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Management of root-knot nematode (*Meloidogyne incognita*) in turmeric

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Abstract
Turmeric root-knot nematode (*Meloidogyne* spp.) is one of the major problem, which causes galls/knots in the rhizomes and causes considerable yield loses. A pot experiment was conducted to manage the root-knot nematode using bioagents, botanicals and chemicals. The maximum plant height (68.75 cm), fresh rhizome weight (308.25 g), dry rhizome weight (62.13) and least number of galls (26.25), lowest soil nematode population (201.75) was found in T_2 - Carbofuron 3G among the individual treatment and it was significantly superior over other individual treatments and control followed by T_3 - *Trichoderma viride* and T_1 - Neem cake. In combination treatments T6- Neem cake + *Paecilomyces lilacinus* performed better with maximum plant height (72.00 cm), fresh rhizome weight (325.00 g), dry rhizome weight (65.00 g) and least number of galls (23.50) and lowest soil nematode population (153.25) which was on par with T5- Neem cake + *Trichoderma viride* when compared with control. In general, all individual and combination treatments performed better and significantly superior to control.

Keywords: root-knot, management, turmeric, *Meloidogyne*.

Introduction
Turmeric is one of the important spice crops grown in India and used to flavour and to colour foodstuffs. It is an herbaceous perennial plant, native to tropical South-East Asia belonging to the family Zingiberaceae. The anti-oxidant attributes of this spice protect against the high energy free radical damage to organic cells. It is regarded as a symbol of well-being and future and is widely used in ceremonies and religious functions. It is known as 'haridra' meaning yellow coloured wood in Sanskrit and is a part of Indian delicacies, health care as well as rites and rituals since time immemorial. The anti-oxidant attributes of this spice protect against the high energy free radical damage to organic cells (Maheshwari *et al.*, 2006) [6].

Turmeric is cultivated mainly in India, Pakistan, Jamaica, Sri Lanka, Indonesia, Bangladesh, Taiwan and China. India is the world's largest producer of turmeric and accounts for 80 per cent of the world production. It is grown in an area of 2.33 lakh hectares with a production of 11.90 lakh metric tons and the productivity of 5.1 metric tons per hectare (Anon, 2015) [1]. In India, turmeric is mainly grown in Tamil Nadu, Karnataka, Assam, Kerala, Maharashtra, Orissa and Andhra Pradesh. Among the major biotic constraints of turmeric, diseases play an important role. Turmeric is severely affected by soil borne and foliar diseases *viz.*, rhizome rot, root-knot nematode, leaf blotch and leaf spot diseases. The root-knot nematode (*Meloidogyne* spp.) is a major problem, which causes galls or knots in the rhizomes and causes considerable yield loses. It also play the way for the secondary infection by different pathogens like *Pythium*, *Fusarium* and *Ralstonia* in the soil (Udo and Ugwuoke, 2010) [12]. There is seldom a single method available, to alleviate nematode problems in the field and a sustainable approach of integrating several tools and strategies, such as crop rotation, soil solarization, application of nematicides, biological control and cultivating resistant varieties. All are needed in a comprehensive manner for effective management.

Material and Methods
The pot experiment was conducted to assess the effect of bio-agents, botanicals and chemicals on plant growth parameters and reproduction of *M. incognita*. The experiment was laid out with the following treatments replicated four times in a Completely Randomized Design (CRD). The ecofriendly components such as oil cakes (Neem cake), biocontrol agents (*T. viride* and *P. lilacinus*), nematicide (Carbofuran 3G) and their combinations as soil

application. Second stage juveniles of nematodes inoculated at the rate of two per gram of soil after 45 days of planting.

The uniform sized mother rhizomes of turmeric variety salem were selected for sowing. Nematode free sterilized mixture of soil and sand filled in 25 cm diameter earthen pots with capacity of 3 kg soil.

Fourty days after planting of turmeric rhizomes the selected treatments were inoculated into the pots individually. After the application of bio-agents into the pots, an average of 2000 freshly hatched J2 of *M. incognita* per kg of soil were inoculated into the individual pot.

Treatment	Details	Dosage
T_1	Neem cake	3.75 g/ 3 kg soil
T_2	Carbofuran 3G	1.5 g / 3 kg soil
T_3	*Trichoderma viride*	15 g / 3 kg soil
T_4	*Paecilomyces lilacinus*	3 g / 3 kg soil
T_5	Neem cake + *Trichoderma viride*	3.75 g + 15g / 3kg soil
T_6	Neem cake + *P.lilacinus*	3.75 g + 3 g / 3 kg soil
T_7	Contol	-

The experiment was terminated after harvesting and observations on growth of turmeric plants were recorded. The population of nematode in soil and root was estimated.

Estimation of nematode population in soil samples

Soil sample of 200 cc was washed thoroughly and processed using combined "Cobb's sieving and Baermann's funnel method" (Ayoub, 1977) [2] as given below.

1. Two hundred cc of soil was taken in 1000 ml beaker and sufficient quantity of water was added to make soil solution.
2. This was stirred thoroughly and allowed to stand for heavier particles to settle down.
3. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh sizes, respectively.
4. Residue from 325 and 400 mesh sieves were collected and poured over a tissue paper spread on a wire gauge and placed on Baermann's funnel.
5. Level of water in the Baermann's funnel was maintained to keep the tissue paper wet and left undisturbed for 48 hr.
6. After incubation of 48 hr, the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting of various plant parasitic nematodes present. Nematode population from this was finally estimated for 200 cc soil.

Estimation of nematode population in root samples

Nematode population in 10 g roots was estimated by Root incubation method (Ayoub, 1977) [2] as explained below:

Procedure

1. Roots were gently washed to remove adhering soil particles.
2. Washed roots were cut into small bits of 2.5 cm and split longitudinally.
3. Then placed over tissue paper spread on a wire gauge and kept in a Petri plate filled with water.
4. Level of water was maintained in Petri plate and left undisturbed for 48 hours.
5. Later, the suspension in the Petri plate was collected and observed for nematodes using stereo-binocular microscope.

Incidence of root-knot nematode was recorded by using the gall index given by (Taylor and Sasser, 1978) which is as follows

Description	Grade
No galls or egg masses	0
1 to 2 galls or egg masses	1
3 to 10 galls or egg masses	2
11 to 30 galls or egg masses	3
31 to 100 galls or egg masses	4
More than 100 galls or egg masses	5

Counting the number of nematodes

The number of nematodes in an aqueous suspension was determined by using a counting dish. A five cm diameter glass Petri plate was used as a counting dish. Squares were made on the outer surface of the bottom of the dish to facilitate counting. A 10 ml volume of aqueous suspension from the beaker was taken and placed into the petriplate. Nematodes were counted in all squares under a stereo-binocular microscope. After counting, the suspension was transferred back to the mother container. Counting of each sample was repeated four times in same manner. The mean number of nematodes per 10 ml was determined by averaging the counts taken.

Results

The data on the effect of various treatments *viz.*, bio agent (*Trichoderma viride* and *Paecilomyces lilacinus*), oilcake (neem cake) and nematicide (carbofuran 3G) on the plant height, number of leaves, shoot weight, root length, fresh rhizome weight and dry rhizome weight and on the development and reproduction of the nematodes are presented in Tables. 1, 2 and 3. The various treatments are abbreviated from T_1 to T_7 as explained under Materials and Methods.

Effect of bio-agents, botanicals and chemicals on plant growth and yield parameters of turmeric cv. Salem infected with *M. incognita*

The results on the effect of soil application of *T. viride*, *P. lilacinus*, neem cake and carbofuran 3G at the time of sowing on various growth and yield parameters of turmeric are presented in Table.1 and 2.

Plant height (cm)

The observations on the effect of different treatments on the plant height of turmeric plants infected with *M. incognita* were recorded at 45, 90 and 180 days after inoculation (DAI).

At 90 and 180 DAI, all the individual and combination treatments had recorded the highest plant height and significantly superior when compared to control. Among the four individual treatments T_2 - Carbofuron 3G performed better (34.00 & 68.75 cm) which was on par with T_3- *Trichoderma viride* (31.75 & 64.75) when compared to control (26.75 & 57.75 cm) respectively. In combination treatments T_6-Neem cake + *Paecilomyces lilacinus* recorded the highest plant height (36.00 & 72.00 cm) at 90 and 180 DAI respectively which was on par with T_5- Neem cake + *Trichoderma viride* (35.00 & 71.50 cm) when compared with control (26.75 & 57.75 cm).

Number of leaves

With respect to number of leaves at 90 and 180 DAI, all the individual and combination treatments performed better over the control. However among individual treatments T_2- carbofuron 3G recorded the highest number of leaves (13.25

& 19.00) respectively. In combination treatments T_6 -Neem cake + *Paecilomyces lilacinus* recorded the highest number of leaves (17.00 & 23.00) which was on par with T_5-Neem cake + *Trichoderma viride* (16.25 & 22.00) when compared with control (8.00 & 12.50).

Root length

Among the individual treatments, the highest root length was recorded in T_2-carbofuron 3G (21.75 cm) which was followed by T_3-*Trichoderma viride* (20.13 cm). In among combination treatments, the highest root length was recorded in T_6-Neem cake + *Paecilomyces lilacinus* (24.13cm) which was on par with recorded T_5-Neem cake + *Trichoderma viride* (23.50 cm).

Fresh and dry shoot weight

All the individual and combination treatments performed better with respect to fresh and dry shoot weight and significantly superior to control. However, carbofuron 3G (205.00 & 38.00 g) performed better among individual treatments and T_5-Neem cake + *Paecilomyces lilacinus* (221.25 &44.13 g) in combination treatments followed by T_6-Neem cake + *Trichoderma viride* (218.75 & 42.75 g) as compared to control (161.25 & 33.13 g) respectively.

Fresh rhizome weight

The highest fresh weight of rhizome recorded in T_2-carbofuron 3G (308.75 g) which was on par with T_3-*Trichoderma viride* (293.50 g) in individual treatments. But T_6-Neem cake + *Trichoderma viride* (326.00 g) recorded highest fresh rhizome weight which was on par with T_6-Neem cake + *Paecilomyces lilacinus* (325.00 g) when compared with control (232.25 g).

Dry rhizome weight

In individual treatments the highest dry rhizome weight was recorded T_2-carbofuron 3G (62.13 g) followed by *Trichoderma viride* (57.25 g) when compared with control (45.93 g). However, among combination treatments T_6-Neem cake + *Paecilomyces lilacinus* recorded the highest dry rhizome weight (65.00 g) which is on par with T_5-Neem cake + *Trichoderma viride* (63.75 g) against control. All the individual and combination treatments performed better over control and were significantly superior.

In general, all individual and combination treatments performed better against control. Among the individual treatments T_2-carbofuron 3G performed better with respect highest plant height at 90 and 180 DAI (34.00 & 68.75 cm), number of leaves (13.25 & 19.00), root length (21.75 cm), fresh & dry shoot weight (205.00 & 38.00 g) and fresh & dry rhizome weight (308.25 & 62.13 g) respectively. However among combination treatments both the treatments performed better and they were on par each other in all growth and yield parameters and significantly superior over control.

Effect of bio-agents, botanicals and chemicals on nematode parameters of *M. incognita* on turmeric cv. Salem

Effect of various treatments on number of galls per root system and nematode population are presented in Table 3.

Number of galls per root system

The data on number of galls per root system with respect to different treatments revealed that all the treatments had performed better in reducing the number of galls per root system. However among the individual treatments T_2-Carbofuron 3G recorded the minimum number of galls (26.25) followed by T_3-*Trichoderma viride* (30.00) and in case of combination treatments T_6-Neem cake + *Paecilomyces lilacinus* (23.50) followed by T_5-Neem cake + *Trichoderma viride* (24.50).

Root gall index

The root gall index in different treatments revealed all treatments record gall index of 3 as against 5 in control.

Number of egg masses per root system

The data on number of egg masses per root system revealed that, all the treatments had performed better against control. However among the individual treatments T_2-Carbofuron 3G recorded the minimum number of egg masses (25.25) followed by T_3-*Trichoderma viride* (29.25) and T_1-Neem cake (29.50) and in case of combination treatments T_6-Neem cake + *Paecilomyces lilacinus* (23.25) followed by T_5-Neem cake + *Trichoderma viride* (23.50).

Egg mass index

The egg mass index at different treatments revealed that all treatments recorded the minimum egg mass index of 3 except *Paecilomyces lilacinus* with egg mass index of 4.

Nematode population in soil

All treatments were effective in reducing the nematode population in soil (200 g) at 180 days after inoculation (DAI). The nematode population vary significantly in all the treatments over control. Among the individual treatments T_2-carbofuron 3G (201.75) recorded the minimum soil population which was on par with *Paecilomyces lilacinus* (214.75) and *Trichoderma viride* (219.00). However, among combination treatments T_6-Neem cake + *Paecilomyces lilacinus* (153.25) recorded least soil nematode population which was on par with T_5-Neem cake + *Trichoderma viride* (156.25) when compared to control (509.75).

In general, all individual and combination treatments performed better against control in terms of nematode parameters were significantly superior over control. Among the individual treatments carbofuron 3G performed better with respect lowest galls, egg masses and soil nematode population of 26.25, 25.25 and 201.75 respectively. However, among the combination treatments T_6-Neem cake + *Paecilomyces lilacinus* performed better with 23.50, 22.25 and 153.25 respectively which was on par with T_5-Neem cake + *Trichoderma viride* as against control.

Discussion

The data on the number of galls and egg masses per root system with respect to different treatments revealed that all the individual and combined treatments had performed better in reducing the galls and egg masses. However among the individual treatments Carbofuron 3G recorded the minimum number of galls and egg masses (26.25 & 22.25) followed by *Trichoderma viride* (30.00 &29.25) and in case of combination treatments neem cake + *Paecilomyces lilacinus* (23.50 & 23.25) followed by neem cake + *Trichoderma viride* (24.50 & 23.50). All treatments were effective in reducing the nematode population in soil (200 g) at 180 days after inoculation (DAI). The nematode population varies significantly in all the treatments over control. Among the individual treatments carbofuron 3G (201.75) recorded the minimum soil population which is on par with *Paecilomyces*

lilacinus (214.75) and *Trichoderma viride* (219.00). However among combination treatments neem cake + *Paecilomyces lilacinus* (153.25) recorded least soil nematode population which was on par with neem cake + *Trichoderma viride* (156.25) when compared with control (509.75).

Among fungal bio agents evaluated against root-knot nematode in turmeric *Paecilomyces lilacinus* (Thom) Samson were found to be quite promising (Eapen *et al*., 2008) [4].

Similar results were reported by Shankarnarayana *et al.* (1999) [8] reduction of the number of galls and egg masses on the root system and nematode population of *Meloidogyne* spp. in soil by application of *T. viride*. Dababat *et al.* (2006) [3] reported that soil with the bio control agents, *T. viride* before transplanting tomato, improved the plant growth by reducing the population of *M. incognita*. Sharma *et al.* (2007) [9] *P. lilacinus* along with the addition of neem cake reduced number of galls, eggs per egg mass by 64 percent each and soil population by 77 per cent. Kannan and Veeravel (2008) [5] reported that highest reduction in soil nematode population, galls per plant and egg mass per 10 g root.

In general all individual and combination treatments performed better against control in terms of nematode parameters. Among the individual treatments carbofuron 3G performed better with respect lowest galls, egg masses and soil nematode population with 26.25, 25.25 and 201.75 respectively. However among the combination treatments neem cake + *Paecilomyces lilacinus* performed better with 23.50, 22.25 and 153.25 respectively which was on par with neem cake + *Trichoderma viride.*

The reason for the reduction in galls and egg masses might be due to the parasitic activity of *Paecilomyces lilacinus* on eggs and all stages of nematodes. The growth and development of *P. lilacinus* kills the nematode by feeding on its body contents and better results obtained along with neem cake. In effect, the *P. lilacinus* acts as a parasite on all stages of the nematode. With respect *Trichoderma viride* the reason for reduction in nematode population might be due to the production of enzyme chitinase by *Trichoderma* spp. which might have caused premature hatching of nematode eggs and could be used in control of nematodes (Nagesh *et al.*, 1998) [7]. Singh and Vinod Kumar (1995) [10] reported that neem cake and carbofuron reduced nematode population.

Table 1: Effect of bio-agents, botanicals and chemicals on plant growth parameters of turmeric infected with root-knot nematode, *M. incognita*

Treatment	Plant height (cm)			No. of leaves			Root length (cm)
	45 DAI	90 DAI	180 DAI	45 DAI	90 DAI	180 DAI	
T_1 - Neem cake	24.50	30.50	62.75	6.00	11.00	17.75	19.75
T_2 – Carbofuron 3G	24.50	34.00	68.75	5.25	13.25	19.00	21.75
T_3 – *Trichoderma viride*	25.50	31.75	64.75	5.75	11.75	17.00	20.13
T_4 - *Paecilomyces lilacinus*	24.00	30.00	64.25	5.75	10.50	17.50	19.50
T_5 - Neem cake + *Trichoderma viride*	24.75	35.00	71.50	5.25	16.25	22.00	23.50
T_6- Neem cake + *Paecilomyces lilacinus*	23.75	36.00	72.00	5.75	17.00	23.00	24.13
T_7 - Control	18.00	26.75	57.75	6.00	8.00	12.50	17.88
S.Em ±	NS	0.85	1.52	NS	0.55	0.45	0.51
CD @ 5%		2.50	4.48		1.61	1.31	1.49

*DAI- days after inoculation

Table 2: Effect of bio-agents, botanicals and chemicals on growth and yield parameters of turmeric infected with root-knot nematode, *M. incognita*

Treatments	Shooot weight (g)		Yield/plant (g)	
	Fresh	Dry	Fresh	Dry
T_1 - Neem cake	174.25	35.88	269.50	54.50
T_2 – Carbofuron 3G	205.00	38.00	308.75	62.13
T_3 – *Trichoderma viride*	176.00	33.75	293.50	57.25
T_4 - *Paecilomyces lilacinus*	182.50	35.25	269.50	54.13
T_5 - Neem cake + *Trichoderma viride*	218.75	42.75	326.00	63.75
T_6- Neem cake + *Paecilomyces lilacinus*	221.25	44.13	325.00	65.00
T_7 - Control	161.25	33.13	232.25	45.93
S.Em ±	2.73	0.89	6.49	1.47
CD @ 5%	8.02	2.62	19.08	4.32

*Replications: 4

Table 3: Effect of bio-agents, botanicals and chemicals on number of galls and egg masses per root system and nematode population of root-knot nematode *M. incognita* in turmeric

Treatment	No. of galls/root system	Root gall index	No. of egg masses/ root system	Egg mass index	Soil nematode population
T_1 - Neem cake	30.50	3	29.50	3	236.00
T_2 – Carbofuron 3G	26.25	3	25.25	3	201.75
T_3 – *Trichoderma viride*	30.00	3	29.25	3	219.00
T_4 - *Paecilomyces lilacinus*	31.25	4	30.25	4	214.75
T_5 - Neem cake+*Trichoderma viride*	24.50	3	23.50	3	156.75
T_6- Neem cake+*Paecilomyces lilacinus*	23.50	3	22.25	3	153.25
T_7 - Control	105.50	5	101.25	5	509.75
S.Em ±	1.18	-	1.39	-	6.05
CD @ 5%	3.48	-	4.08	-	17.80

*Replications: 4

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