Integrated approach for management of root-knot nematode (Meloidogyne incognita) in bitter gourd (Momordica charantia)

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ABSTRACT

Field trials were conducted to manage root-knot nematode (*Meloidogyne incognita*) infesting bitter gourd (*Momordica charantia* L.). Four summer ploughings in May-June, 2014 at 15±2 days interval caused 67% reduction in *M. incognita* population. To develop an ecofriendly nematode management module organic amendment (neem cake, *Azadirachta indica* at 1.5 t/ha) and talc based preparations of bio-agents (*Pseudomonas flourescens* and *Trichoderma harzianum*) as seed treatment (10 g/kg) and soil application (10 kg/ha) were integrated with summer ploughings as pre-treatment. Carbofuran 3G at 1 kg a.i./ha was kept as control. The treatments were: T₁- Control (Carbofuran); T₂-Neem cake; T₃- *P. flourescens* (seed treatment (10 g/kg seed as well as soil application at 10 kg/ha); T₅- T₂+T₃+T₄; T₆-Farmers' practice. The best protection of bitter gourd root-knot disease was achieved through integrated application of neem cake, *P. flourencens* and *T. harzianum*. Significantly better results (in terms of vine height and reduction in *M. incognita* population) were recorded under consequent second year trial (84 and 94% respectively) as compared to first year trial (47 and 73% respectively). It also enhanced marketable yield of bitter gourd significantly (P>0.5) (up to 108%) in the second season trial, as compared to farmers' practice. It is suggested that integrated approach using organic amendment with bioagents to manage root-knot disease of bitter gourd under natural infestation is environmental friendly and beneficial to growers/farmers' having small holdings of land.

Key words: Bio-agents, Bitter gourd, Integrated Management, Meloidogyne incognita, Neem cake

Bitter gourd is animportant vegetable crop which is also used as aherbal medicine (nutraceutical) for human and animals (Poolperm and Jiraunokoorskul 2017). Due to its increasing demand in urban as well as local markets, farmers are required to grow bitter gourd crop continuously season after season. Among root-knot nematode species Meloidogyne incognita is major pest that severely affects quality and quantity of produce (Anwar and McKenry 2010) and has high pathogenic potential on bitter gourd (Momordica charantia L.) (Singh et al. 2012). It is one of the most important group of plant parasitic nematodes attacking nearly every crop grown and have been reported to cause an annual loss of ₹ 547.5 million in cucurbits (Jain et al. 2007). Kaur and Pathak (2011) reported 38-48% yield losses in bitter gourd due to M. incognita. Mono-cropping of bitter gourd in local farming system in India leads to the accumulation of root-knot nematode disease. In India, the efficacy of current strategies for management of root-knot disease is limited and bitter gourd production is achieved

through chemical pesticides. In developing countries like India, integrated nematode management is required, as profit margins and environmental consideration preclude the use of chemical control (Singh 2013).

The present investigation utilized neem cake (Azadirachta indica), a plant growth promoting rhizobacteria (Pseudomonas fluorescens) and an antagonistic fungus (Trichoderma harzianum) to develop an integrated nematode management module, because often any biological control of root-knot nematode is gaining wide acceptance among growers, but lack of awareness and consistent field efficacy data on currently available bio-pesticides against this hidden enemy decelerates the adoption among farmers and growers. There is no information on the combined use of these three promising components for the management of M. incognita infesting bitter gourd. The objective of the present study was to develop an integrated nematode management module using eco-friendly components, for controlling the losses caused by M. incognita to bitter gourd production.

MATERIALS AND METHODS

Experiment was conducted at Kushinagar (Uttar Pradesh), India on a farmer's field located at coordinates 26.739879°N and 83.88697°E, where symptoms of wilting and dying were observed in bitter gourd crop. The root

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systems of the plants were examined and found to be heavily infected with root-knot nematode (100% of root system) forming large amalgamating root galls which was identified as root-knot nematode.

Experimental design

Four summer ploughings in May-June, 2014 at 15 ± 2 days interval were applied as pre-treatment to bring down the *M. incognita* population. The experimental design was a factorial randomized complete block design (RCBD) for three main factors (2 × 2 × 2). The trial (*Kharif* season) comprised six treatments, i.e. T_1 - Control (carbofuran alone); T_2 - Neem cake alone (1.5 t/ha); T_3 - *P. flourescens* (seed treatment (10 g/kg seed as well as soil application at 10 kg/ha); T_4 - *T. harzianum* (seed treatment (10 g/kg seed as well as soil application at 10 kg/ha); T_5 - T_2 + T_3 + T_4 ; T_6 -Farmers' practice (FP) and repeated in the same plot in the following *rabi* season with three replications.

One plant of bitter gourd cv. Pusa Do Mousmi per hill and 16 plants/plot $(7.0 \text{ m} \times 5.5 \text{ m})$ was maintained. As per experimental layout, plots were amended with neem cake 10 days before sowing of seeds of bitter gourd and application of bio-agents to ensure decomposition of neem cake. Stacking of bitter gourd vine with help of bamboo sticks and nylon wire was maintained to ensure good establishment of plants. All standard good agronomic practices were adopted for better crop.

Data collection

Data on marketable yield (from first to last picking of fruits), plant growth parameters and nematode multiplication at final harvest of crops was recorded. An average of ten plants was considered for record of each parameter. The root gall index was recorded based on the percentage of infested root system with galls (Hussy and Janssen 2002), where 0 = no galling; 1, trace infection with a few small galls; $2.5 \le 25\%$ root galled; 3, 26 to 50 %; 4, 51 to 75% and 5, >75% roots galled.

Establishment of bio-agent

The colony forming units (cfu/g soil) of both the bio-agents after harvest was counted by serial dilution (up to 10⁻⁶) and plating technique on Petri plates containing appropriate media followed by incubation at 25±2°C for 15 days. The developed colonies were then counted and calibrated perml.

Statistical analysis

The data were analyzed for comparison of means, using SAS Version 12.0. Three way analysis of variance (ANOVA) was performed on the collected data and mean separation with Tukey's grouping and HSD adopted to compare means among treatments. The analysis of data

was carried out using PROC GLM of SAS with Random statement with test option. Data for 2 years were analyzed separately to test the homogeneity of error of variances; since this value was significant, the combined analysis was not performed. The significance was considered at $P \le 0.05$.

RESULTS AND DISCUSSION

Pretreatment

The initial population of root-knot nematode was recorded very high (7.2 J₂/g soil) under naturally infested field of bitter gourd crop. Few plants recorded with all roots severely knotted with no root system and plants usually die. Summer ploughing reduced M. incognita population by up to 67% (2.4 J_2/g soil). In the plots where only fallowing was applied, a reduction of 27% in M. incognita population $(5.7 \text{ J}_2/\text{g soil})$ was recorded. A gradual increase in reduction of M. incognita population was recorded up to fourth ploughing. The maximum reduction was recorded after third ploughing (34.84%). The reductions in nematode population significantly lower (26.35%) after fourth ploughing, which was recorded at the time of sowing of bitter gourd (Fig 1). This study demonstrated the availability of effective ecofriendly integrated options for the management of M. incognita on bitter gourd. Fallowing and summer ploughing during summer months as pretreatment is an ecofriendly good option to bring down M. incognita population in a heavily infested field. Jain and Bhatti (2008) also recorded a significant decline in the population of M. javanica and increased yield of tomato through summer ploughings.

Biological control of root-knot nematodes by microbial antagonists has been considered a more natural and environmentally acceptable alternative to chemical pesticides/nematicides. Strains of *P. flourescens* and *T. harzianum* are able to antagonize and suppress root-knot nematode, *Meloidogyne* spp. (Suarez *et al.* 2004, Singh and Singh 2012). Commercial products of *P. flourescens* and *T. harzianum* are available in the market which may be more adaptive and less expensive. Increase in the efficacy

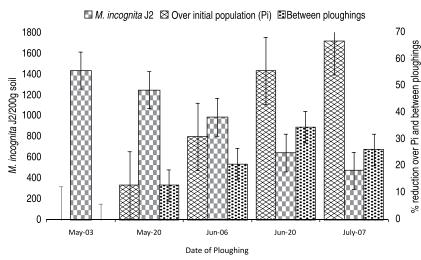


Fig 1 Effect of summer ploughings on the population of M. incognita J2 during 2014.

Table 1 Effect of treatments on plant height, no. of fruits, yield and nematode multiplication in July-December, 2014 (First year trial)

Treatment	Plant height (cm)	No. of fruits/ plant	Yield/plot (kg)	Yield/ha (t)	Percent galling (root-knot)	G.I & {R.F}	RKN population in (200cc)
T1-Carbofuran (Control)	239.13** (15.50±0.12)***c [+11.39]	16.75 (4.19±0.28)b	31.10 (5.66±0.30) cd	8.35# (3.05±0.15)c [+8.02]	17.00 (24.13±2.11)##c [-79.70]	2 {0.37}	174.75 (13.22±0.14)d [-90.40]
T2-Neem cake	241.18 (15.56±0.11)c [+12.32]	19.25 (4.49±0.14) ab	36.30 (6.02±0.10) bc	9.41 (3.22±0.05)bc [+21.73]	36.25 (36.97±1.44)b [-56.72]	3 {0.63 }	298.5 (17.30±0.24)b [-74.51]
T3-Pseudomonas flourescens (Pf)(ST+SA)*	270.30 (16.47±0.07)b [+25.88]	22.00 (4.79±0.13)a	40.17 (6.49±0.08) ab	10.98 (3.46±0.04)a [+42.04]	37.50 (37.70±1.92)b [-55.22]	3 {0.59}	281.25 (16.80±0.16)b [-63.88]
T4-Trichoderma harzianum (Th)(ST+SA)	279.58 (16.75±0.12)b [+30.20]	20.50 (4.63±0.15)a	37.50 (6.34±0.15) ab	10.46 (3.39±0.07)a [+35.32]	38.75 (38.41±2.51)b [-53.73]	3 {0.60}	286.50 (16.96±0.11)b [-63.21]
T5-Pf+Th + Neem cake	316.05 (17.80±0.22)a [+47.18]	23.00 (4.90±0.11)a	46.61 (6.75±0.13)a	11.90 (3.60±0.06)a [+53.94]	16.25 (23.58±1.93)c [-80.60]	2 {0.44 }	209.25 (14.50±0.20)c [-73.13]
T6-Farmer's practice (FP)	214.73 (14.69±0.07)d [0.00]	14.50 (3.39±0.13)c	28.00 (5.47±0.19) d	7.73 (2.95±0.09)c [0.00]	83.75 (66.61±2.93)a [0.00]	5 {1.64}	778.75 (27.92±0.31)a [0.00]
Tukey's HSD @0.05	0.39	0.53	0.54	0.27	6.25		0.65

FP – Farmer's practice; G.I. – Gall index; R.F. Reproductive factor and presented in $\{\}$ parentheses and calculated by dividing final population (Pf) of *M. incognita* by the initial population (Pi); *(ST+SA) Seed treatment + soil application; **Original mean value; *** Value presented in parentheses () are square root transformed value at $\sqrt{x}+0.5\pm SE$ (standard Error); #Yield t/ha calculated by multiplying factor; ##Value presented in parentheses () are Arc Sign transformed value at deg (asin (sqrt (x/100))); Values presented in parentheses [] are % increase (+) or decrease (-) over farmer's practice. Mean in each column with different superscript letter differ significantly and values in the same column followed by the same letter(s) do not differ (Tukey's HSD at 0.05).

of these bio-agents appears possible when these bio-agents are integrated with organic amendments such as oil cakes (Verma *et al.* 2005, Singh and Singh 2012, Singh 2013) and this has been demonstrated in the present investigation. It is also recorded in the present study that neem cake enhanced plant growth and yield during consequent second season's trial. Addition of neem cake changes the soil environment and adversely affects the life cycle of nematode and also enables the plants to resist nematode attack and helps in proliferation of the bio-agents (Singh 2013).

Field experiment (2014)

The results recorded during first year trial were presented in Table 1. In general, M. incognita adversely affected the plant growth and caused significant (P>0.05) reduction in plant health in terms of vine height, number of fruits and marketable yield of bitter gourd under farmer's practice (FP). Under FP, M. incognita caused heavy galling up to 83.7% on roots (gall index-5) of bitter gourd. Individually, both tested bio-agents, P. flourescens and T. harzianum (T_3 and T_4) caused significant (P>0.05, Tukey's HSD=2.06) improvement in plant health which was 25.8% and 30.2% respectively, greater than the treatment T_1 (carbofuran/control) (11.3%). Interestingly, the integrated application of neem cake, P. flourescens and T. harzianum (T_5) was found to be the best treatment (P>0.05, Tukey's HSD=3.11) that resulted in the greatest enhancement in plant height, i.e. 47.2% and yield (P>0.05, Tukey's HSD=1.28),

i.e. 53.9% as compared to (FP). This treatment (T₅) also significantly (P>0.05) suppressed number of M. incognita in the soil (73%) as well as on roots (number of galls), i.e. 80%. Individually neem cake, P. flourescens and T. harzianum did not show any significant difference (P<0.05) in M. incognita population. However, integration of all the tested management components (T₅) caused greater (up to 73%) reduction in M. incognita J2 population which was significantly less than control (T₁) and recorded up to 90%. The reproductive factor (P_f/P_i) was highest (1.64) under FP (T_6) and lowest (0.37) in the plots that received carbofuran (T_1) followed by 0.44 under combined application (T_5) with all management components. The net yield from FP was up to 7.7 t/ha in the first year which did not show any significant (P<0.05) difference with carbofuran (T₁-control) and neem cake (T_2) , however, apparently latter showed higher yield 8.3 and 9.4 t/ha respectively. The highest marketable yield was recorded 11.9 t/ha with the treatment (T₅) where all the management components were applied. The yield obtained from treatments T_3 , T_4 and T_5 did not show any significant (P<0.05) difference.

Field experiment (2015)

The results of second year trial are presented in Table 2. During second year trial neem cake (T₂) showed better response to increase vine height and yield when compared to previous season's data, however, did not contribute to suppress nematode multiplication as much as other

Table 2 Effect of treatments on plant height, no. of fruits, yield and nematode multiplication in January to June, 2015 (Second Year Trial)

Treatment	Plant height (cm)	No. of fruits/plant	Yield/plot (kg)	Yield/ha (t)	Percent galling (root-knot)	G.I & R.F	Nematode population (200cc)
T1-Carbofuran (Control)	248.60** (15.79 ± 0.38)***d [+33.66]	17.00 (4.22 ± 0.28)c	$ \begin{array}{r} 34 \\ (5.88 \pm 0.40) \\ b \end{array} $	9.07# (3.16 ± 0.20)b [+46.29]	8.75 (17.12 ± 1.01)##c [-90.67]	1 {0.31}	$54.75 (7.45 \pm 0.25)d [-94.92]$
T2-Neem cake	$281.00 (16.78 \pm 0.41)c [+51.08]$	22.25 (4.80 ± 0.26)ab	$ 41 (6.46 \pm 0.30) ab $	10.93 (3.44 ± 0.15) ab $[+76.29]$	$31.25 (33.93 \pm 1.45)b [-66.66]$	2 {0.60}	$ 178.5 (13.39 \pm 0.31)b [-83.45] $
T3-Pseudomonas flourescens (Pf) (ST+SA)*	290.75 (17.08 ± 0.12)bc [+56.32]	22.25 (4.82 ± 0.13)ab	$44.50 \\ (6.73 \pm 0.18) \\ a$	11.87 (3.58 ± 0.09)a [+91.45]	$ 27.50 (31.45 \pm 2.09)b [-70.67] $	2 {0.29}	$81.25 (9.06 \pm 0.29)c [-92.47]$
T4-Trichoderma harzianum (Th) (ST+SA)	296.53 (17.25 ± 0.13)b [+59.42]	20.75 (4.66 ± 0.15)bc	41.50 (6.51 ± 0.21)ab	11.07 (3.47 ± 0.10)ab [+78.55]	23.75 (16.35 ± 0.97)c [-74.67]	2 {0.30}	86.5 $(9.34 \pm 0.20)c$ [-91.98]
T5-Pf+Th + Neem cake	343.55 (18.56 ± 0.22)a [+84.70]	26.50 (5.24 ± 0.13)a	48.50 (7.02 ± 0.23)a	$12.93 (3.73 \pm 0.11)a$ [+108.55]	8.00 (16.35 ± 0.97)c [-91.46]	1 {0.28}	59.25 $(7.74 \pm 0.37)d$ [-94.51]
T6-Farmer's practice (FP)	$ 186.00 (13.67 \pm 0.24)e [0.00] $	12.25 (3.63 ± 0.14)d	23.25 (4.92 ± 0.11)c	6.20 (2.68 ± 0.06)c [0.00]	93.75 (79.68 ± 6.02)a [0.00]	5 {1.36}	$ 1078.75 (32.86 \pm 0.26)a [0.00] $
Tukey's HSD @0.05	0.91	0.52	0.78	0.39	9.01		0.90

FP – Farmer's practice; G.I. – Gall index; R.F. Reproductive factor and presented in $\{\}$ parentheses and calculated by dividing final population (Pf) of M. incognita by the initial population (Pi); *(ST+SA) Seed treatment + soil application; **Original mean value; *** Value presented in parentheses () are square root transformed value at $\sqrt{x}+0.5\pm SE$ (standard Error); #Yield t/ha calculated by multiplying factor; ##Value presented in parentheses () are Arc Sign transformed value at deg (asin (sqrt (x/100))); Values presented in parentheses [] are % increase (+) or decrease (-) over farmer's practice. Mean in each column with different superscript letter differ significantly and values in the same column followed by the same letter(s) do not differ (Tukey's HSD at 0.05).

management components (Fig 2). Under T_5 a gradual increase was recorded in vine height up to 343.6 cm (84.7%) in second season trial, whereas, vine height was 316.1 cm (47.2%) during first year trial with their corresponding FP treatment. The same trend was also recorded in case of

marketable fruit yield which was increased up to 108% at the end of second year trial. Although it is highest among all treatments, it did not differ with other treatments except control (T₁) and FP (T₆). Maximum reproductive factor was 1.36 with highest gall index 5 with FP (T₆). Suppression in soil population of M. incognita was also higher during the second year trial in comparison to previous season trial which was recorded below threshold economy level i.e. 1.0 J_2/g soil. Integrated use of bio-agents with neem cake (T₅) reduced nematode population up to 94.5% and did not show any significant (P<0.05, Tukey's HSD=0.29) difference with control (T_1) (carbofuran) treatment (94.9%). Interestingly, reproductive factor was also recorded less (0.28) in T_5 compared to T_1 (0.31). Under T_5 treatment, this season's crop produced 12.9 t of marketable fruit yield/ha, which was higher than

the application of other treatments which was recorded in the range of 6.2 to 11.8 t/ha.

In the present investigation, neem cake was applied 10 days prior to sowing to enable decomposition. Neem cake produces nematode suppressive active ingredients in soil

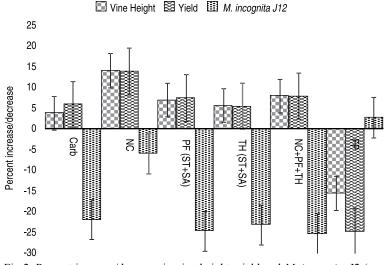


Fig 2 Percent increase/decrease in vine height, yield and M. incognita J2 (n \times 10) over first season crop,

Table 3 Population of bio-agents in different treatments in the field after harvesting of consecutive second year bitter gourd

Treatment	Bio-agents population in soil after first and consequently second season trial							
	Pseudomonas floui	rescens (cfu/g soil) ×10 ³	Trichoderma harzianum (cfu/g soil) ×10 ³					
	Year 2014	Year 2015	Year 2014	Year 2015				
T1-Carbofuran (Control)	0.00	0.00	0.00	0.00				
	$(1.00\pm0.00)b$	$(1.00\pm0.00)c$	$(1.00\pm0.00)c$	$(1.00\pm0.00)c$				
T2-Neem cake	0.00 (1.00±0.00)b	0.00 (1.00±0.00)c	0.00 (1.00±0.00)c	0.00 (1.00±0.00)c				
$T3-\textit{Pseudomonas flourescens} \ (Pf)(ST+SA)*$	3.60 (2.14±0.08)a	5.77 (2.60±0.05)b	0.00 (1.00±0.00)c	0.00 (1.00±0.00)c				
T4-Trichoderma harzianum (Th)(ST+SA)	0.00 (1.00±0.00)b	0.00 (1.00±0.00)c	3.20 (2.05±0.04)b	5.57 (2.56±0.07)b				
T5-Pf+Th + Neem cake	3.83 (2.20±0.03)a	6.87 (2.81±0.03)a	3.73 (2.17±0.07)a	8.23 (3.04±0.07)a				
T6-Farmer's practice (FP)	0.0 (1.00±0.00)b	0.00 (1.00±0.00)c	0.00 (1.00±0.00)c	0.00 (1.00±0.00)c				
Tukey's HSD @0.05	0.10	0.08	0.09	0.12				

Cfu – colony forming unit; Value presented in parentheses are square root transformed value at $\sqrt{x+0.5\pm SE}$ (standard Error); Mean in each column with different superscript letter differ significantly and values in the same column followed by the same letter(s) do not differ (Tukey's HSD at 0.05).

during decomposition, a good soil texture and environment to ensure proliferation of bio-agents (Singh 2013). It may have improved the organic composition of the soil by providing a more nutritive and porous substrate at the root zone, leading to the promotion of biomass and yield (Asraf and Khan 2010). Similar, combination treatments using bioagents and neem cake reduced the nematode multiplication and increase the yield of chilli (Singh and Singh 2012) and eggplant (Singh 2013).

Two mechanisms of action are thought to be responsible for the reduction in nematode infection on bitter gourd. T. harzianum directly parasitized eggs and juveniles of M. incognita in soil and suppressed their penetration in roots through increase in chitinase (Sahebani and Hadavi 2008), protease activity leading to a lower disease intensity (Saurez et al. 2004) and improved plant growth (Campbell et al. 2006, Singh and Singh 2012, Singh 2013). It induced plant defense mechanisms leading to systemic resistance (Jegathambigai et al. 2011). P. fluorescens also showed antagonistic action to reduce nematode activity by induced systemic resistance (Kloepper et al. 2004). Timper et al. (2009) reported that activities of defense enzymes peroxidase, phenylalanine ammonia lyase, chitinase and catalase were significantly higher in P. fluorescens treated tomato root tissues challenged with M. incognita.

Results showed successful establishment of both biological control agent, i.e. *P. fluorescens* and *T. harzianum*: a significant difference (P>0.05) was recorded among treatments. Both the bio-agents were isolated from the rhizosphere of the experimental sites at the time of final harvesting of fruits, i.e. 145 days after sowing. Significantly (P>0.05) higher number of colony forming units (cfu) were recorded with neem cake amended soil compared with treatments that did not receive neem cake (Table 3). A

significant increase (P>0.05) in cfu/g was recorded among treatments during second year trial compared to previous year trial. There was also significant reduction in the rootknot nematode population in roots and soil around bacterized plants (Anita et al. 2004). Thus, the combined use of bioagents (P. flourescens and T. harzianum) having different mode of action with neem cake amended soil gave the best control of root-knot disease of bitter gourd. Nematode suppressive effects of the tested management components in T₅ also enhanced the growth and yield of bitter gourd: due to improved soil fertility by addition of neem cake and farm yard manure and release of growth hormones by both the bio-agents. The ability of P. fluorescens and T. harzianum to manage M. incognita seems to be increased by integration with neem cake as evidenced by more number of colony forming units recorded under field, and these are regarded as reduced-risk of bio-rational products as there are no reports of their ill effects (Singh 2013).

It is concluded that pretreatment (fallowing and summer ploughing), application of neem cake and biological control agents (*P. fluorescens* and *T. harzianum*) with diverse modes of action is an effective strategy for the management of root-knot disease of bitter gourd caused by *M. incognita* under naturally infested field. It benefits small and marginal farmers.

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