Maintenance of *Meloidogyne enterolobii*

The root - knot nematode, (*M. enterolobii*) culture was maintained in susceptible tomato plants variety Pant T-3. Sterilized soil mixture was filled in 5kg capacity plastic pots. Three weeks old seedlings of tomato plants were transplanted in these pots. After one week of plant establishment, 4-5 egg mass containing 400-600 eggs in each egg mass were inoculated in the vicinity of tomato roots by removing surface soil. These pots were kept in glass house under controlled conditions and watered when required. Extensive root knots were formed within two months. These plants were maintained throughout the investigation as mother plants for test nematode.

Treatments preparation

(i) Soil mixture preparation: A mixture of soil was thoroughly mixed and steam sterilized at 120°C for 120 minutes. The soil was then filled into previously sterilized pots (1kg capacity).

(ii) Collection of infested soil: Soil was collected from the mother culture of *Meloidogyne enterolobii* which were previously maintained on tomato plant by uprooting the plants.

(iii) Counting of initial population: Population of nematodes of the pot mixture was counted under Magnus Inverted light microscope before establishing the experiment.

(iv) Biofumigation: *Brassica juncea* and *Raphanus sativus* tissue maceration and incorporate in soil.

Mustard plants (*Brassica juncea* -Variety Kranti) and Raddish plants (*Raphanus sativus*- Variety Japanese white) grown in the field. It was uprooted at 50 per cent flowering stage, taken to the glass house and and washed thoroughly with tap water to remove adhering soil. Plants were chopped into small pieces seperately. The chopped plant parts were mixed into soil @ 100g/pot to the top 5 cm layer of soil as per treatments. In control there was no incorporation of *B. juncea* and *R. sativus* in the soil. After filling the pots with desired treatments, water was added to the pots in order to hydrolyse the glucosinolate present in the plant parts. Each biofumigated pot was then tightly covered with a transparent polythene sheet for two weeks. After removing polythene sheet, soil was loosened.

(v) Application of Biocontrol agent

- Soil treatment: Biocontrol Agent @ 1kg/q vermicompost is mixed 15 days prior to it's application in the soil so that it can get multiplied into the compost.
- Seed biopriming: Seeds are mixed with the formulated BCAs @10g/kg. Water sprinkled while mixing bioagents. These were placed on

plastic sheet as a heap. Covered with moist sacks and incubated under moist conditions for 24 to 48h before sowing.

- Root Dip Method: Used for the treatment of tomato seedlings. Pant T-3 a susceptible variety is used for experimental purpose. Before transplantation of seedlings, roots were dipped in bioagent suspension @10g/l water for 24 hrs. Next day seedlings were transplanted.
- Soil drenching: Solution of Biocontrol Agent was mixed in water @10g/l water and soil drenching is done at root at 15 days interval.

(vi) Application of Carbofuran: The Carbofuran (nematicide) was thoroughly mixed into the soil in the plastic pots with dose of 6.15mg/kg soil before transplanting the tomato seedlings.

Pots were filled with according the treatment. 3-week - old tomato seedlings (Pant T-3) were transplanted singly in each of treated pot. Each treatment was replicated thrice and equal numbers of non-treated replicates served as the control. Plants were uprooted after 45 days. Following Observations to be taken after 45 days of transplanting: Root length and Shoot length; Fresh shoot and fresh root weight; Dry shoot and dry root weight; Number of gall formation; Population of nematodes present in each treatment.

The nematodes from the soil were extracted by sieving and decanting methods (Barker, 1985).

The treatments included: T1-Biofumigation (Mustard); T2-Biofumigation (Radish); T3-*Trichoderma asperellum* 14; T4-Ta14+Biofumigation (M); T5- Ta14+Biofumigation (R); T6- *Pseudomonas flourscens* 173; T7-Psf173+Biofumigation (M); T8- Psf173+ Biofumigation (R); T9-Pant Biocontrol Agent-3; T10- PBAT3+Biofumigation (M); T11- PBAT3+ Biofumigation (R); T12- Commercial formulation (CF- *Paecilomyces*); T13- CF+Biofumigation (M); T14-CF+Biofumigation (R); T15- Standard Chemical check (Carbofuran); T16- Polysheet mulching alone; T17- Control (untreated)

*Pant Biocontrol Agent-3 (PBAT3) is a consortium of *Trichoderma asperellum* and *Pseudomonas fluorescens* developed at GBPUA&T.

*Treatments (T3, T6, T9, T12, T15 and T17) not covered with polythene sheet remaining treatments covered with polysheet for biofumigation.

*The data generated from the glasshouse and laboratory experiment using completely randomized design were analyzed statistically by STPR software (GBPUA&T statistical software).

RESULTS AND DISCUSSION

Morphological identification of nematode (*Meloidogyne enterolobii*)

After extraction of nematodes using Cobb's Sieving and Decanting Technique, isolation of female and egg masses from the infected root, identification of nematode was done under Magnus Inverted light microscope at 10X and 40X magnification.

Morphological characters of root knot nematode (*M. enterolobii*)

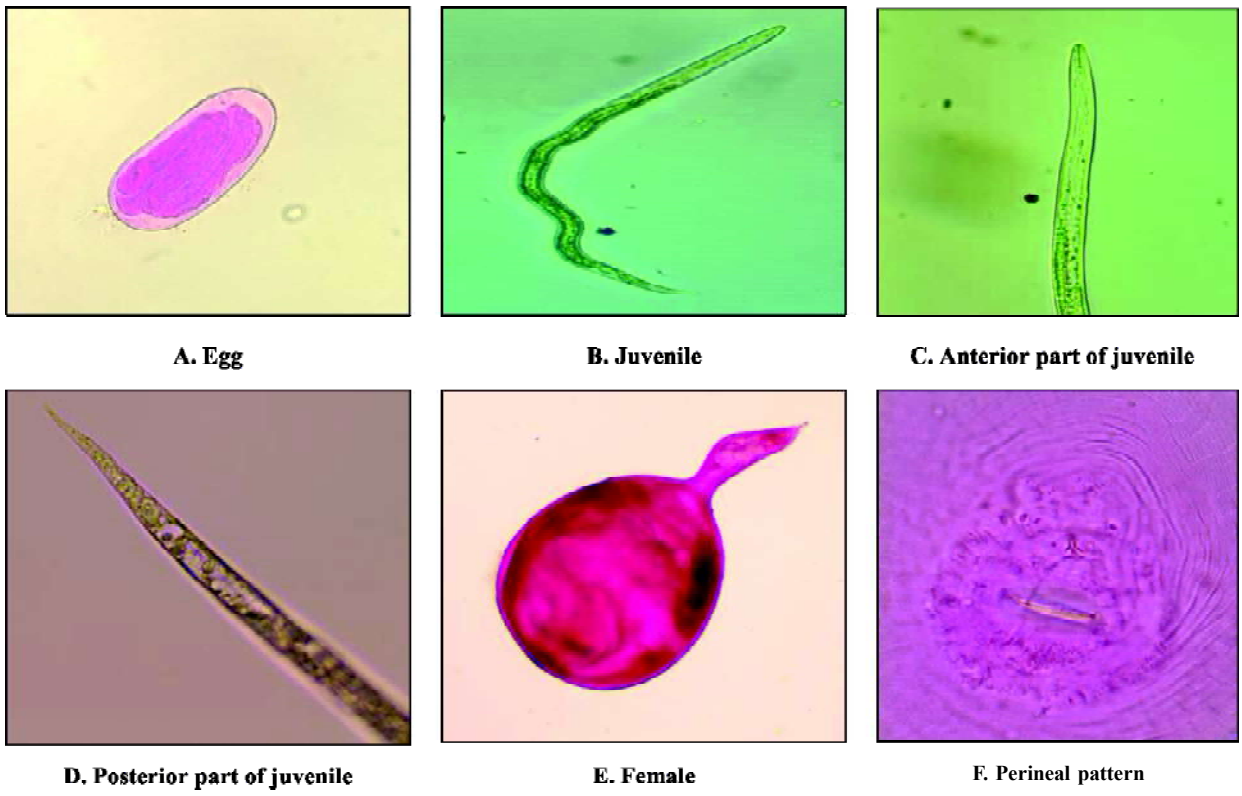
Stages of nematode	Characters found
Egg	Eggs were located on the root surface laid by the female in a gelatinous sac. Females produce approximately 400–600 eggs (Plate-1a-A).
Juvenile	Juveniles were vermiform, the tail of second stage juvenile was relatively thin, long and rounded ending portion and with hyaline region, and sometimes with a lobed terminus (Plate-1a-B-D).
Female	Females were characteristically pear-shaped pearly-white in colour. The perineal pattern of adult females showed an oval shape with dorsal arch, usually high and round, weak lateral lines and large phasmids. There was a lip region not annulated and an elongated neck. Tail tip was marked with prominent, coarse, well separated striae (Plate-1a-E-F).
Male	Males had annulated vermiform body, while a slightly set off head region, rounded tail and spicule located on the tail tip (Plate-1b-A-C).

These results are in accordance with the results of Yang and Eisenback (1983), they described the similar characteristic features of *M. enterolobii*. The

perineal pattern of the female is usually oval shaped, the striae are fine to coarse, the dorsal arch is moderately high and usually rounded, and the phasmids are large. Other characteristics features of *Meloidogyne enterolobii*, *viz*., the stylet knobs in females are divided longitudinally by a groove so that each knob appears as two. The mean distance of the excretory pore to the anterior end in the female is 62.9 µm. Males have a large, rounded labial disc that fuses with the medial lips to form a dorso-ventrally elongate head cap. Anonymous (2014) found that the eggs are laid by the female in a gelatinous sac near the root surface females produce around 400–600 eggs. Kumar and Rawat (2018) studied the perineal pattern of the female placing on the glass slides with a drop of water. Posterior region was cut with the help of a sharp blade. It is then cleaned, covered with neat cover slip and sealed. The prepared perineal pattern of adult females showed an oval shape, dorsal arch usually high and round, weak lateral lines sometimes present, large plasmids, typical characters of the species *M. enterolobii*. There is also a lip region not annulated and an elongated neck. Tail tip was marked with prominent, coarse, well separated striae.

Pathogenicity test

The pathogenicity test was conducted to establish the association of host with nematode to cause disease. Tomato seedlings were transplanted in the sterilized soil which was artificially inoculated by the J2 of *M. enterolobii*@ 2000 J2/pot of soil as described in material and methods. After 45 days of inoculation, the tomato plants showing typical symptoms of stunted growth and yellowing of leaves were uprooted and the pathogenicity test was confirmed by visualizing the gall formation in roots. These galls were cut open with the help of dissecting needles and females of *M. enterolobii* were examined under the microscope for the confirmation of test



A. Egg **B. Juvenile** **C. Anterior part of juvenile**

D. Posterior part of juvenile **E. Female** **F. Perineal pattern**

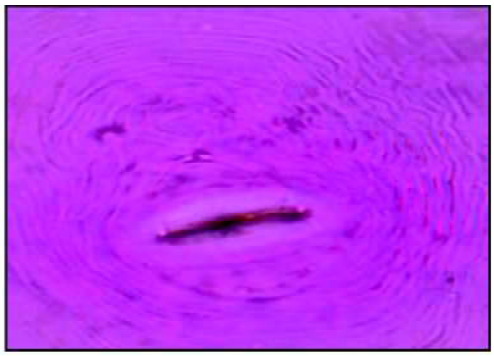
Plate 1a: Morphological identification of nematode *(Meloidogyne enterolobii)*

A. Male B. Anterior part of Male C. Posterior part of Male

Plate 2b: Morphological identification of nematode *(Meloidogyne enterolobii)*

A. Above ground wilting symptoms **B. Infected root with galls formed**

C. Egg mass and female attach with root



D. Perineal pattern of female *(Meloidogyne enterolobii)*

Plate 3: Pathogenecity test

nematode (Plate 3).

Symptomatology

Plants infected by *M. enterolobii* showed above-ground symptoms such as stunted growth, temporary wilting and chlorosis. The root system was poorly developed showing typical symptoms of this nematode with the formation of root galls found below ground which can be large in size and numbers (Cetintas *et al*., 2007). In severe infestation, roots are distorted by the formation of small and large multiple galls and devoid of fine roots. *M. enterolobii* affects growth, yield, lifespan and tolerance to environmental stresses of infested plants. Overall, damage due to *M. enterolobii* may consist of reduced quantity and quality of yield. It causes susceptibility of the host plant leading to the entry of secondary pathogen described as "Indirect damage" such as being described for *Fusarium solani* on guava.

After identification and pathogenicity test of nematode (*Meloidogyne enterolobii*), a pot experiment was carried out during 2018-19 in glasshouse of Department of Plant Pathology. Effects of biofumigants (Mustard- *Brassica juncea* and Radish-*Raphanus sativus*) and biocontrol agents (*Trichoderma asperellum* and *Pseudomonas fluorescens*) in different combinations applied @ 10g/l water through different application methods *viz.,* soil treatment, seedling dip and soil drenching etc. were studied for the management of *Meloidogyne enterolobii* is presented in (Table 1 & 2).

Effect of Biointensive approaches on plant growth and biomass

Effect of biofumigation and biocontol agents on plant growth parameters *viz*., shoot length, root length, fresh weight and dry weight (root, shoot) of tomato plants were recorded.

Effect on root and shoot length

The effect of various treatments on tomato root and shoot length was recorded at 45 days after transplantation and the data presented in the Table 1.

Results revealed that root length recorded ranges between 11.50 cm to 25.67 cm in different treatments. Maximum root length was recorded with Ta14 (25.67 cm), followed by PBAT3+Biofumigation(R) (25.60

Table 1: Effect of Biocontrol Agents and Biofumigation on the growth parameters of tomato under glasshouse conditions

S. No.	Treatments	Length (cm)		Fresh weight (g)		Dry weight (g)	
		Root	Shoot	Root	Shoot	Root	Shoot
1.	Biofumigation(M)	17.67	67.33	2.54	14.60	0.67	5.23
2.	Biofumigation(R)	17.70	72.67	2.30	20.87	0.68	4.37
3.	*Trichoderma asperellum* 14	25.67	93.67	4.50	27.50	0.91	7.40
4.	Ta14+Biofumigation(M)	19.17	91.00	5.07	28.77	0.85	7.17
5.	Ta14+Biofumigation (R)	19.17	84.33	4.24	23.53	0.77	6.87
6.	*Pseudomonas fluorescens* 173	17.70	82.00	4.23	23.23	0.80	6.87
7.	Psf173+Biofumigation(M)	17.40	84.33	4.36	22.03	0.93	7.53
8.	Psf173+Biofumigation(R)	22.27	68.33	6.48	28.83	1.13	7.60
9.	Pant Biocontrol Agent-3	23.50	101.33	6.92	33.37	1.55	8.70
10.	PBAT3+Biofumigation(M)	25.50	96.33	7.38	36.07	1.73	10.17
11.	PBAT3+Biofumigation(R)	25.60	97.67	7.15	34.60	1.60	8.00
12.	Commercial Formulation (CF- *Paecilomyces*)	19.17	62.67	5.51	27.60	0.73	4.33
13.	CF+Biofumigation (M)	24.00	90.67	6.06	30.97	0.77	5.13
14.	CF+Biofumigation (R)	23.43	78.33	5.79	27.77	0.77	5.73
15.	Standard chemical check (Carbofuran)	18.27	67.00	6.41	26.37	0.70	4.97
16.	Polysheet mulching alone	13.00	65.33	2.62	19.20	0.63	3.60
17.	Control (untreated)	11.50	59.00	1.61	12.57	0.50	3.30
	C.D. at 5%	2.45	8.08	0.38	3.40	0.09	0.55
	SE (m)	0.85	2.79	0.13	1.18	0.03	0.19
	C.V.	7.35	6.05	4.63	7.91	5.37	5.27

cm) which did not differ significantly from other treatments *viz.,* PBAT3+Biofumigation(M) (25.50 cm), CF+ Biofumigation (M) (24.00 cm), Pant Biocontrol Agent-3 (23.50 cm) and CF+ Biofumigation (R) (23.43 cm). However, minimum root length was recorded in control (11.50 cm), which was at par with the Polysheet mulching alone (13.00 cm). The shoot length range observed among all the treatment was 59.00 cm to 101.33 cm. Maximum shoot length was observed in pot having the treatment Pant Biocontrol Agent-3 (101.33 cm) followed by the PBAT3+ Biofumigation(R) (97.66 cm) which were at par with the PBAT3+Biofumigation(M) (96.33 cm) and Ta14 (93.66 cm). However, minimum shoot length was recorded with the control (59.00 cm), which did not differ significantly from CF (*Paecilomyces*) (62.66 cm), Polysheet mulching alone (65.66 cm) and Standard chemical check (Carbofuran) (67.00 cm).

Effect on fresh root and fresh shoot weight

The effect of various treatments on tomato fresh root and fresh shoot weight were recorded at 45 days after transplanting and the data presented in the Table 1. The fresh root weight was found in the range of 1.61 g to 7.38 g among all the treatments. Maximum fresh root weight obtained from PBAT3+Biofumigation (M) (7.38 g) followed by PBAT3+Biofumigation(R) (7.15 g) which was statistically similar to the each other. However, minimum fresh root weight was observed in control (1.61 g). The fresh shoot weight observed in the range of 12.57 g to 36.07 g. Maximum fresh shoot weight was observed in PBAT3+Biofumigation(M) (36.07 g) which was at par with the PBAT3+Biofumigation(R) (34.60 g) and Pant Biocontrol Agent-3 (33.37 g). While, minimum fresh shoot weight was observed in case of control (12.57 g) which was statistically at par with Biofumigation(M) (14.60 g).

Effect on dry root and dry shoot weight

The effect of various treatments on tomato dry root and dry shoot weight were recorded at 45 days after transplanting and the data presented in the Table 1. The dry root weight recorded ranged from 0.50 g to 1.73 g. Maximum dry root weight was found in PBAT3+Biofumigation(M) (1.73 g) followed by PBAT3+Biofumigation(R) (1.60 g), which were significantly different from each other. However,

Table 2: Effect of Biocontrol Agents and Biofumigation on galls formation and soil population of nematodes on tomato under glasshouse conditions

S. No.	Treatments	No. of Gall Formed	Final Population (Pf)	Reproduction Factor (Pf/Pi)
1.	Biofumigation(M)	29.00	2017.00	0.35
2.	Biofumigation(R)	26.00	1951.00	0.33
3.	*Trichoderma asperellum* 14	41.00	2751.00	0.47
4.	Ta14+Biofumigation(M)	27.00	1844.00	0.31
5.	Ta14+Biofumigation(R)	25.67	1753.00	0.30
6.	*Pseudomonas fluorescens* 173	32.67	2559.00	0.44
7.	Psf173+Biofumigation(M)	22.67	1797.00	0.31
8.	Psf173+Biofumigation(R)	23.00	1855.00	0.32
9.	Pant Biocontrol Agent-3	23.33	2072.00	0.36
10.	PBAT3+Biofumigation(M)	18.33	1457.00	0.25
11.	PBAT3+Biofumigation(R)	22.00	1697.00	0.29
12.	Commercial Formulation (CF- *Paecilomyces*)	34.00	2574.00	0.44
13.	CF+ Biofumigation(M)	25.33	2395.00	0.41
14.	CF+ Biofumigation(R)	25.00	2298.00	0.39
15.	Standard chemical check (Carbofuran)	15.00	1291.00	0.22
16.	Polysheet mulching alone	45.00	5257.00	0.90
17.	Control (untreated)	77.00	9728.00	1.67
	C.D. at 5%	1.81	-	-
	SE (m)	0.63	-	-
	C.V.	3.60	-	-

*Initial population (Pi) of nematodes was 5821.00

minimum dry root weight was found in control (0.50 g). Dry shoot weight was 3.60 g to 10.17 g in different treatments. Maximum dry shoot weight was observed in PBAT3+Biofumigation (M) (10.17 g) followed by Pant Biocontrol Agent-3 (8.70 g), which were significantly different from each other. While, minimum dry shoot weight was recorded in control (3.30 g) which did not significantly differ from Polysheet mulching alone (3.60 g).

Effect of Biocontrol Agents and Biofumigation on gall formation and soil population of nematode on tomato under glasshouse conditions.

Effect of biofumigation and biocontol agents on infestation of root knot nematodes on the tomato plants were recorded after 45 days of transplanting presented in the Table 2.

Effect on gall formation and soil population of nematodes

Effect of various treatments on gall formation on the tomato plants and final nematode population were evaluated under glass house conditions at 45 days after transplanting, results presented in Table 2.

Pots treated with Standard chemical check (Carbofuran) (15.00 galls per root system) showed minimum number of galls followed by PBAT3+Biofumigation(M) (18.33 galls per root system) which were statistically differ with each other. However, the maximum numbers of galls were found in control (77.00 galls per root system). Pots treated with Standard chemical check (Carbofuran) and showed minimum number of nematode population (1291.00) and multiplication (reproduction factor 0.22) followed by PBAT3+Biofumigation(M) (nematode population 1457.00 and reproduction factor 0.25) However, maximum number of nematodes population (9728.00) and multiplication (reproduction factor 1.67) was recorded in control.

The results of the present study showed that application of the combination of these biointensive techniques using biofumigation of soil with *Brassica juncea* and *Raphanus sativus* followed by application of biocontrol agents *viz., Trichoderma* and *Pseudomonas* (soil, seed and root treatment and drenching) improved tomato plant growth significantly. Shankar *et al.* (2011) reported that plant inoculated with bacterium *(Pseudomonas aeruginosa)* significant increase in plant height, shoot weight and root weight. Incorporated crushed cabbage (*Brassica oleracea*) leaves into the soil under greenhouse conditions @ 2.5, 5 and 10 g per pot at 10 days before transplanting tomato cv. Super Strain B. They observed that amending soil with crushed cabbage leaves enhanced plant growth criteria of tomato (Youssef *et al.,* 2013). Bioagents BioAct WG and *T. viride* strain T6 alone and in combination significantly promoted the growth of plants compared with the control. Root length was observed significantly different between the treatments. It was greatest in treatments *T. viride* strain T6 alone and BioAct WG + *T. viride* (Yankova *et al.,* 2014). The data on plant growth parameters were recorded 8 weeks after inoculation. Treatment of soil with Carbofuran 3G @ 9 g/m^2 manifested best results of plant height (33.84 cm), fresh weight of shoot (21.69 g), dry weight of shoot (5.22 g), fresh weight of root (12.84 g) and dry weight of root (1.32 g) *Trichoderma* increased the growth of roots and there was a distinct increase in the dry weight of roots (Jena *et al.,* 2017).

The results of the present study showed that Biofumigation of soil with *Brassica juncea* and *Raphanus sativus* followed by application of biocontrol agents *viz*., *Trichoderma* and *Pseudomonas* (soil, seed and root treatment and drenching) significantly controlled the root knot nematode *M. enterolobii*. Application of the combination of these biointensive techniques decreased the number of gall formation and nematode population in the soil. Uses of biofumigants and biocontrol agents have been reported to suppress the activity and kill root knot nematodes. (Sharon *et al*., 2001) observed that in greenhouse experiments, root galling was reduced and top fresh weight increased in nematode-infected tomatoes following soil pre treatment with *Trichoderma* peat-bran preparations. The largest net reductions in nematode populations were noted

between incorporation of the *Brassica* species and the vegetable crop planting in most years (Monfort *et al.,* 2007). The *T. harzianum* BI have significant potential as biocontrol agent against the root-knot nematode *M. javanica* in greenhouse experiment. Inoculation of tomatoes with *T. harzianum* can significantly reduce population of this nematode and disease severity (Sharon *et al.* 2007). Suitable rate of *T. harzianum* BI for suppressing nematode activities such as nematode infection, egg mass production and number of eggs per egg mass was observed in 10^6 spores/ml concentration (Sahebani and Hadavi, 2008). The maximum decreases in GI (gall indices) and EMI (egg mass indices) were recorded with the treatment of *P. fluorescens* or Furadan in comparison to the inoculated control. Application of *T. harzianum* was also found to be effective against the nematode but less so than *P. fluorescens* or Furadan. In tobacco cultivar RK-18 P8, greatest decrease in the nematode population was recorded with Furadan (350%), followed by *P. fluorescens* (103%), *T. harzianum* (99.4%) and Phorate (40.4%) in comparison to the initial population (Khan and Haque, 2011). The incorporation of soil pots with mustard pow-der at all different doses 3, 5 per cent (48 h and one week before nematode inoculation) and 7% of soil weight significantly reduced all related nem-atode parameters compared to treated plants with nematode alone. All nematode parameters i.e., number of galls/root system, root galling in-dex, number of egg masses/root system as well as number of juveniles/250 g soil showed high re-duction with mixing the soil pots with mustard at 5 per cent one week before nematode inoculation fol-lowed by 5 per cent before 48h nematode inoculations (Salem and Mahdy, 2015).

CONCLUSION

Ecofriendly management techniques like Biological control and Biofumigation are the best option to avoid hazards of chemical pesticides. The present investigations were carried out to study bio-intensive management of *Meloidogyne enterolobii* causing root-knot in tomato. The different treatments of Biocontrol agents (*Trichoderma asperellum* (Ta-14), *Pseudomonas flourescens* (Psf-173), PBAT 3 (consortium of Ta14 & Psf173) and *Paecilomyces lilacinus* (commercial) with the combination of Biofumigation (*Brassica juncea* & *Raphanus sativus*). Nematicide carbofuran was used for the study. Among all the treatments combination of consortium of *Trichoderma asperellum* and *Pseudomonas fluorescens* + Biofumigation with Mustard resulted in maximum fresh root (7.38g) and shoot weight (36.07g) with minimum number of root galls (18.33galls per root system) as compared to the combination of other treatments under glasshouse conditions.

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