Development of an integrated approach for managing root-knot disease on chili (*Capsicum annum* L.) under field conditions

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Summary. A 2-year field study was conducted to develop a field application method using eco-friendly means for controlling root-knot disease of chili. The test sites at the Indian Institute of Vegetable Research, Varanasi, UP, India were heavily infested (up to 5.2 juveniles g^{-1} soil) with root-knot nematode, *Meloidogyne incognita*. As a pretreatment, three deep summer ploughing at 15 days interval caused a reduction of 53.8% of the initial population levels of *M. incognita*. The integration of soil amendment (neem cake at 20 t ha^{-1}), seed treatment (6 g kg^{-1} seed) and/or soil application with a talc preparation of fungal biological control agents (*Pochonia chlamydosporia* (2×10^7 cfu g^{-1}) at 12 kg ha^{-1} and *Trichoderma viride* (2.8×10^6 cfu g^{-1}) at 12 kg ha^{-1} were tested. Neem cake was applied 10 days prior to sowing seeds in nursery beds and/or application of biological control agents to the main field as and when required as per experimental layout. Application of any of the tested components significantly reduced the incidence of root-knot disease in both years compared with the controls. Combined applications of any two components caused better recovery in plant health compared with individual treatments and/or controls. The best protection of disease, in terms of reduction in number of galls (58%) and reproductive factor (P_f/P_i (R) = 0.3), was achieved through integration of neem cake, *P. chlamydosporia* and *T. viride* (reduced half dose). It also enhanced marketable chili yield by up to 54%. Re-isolation of both fungi at harvest showed that they were successfully established in the rhizosphere of chili plants up to the termination of experiment.

Key words: Biological control, chili, *Meloidogyne incognita*, neem, *Pochonia chlamydosporia*, *Trichoderma viride*.

Chili pepper (*Capsicum annum* L.) is being grown worldwide as one of the most important vegetables and spice crops for its multipurpose uses. Several pests and disease influence the growth and development of the chili crop, ultimately leading to poor yield. Vegetable production is not possible in the tropics and subtropics without considering the nematode pests (Sikora & Fernandez, 2005). Under the conditions of farming in tropical and subtropical regions, alternatives to current practices are desperately needed (James *et al*., 2006; Rosendahl *et al*., 2008). Root-knot nematode, *Meloidogyne* spp., has been considered the most damaging of ten economically important genera of plant-parasitic nematodes (Sasser & Freckman, 1987). Root-knot disease caused by *Meloidogyne incognita* is the most frequently encountered disease of chili and is one of the limiting factors affecting the production of chili in India; the national loss due to this nematode in chili was up to 12.9%, equivalent to 3.9 million US $ per year (Jain *et al*., 2007).

The efficacy of current strategies for management of root-knot nematode is limited, except for chemical nematicides. However, most of the chemical nematicides are either banned or very expensive, and have adverse effects on human health, ground water and the environment (Zukerman & Esnard, 1994). Research on the use of antagonistic micro-organisms has received increasingly greater attention during last two decades (Stirling, 1991; De Leij & Kerry, 1991; Rao *et al*., 2004a, b; Hallman *et al*., 2009; Tariq *et al*., 2009; Singh & Mathur, 2010a, b). Identifying a suitable option for managing root-knot nematodes and their destructive impact is therefore a key consideration, not only with regard to the environment and human health but also in relation to the imminent removal from use of harmful

cemicals (Affokpon *et al*., 2010). Biological control agents could be an effective part of eco-friendly integrated nematode management. However, biological control agents often are not thought of as acceptable alternatives for pesticides. Reasons for this include the lack of broad spectrum activity, inconsistent performance in the field and slower action when compared with pesticides. One of the strategies for overcoming inconsistent performance is to combine the disease-suppressive activity of two (or more) beneficial bio-agents (Mayer & Roberts, 2002), which have different modes of action on various life stages of the nematodes (Singh & Mathur, 2010 a, b). Such combinations have potential for more extensive colonisation of the rhizosphere, and more consistent expression of beneficial traits under a broad range of soil conditions than strains applied individually (Mayer & Roberts, 2002). However, some studies have also demonstrated the negative effects of mixed isolate applications (Oyekanmi *et al*., 2007); with incompatibility between microorganisms causing a combination to be less effective than single isolate application (Meyer *et al*., 2001). Consequently, the assessment and identification of suitable combinations are important. Hence, development of bio-rational soil treatment for effective management of root-knot nematode is highly desirable.

The use of biological control agents with cultural practices is an obvious alternative and environmentally attractive option for managing root-knot nematodes. Thus, the present field study was planned to manage root-knot disease caused by *M. incognita* by combining neem cake and fungal biological control agents. It is an established fact that soil amendments such as neem cake have the capacity to influence and modify the relationship between plant and nematodes, besides helping to build-up soil fertility (Mishra & Mojumder, 1995). In the present study, we first examined whether toxic compound produced by one and/or both fungal control agents kills the mobile or infective second-stage juveniles (J2) of the root-knot nematode, *M. incognita*, in soil before penetration. Second, we examined whether an egg parasitic fungus could act against nematodes that avoided control, and penetrated hosts, developed and completed their life cycle to produce eggs. The main objective of this study was to evaluate the efficacy of a talc based preparation of indigenous fungal bio-agents, *Pochonia chlamydosporia* (PC-01) and *Trichoderma viride* (TV-04) alone and in combination with neem cake (10 days prior to application of biological control agents) in suppression of root-knot disease of chili under naturally infested field condition. The tested isolates of *P. chlamydosporia* and *T. viride* were previously isolated from the egg masses of *M. incognita* and tested *in vitro* and *in vivo* (Goswami *et al*., 1998; Dhawan & Singh, 2008). In addition, we evaluated the combination of both bio-agents (reduced half dose) with neem cake for developing an integrated approach for managing the disease on chili *cv.* Pusa Sadabahar (PS).

MATERIALS AND METHODS

Two field experiments were conducted to develop an integrated approach for the management of root-knot disease caused by *M. incognita*. Heavy infestation of root-knot nematode *M. incognita* on vegetable crops (up to 5.2 J2 g^{-1} soil) was reported at the Indian Institute of Vegetable Research, Varanasi, UP, India (Singh *et al*., 2009). To overcome this heavy infestation integration of cultural and non chemical methods were tried. Two-week old freshly extracted neem kernel cake was obtained from a local oil mill. This neem kernel was ground to prepare a fine powder. The nutritional value of the organic amendment was: nitrogen 3.5%, phosphorous (P_2O_5) 0.2%, potash (K_2O) 1.8%, neem oil 6%, moisture 10% and sand silica 8%. Talc-based formulations of both biological control agents, PC-01 (2×10^7 cfu g^{-1}) at 12 kg ha^{-1} and TV-04 (2.8×10^6 cfu g^{-1}) at 12 kg ha^{-1} alone and in combination with neem cake at 20 t ha^{-1} was thoroughly mixed in the soil manually as per layout plan. Treatments with half dose of both biological control agents at 6 kg ha^{-1} in combination with neem cake were also tried. The experiment comprises eight treatments T1-Control (untreated), T2-PC alone, T3-TV alone, T4-neem seed cake alone, T5-PC + TV, T6-PC + neem seed cake, T7-TV + neem seed cake and T8-PC (reduced half dose)+ TV (reduced half dose) + neem seed cake. The same treatments were also used for nursery beds sowing, including seed treatments with the biological control agents at 6 g kg^{-1} seed as per experimental layout.

The main field was exposed to sunlight and kept fallow for at least 2 months (May-June) as the pretreatment. The field was then prepared by applying standard agronomic practices and was divided into equal size micro plots of 10 m^2 (4 × 2.5 m). The neem cake was applied 10 days prior to application of fungal biological control agents. Chili cv. PS was grown in a nursery on a bed which was solarized (as pretreatment) for 21 days in May and June with 25 µ thick polyurethane transparent sheet; at intervals of 10 days the soil was turned manually. The nursery beds and the main field were treated

Table 1. Effect of integration of neem cake, *Pochonia chlamydosporia* and *Trichoderma viride* on the colonisation of biological control agents on seedlings at the time of transplanting and under field conditions at the time of harvest of the experiment

Treatment	Number of cfu g^{-1} of Seedlings roots		Number of cfu g^{1} of root at the time of harvest of experiment		Number of cfu g^{1} soil at the time of harvest of experiment		% egg parasitization by PC	% mortality of TV larve
	PC	TV	PC	TV	PC	TV		
Control	0.0 (1.0±0.0)^{e}*	0.0 (1.0±0.0)^{d}	0.0 (1.0±0.0)^{e}	0.0 (1.0±0.0)^{d}	0.0 (1.0±0.0)^{d}	0.0 (1.0±0.0)^{e}	0.0 ^{c} {0.0}**	0.0 {0.0}^{b}
Trichoderma viride alone	0.0 (1.0±0.0)^{e}	21810 (147.7±1.3)^{b}	0.0 (1.0±0.0)^{e}	14479 (120.3±1.1)^{c}	0.0 (1.0±0.0)^{d}	14846 (121.8±0.3)^{d}	0.0 {0.0}^{c}	55 {47.9±1.00}^{a}
Pochonia chlamydosporia alone	18599.7 (136.4±0.7)^{b}	0.0 (1.0±0.0)^{d}	20463 (143.0±0.9)^{c}	0.0 (1.0±0.0)^{d}	16649.3 (129.0±0.6)^{c}	0.0 (1.0±0.0)^{e}	47.2 {40.2±0.2}^{b}	0.0 {0.0}^{b}
Neem cake Alone	0.0 (1.0±0.0)^{e}	0.0 (1.0±0.0)^{d}	0.0 (1.0±0.0)^{e}	0.0 (1.0±0.0)^{d}	0.0 (1.0±0.0)^{d}	0.0 (1.0±0.0)^{e}	0.0 (0.0}^{c}	0.0 {0.0)^{b}
T. viride+ *P. chlamydosporia*	18428.0 (135.8±0.9)^{b}	19190 (138.5±1.2)^{c}	19479 (139.6±0.5)^{d}	15367.7 (124.0±0.9)^{b}	17569 (132.6±0.4)^{b}	16526.3 (128.5±1.1)^{c}	41.5 {40.1±0.2}^{b}	53.3 {46.9±1.5}^{a}
T. viride + neem cake	0.0 ^{c} (1.0±0.0)	25530 (152.3±1.1)^{a}	0.0 (1.0±0.0)^{e}	17048 (130.6±0.6)^{a}	0.0 (1.0±0.0)^{d}	21483.7 (146.6±0.8)^{a}	0.0 {0.0}^{c}	54.4 {47.5±2.6)}^{a}
P. chlamydosporia +neem cake	20560.0 (143.4±0.7)^{a}	0.0 (1.0±0.0)^{d}	22584 (150.3±0.7)^{a}	0.0 (1.0±0.0)^{d}	18463 (135.9±0.9)^{a}	0.0 (1.0±0.0)^{e}	44.6 {41.9±0.2}^{a}	0.0 {0.0}^{b}
T. viride + *P. chlamydosporia* +Neem cake	20532.0 (143.3±0.8)^{a}	23418.7 (149.7±2.5)^{ab}	21423 (146.4±0.8)^{b}	17057.3 (130.6±0.3)^{a}	18426 (135.7±0.8)^{a}	17521.3 (132.36±1.3)^{b}	41.6.3 {43.4±0.1}^{a}	57 {49.0±1.0}^{a}
CD at 0.05	1.8	3.5	1.3	1.9	1.5	1.9	1.5	3.2
SEm	0.59	1.1	0.43	0.61	0.49	0.63	0.15	1.04

Note- Means in each column with different letters differ significantly ($p<0.05$).
All values presented in table are average of three replications.
PC- *Pochonia chlamydosporia*, TV-*Trichoderma viride.*
*Values presented in ( ) are square root transformed values ± standard error.
**Values presented in { } are angular transformed value ± standard error.

with same method and doses as per protocol. Twenty-eight day-old seedlings were removed from the nursery bed and transplanted to the main field. A distance of 45 cm (row to row) and 45 cm (plant to plant) was kept to maintain 40 plants per plot.

Soil samples were taken from the treated plots before and after harvest and analysed for nematode population counts, in order to determine the initial and final population of nematodes (P_i and P_f). Nematode population from soil was assessed by using Cobb's sieving and gravity method followed by Baerman's funnel technique (Southey, 1986). Fifteen weeks after planting ten randomly selected plants per plot were carefully removed and the adhering soil washed off for assessment of root galls using a stereoscopic microscope. Root systems were rated for nematode induced galling on the scale of 0-4 as follows: 0 = no galls, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100% roots with galls. Eggs were extracted from roots and their number estimated using the sodium hypochlorite method of Hussey & Janssen (2002). The reproductive factor (R), where $R = P_f/P_i$ was determined. At harvest, ten randomly selected plants per plot were measured for the following parameters: plant height from the soil surface to the tip of top leaf and its weight, root length and weight, and fruit yield. For estimation of marketable fruit yield, the fully mature green fruits of chili were harvested and weighed.

Colonisation of roots by *P. chlamydosporia* and *T. viride* were also recorded. The root system was gently washed to remove adhering soil, dried using blotting paper, weighed and cut into small pieces (3-4 mm). Sub samples (1 g) of roots were taken randomly and root pieces plated on a semi selective media developed by Kerry *et al*. (1993) and potato dextrose medium respectively for PC and TV. The Petri plates were then incubated at 25±2ºC in a BOD

incubator for 15 days. Both the fungi were also re-isolated from treated soil using soil dilution plating technique. The dilutions were prepared up to 10^{-6} and 0.1 ml of each 10^{-5} and 10^{-6} dilutions were spread on Petri plates containing potato dextrose agar medium. The plates were incubated at 25±2ºC in a BOD incubator for 15 days. The resulting colonies were then counted and calibrated to 10^{-6} cfu ml^{-1}. Both the fungi isolated from roots as well as from soil were sub-cultured and purified to compare with tested isolates of PC and TV.

Preparation of talc based formulation. Both isolates of antagonistic fungi were previously isolated from egg masses of *M. incognita*. These were purified with repetitive sub-culturing and tested *in vitro* and *in vivo* (Goswami *et al*., 1998; Dhawan & Singh, 2008). Potato dextrose (PD) broth medium was used for mass multiplication of both the tested fungi. Autoclaved (at 15 psi for 20 min) PD broth was inoculated with each of the tested fungal isolates separately for mass multiplication. Each flask was then incubated at 25±2ºC in an incubator for 21 days. Thereafter, both broth and fungal mat were mixed thoroughly with the help of an electric mixer and grinder. This fungal biomass (200 ml) was then mixed with talc powder (770 g) as carrier material. To this, fine powder of chickpea pod waste (2% w/w) and carboxymethyle cellulose (1% v/w) were added to the carrier material as a carbon source and sticking agent, respectively. All the contents were remixed manually and dried under shade at room temperature. This formulation (material) was then packed in polythene bags and stored at 4ºC. Over a 2 weeks period, it was applied in the respective treatments. The spore loads of each tested fungi was estimated in diluted samples using a haemocytometer.

Data analysis. The experimental design was Randomised Complete Block Design (RCBD). Each treatment was replicated three times and repeated twice. Similar results were achieved in two consecutive years; therefore, data obtained from both the trials was pooled, analysed and subjected to analysis of variance using SPSS ver. 12.0 and means separated using Duncan's Multiple Range Test (Gomez & Gomez, 1984) at 5% significance level.

RESULTS

In the present investigation fallowing and summer ploughing (as a pretreatment) reduced the initial *M. incognita* population by up to 53.8% which had reduced to 2.4 J2 g^{-1} soil at the time of transplanting. The seedlings obtained from the treated nursery beds were vigorous compared with the controls. Colonisation of seedling roots by both the biological control fungi was recorded (Table 1). However, the assessments of fungal colony at nursery bed were not done. Re-isolation and colonisation of the biological control agents from the rhizosphere of chili plants and roots from main experimental field were also studied at the termination of experiments. Data presented in Table 1 showed successful establishment of fungi both in the rhizosphere and roots of the chili plants. Also the fungi were antagonistic against *M. incognita*, either parasitising eggs and/or caused juvenile mortality when examined after harvesting the experiment. Up to 47.2% of the eggs were parasitised by PC. The percent mortality caused by TV was up to 57%. Roots of the seedlings were colonised by both PC and TV up to 2.0×10^4 and 2.5×10^4 cfu g^{-1} root, respectively, at the time of transplanting to the main field. The bio-agents were also isolated from the rhizosphere of the main experimental sites (up to 2.2×10^4 cfu g^{-1} in case of PC treated soil and up to 1.7×10^4 cfu g^{-1} in case of TV treated soil) when experiments were terminated 100 days after transplantation. The amount of colony forming units was significantly higher in the treatment that received the biological control agent and neem cake application compared with treatments that did not received neem cake.

The data on plant health, yield and *M. incognita* multiplication and development are presented in Tables 2 and 3. Application of neem cake and both biological control agents significantly suppressed number of *M. incognita* in soil as well as on roots of chili. Significant reductions in number of galls were recorded between treated and untreated plants. Application of any two management components was more effective than the individual component in reducing nematode multiplication. However, a significant difference ($P < 0.05$) was recorded compared with control. Only PC seems to be effective in reducing the number of eggs per egg mass. PC reduced eggs of *M. incognita* per egg mass by up to 62% and did not show any significant difference with the combined applications, PC + TV and/or neem cake. The magnitude of the recovery of each growth character varied with the treatment. Combined application of PC and TV with or without neem cake showed better results in improving the plant health compared with other treatments (Table 2). The integrated use of neem cake and reduced half dose of the bio-agents resulted in maximum recovery in plant health (up to 69% in terms of shoot length) with a significant reduction in nematode multiplication and development (up to 85% in terms of soil population – Table 3).

Data for root gall rating of *M. incognita* showed highly significant differences ($P < 0.05$) between individual and/or combined applications, particularly galls in treatments with biological control agents were reduced compared with control plants. Root

Table 2. Effect of integration of biological control agents and neem cake on the plant health of chili infested by root-knot nematode, *Meloidogyne incognita.*

Treatments	Plant growth parameters				
	Length (cm)		Weight (g)		Fruit yield (kg/plot)
	Shoot	Root	Shoot	Root	
Control untreated	*25.5±1.3^{f}	7.4±0.3^{e}	19.6±0.9^{e}	6.8±0.2^{c}	7.0±0.1^{e} **[7.0]***
Trichoderma viride alone	63.4±1.7^{c} **(+59.8)	13.7±0.4^{c} **(+46.5)**	35.9±1.1^{cd} **(+45.6)**	9.8±0.3^{b} **(+30.3)**	11.1±0.9^{d} **(+36.7) [11.1]**
Pochonia chlamydosporia alone	57.0±1.6^{d} **(+55.3)**	13.1±0.6^{c} **(+43.7)**	34.5±0.9^{d} **(+43.3)**	9.2±0.5^{b} **(+26.3)**	10.5±0.1^{d} **(+33.2) [10.5]**
Neem cake alone	49.6±1.2^{e} **(+48.6)**	8.8±0.3^{d} **(+16.5)**	33.1±0.9^{d} **(+40.9)**	8.7±0.3^{bc} **(+21.4)**	10.9±0.1^{d} **(+35.8) [10.9]**
T. viride+ *P. chlamydosporia*	72.9±2.5^{b} **(+65.0)**	16.6±0.5^{ab} **(+55.7)**	43.3±0.5^{b} **(+54.9)**	11.9±0.4^{a} **(+43.2)**	13.0±0.1^{c} **(+46.1) [13.0]**
T. viride + neem cake	66.8±1.9^{c} **(+61.8)**	15.9±0.6^{b} **(+53.93**	38.1±0.6^{c} **(+48.6)**	8.9±2.4^{bc} **(+23.9)**	12.1±0.1^{b} **(+41.8) [12.1]**
P. chlamydosporia +neem cake	62.7±1.7^{cd} **(+59.3)**	16.4±0.4^{ab} **(+55.1)**	33.2±0.8^{d} **(+41.0)**	10.0±0.6^{ab} **(+32.2)**	12.0±0.2^{c} **(+41.7) [12.0]**
T. viride + *P. chlamydosporia* +Neem cake	82.1±1.4^{a} **(+68.9)**	17.3±0.2^{a} **(+57.4)**	50.9±1.8^{a} **(+61.6)**	12.3±0.2^{a} **(+44.9)**	15.2±0.1^{a} **(+53.8) [15.2]**
CD at 0.05	4.9	0.9	3.2	2.4	3.2
Sem	1.68	0.31	1.08	0.82	1.60

Note: means in each column with different letters differ significantly (P<0.05)
* Figures presented in table are mean values ±Standard Error
** Figures presented in parentheses ( ) and bold are percentage increase (+) or decrease (-) over their respective control
*** Figures presented in [ ] are yield t ha^{-1}

galling in plots treated with all three management components caused 58% suppression of gall formation. No statistically significant difference (P > 0.05) was obtained in gall rating among individual applications. Similarly, dual application did not differ significantly. Data on root and shoot weight showed that combined application of management components did not differ significantly (Table 2). The reproduction factor of various treatments was highest (1.8) for untreated control plots and lowest (0.3) in the plots that received combined treatment of neem cake 10 days prior to application of reduced doses of tested biological control agents (Table 3).

Although a significant difference was recorded in yield between treated and untreated plants, the yield with untreated plots was only up to 7.0 t ha^{-1}. The yield from plots with individual application of any component (PC, TC, and neem cake) or combined dual application of any two (PC + TV, PC + Neem cake and TV + neem cake) did not differ significantly at P < 0.05 (Table 2). However, the recovery in marketable yield (mature green fruits) was from 33.2% to 46.1% in these treatments. The maximum recovery (54%) with the highest yield (15.2 t ha^{-1}) was recorded with the combination of neem cake and biological control agents (PC and TV at reduced dose) (Table 2).

DISCUSSION

The biological control agents and neem cake in the present investigation were found to improve the health and yield of chili plants and reduce the multiplication of *M. incognita* compared with the untreated control. The explanations for these results may be due to the antagonistic activity of biological control agents, which induced reductions in nematode populations (Kerry *et al*., 1993; Spiegel & Chet, 1998; Rao *et al*., 2004a, b; Kumar & Khanna, 2006; Jegathambigai *et al.,* 2011) and addition of neem cake which changes the soil environment and adversely affected the life cycle of nematode and

Table 3. Effect of integration of biological control agents and neem cake on multiplication and development of root-knot nematode, *Meloidogyne incognita*, infesting chili.

Treatment	Root gall rating	Number of galls/ root system±S.E	Number of egg mass/root system±S.E	Number of eggs/egg mass	Soil population /200 ml soil	$R=P_f/P_i$
Control untreated	4.8[a]	62.8±2.8[a]	41.0±4.3[a]	237±22.3[a]	882.6±42.0[a]	1.8[a]
Trichoderma viride alone	2.6[c]	43.3±4.4[b] **(-31.0)**	26.8±2.1[bcde] **(-22.7)**	182.5±9.4[b] **(-23.0)**	494.5±44.6[c] **(-43.9)**	1.0[c]
Pochonia chlamydosporia alone	2.4[c]	49.5±2.5[c] **(-21.1)**	23.0±21[cdef] **(-28.7)**	89.8±7.3[d] **(-62.1)**	522.5±17.5[bc] **(-40.8)**	1.1[b]
Neem cake alone	3.1[b]	40.3±3.9[c] **(-35.9)**	31.0±4.2[b] **(-15.9)**	198.0±8.6[b] **(-16.5)**	528.0±20.9[bc] **(-40.2)**	1.1[b]
T. viride+ *P. chlamydosporia*	1.7[d]	33.3±2.9[bc] **(-47.0)**	19.0±2.1[ef] **(-35.2)**	70.8±3.4[d] **(-70.2)**	407.5±26.9[d] **(-53.8)**	0.9[d]
T. viride + neem cake	1.2[e]	31.0±1.7[d] **(-50.6)**	30.5±3.3[bc] **(-16.7)**	155.5±6.7[c] **(-34.4)**	320.0±25.2[d] **(-63.8)**	0.7[f]
P. chlamydosporia +neem cake	1.4[d]	32.0±2.8[d] **(-49.0)**	28.0±4.0[bcd] **(-20.7)**	77.5±4.6[d] **(-67.3)**	370.5±21.3[d] **(-58.0)**	0.8[e]
T. viride + *P. chlamydosporia* +Neem cake	0.7[f]	26.5±2.7[e] **(-57.8)**	12.3±1.3[f] **(-45.8)**	54.3±7.7[e] **(-77.1)**	131.0±20.1[e] **(-85.2)**	0.3[g]
CD at 0.05	0.4	6.7	7.8	27.2	49.2	0.2
SEm	0.16	2.27	2.68	9.20	16.63	0.06

Note: Means in each column with different letters differ significantly ($p<0.05$)

* Figures presented in table are mean values ±Standard Error

** Figures presented in parentheses ( ) and bold are percentage increase (+) or decrease (-) over their respective control.

also enables the plants to resist attack (Mishra & Mojumder, 1995). Similar combination treatments, using neem cake and farmyard manure, reduced the nematode population and increased yield of mulberry (Murugesh & Mahalingam, 2008). Results of the present research suggest that treatments of nursery beds helped to obtain healthy vigorous seedlings. In addition, seed treatment and soil application of both PC and TV helps seedlings to escape the initial nematode infestation.

Both the isolates of the tested antagonistic fungi were isolated from the egg mass of *M. incognita*. The observation that fungi isolated directly from root-knot nematode egg masses, as opposed to isolation from soil, tended to show higher levels of antagonism indicates that fungal origin is likely to be an important criterion when obtaining biological control agents (Affokpon *et al.*, 2010; Singh & Mathur, 2010a). A number of studies have also demonstrated that root colonisation by *Pochonia* spp or *Trichoderma* spp. frequently enhances root growth and development, crop productivity, resistance to abiotic stress and uptake and use of nutrients (Chang *et al*., 1986; Spiegel & Chet, 1998; Sharon *et al*., 2001; Howell, 2003; Harman *et al*., 2004; Rao *et al*., 2004a, b; Sahebani & Hadavi, 2008).

The results suggest that there is a significant reduction in *M. incognita* eggs and juveniles populations when chili plants are grown in neem cake amended soil in the presence of PC and TV at reduced dose levels. The nematode build up under the reduced half dose treated plots was low. The post application of TV seems to be effective in reducing the number of galls and number of juveniles present at the termination of experiments. The better performance of fungal biological control agents may be due to the specific mode of action of PC, a well known egg parasite that attacks nematode eggs in the soil, and TV, which produces a toxin fatal to those *M. incognita* juveniles that were still able to hatch. Later in the vegetation period PC attacked eggs produced by females, which had avoided the initial controls and completed their life cycle. These findings are in agreement with the earlier reports of Singh & Mathur (2010b) on tomato using *Acrimonium strictum* (toxic and egg parasitic) and *Aspergillus terreus* (toxic). Additionally, neem cake added to soil and chickpea pod waste added to talc preparation provided a suitable base and more favorable environment for fungal proliferation and increased the efficiency of both the biological control agents. Effective control of *M. incognita* might be due partly to the reduction

in nematode population by the toxic and egg parasitic action of fungal biological control agents, and partly due to the fact that the soil was amended with neem cake. The ability of PC and TV to manage *M. incognita* seems to be increased by integration with neem cake. Root-knot nematode infestation stunted maximum untreated plants and reduced net yield. Verma *et al*. (2005) also found that combination of *Paecilomyces lilacinus*, *T. harzianum* and neem cake significantly reduced the nematode population, resulting in greatest shoot length in pointed gourd. Root gall ratings were significantly lower in the present study in response to combined application.

Data of the number of colony forming units demonstrated that there was considerable multiplication of both the fungal biological control agents in the treatment where neem cake was applied as compared with the treatments where neem cake was not applied. Thus, the applied agents proliferated well in the rhizosphere of chili plants in the presence of neem cake. No tested fungus was found in the control treatment. Similar results were recorded by Nagesh & Janakiram (2004) who recorded that *P. chlamydosporia* established better in neem cake amended beds. Chen *et al.* (1998) reported that biological control appears to be a promising strategy that can be integrated into a management programme to reduce the nematode population and minimise the yield loss. In the present research, root-knot eggs and J2 numbers were significantly lower with combined applications of biological control agents. Further study is needed to determine the exact mechanism of nematode suppression and the effect of neem cake on biotic and abiotic changes in soil.

The current study provides evidence that combinations of biological control agents having different modes of action may have a future for management of plant-parasitic nematodes in vegetable production by increasing the productivity. It is also notable that tested isolates of both biological control agents, PC and TV, were compatible under the conditions used in the present studies. The integration of neem cake 10 days prior to application of talc preparations of PC and TV in nursery as well as on the main field can be considered as a strong candidate for management of root-knot disease of chili caused by *M. incognita*. Finally the individual effect of management components was maximised when both fungi and neem cake were apply in an integrated manner both in the nursery and the main field after transplanting. Accordingly, this package of management practices could be a safe and eco-friendly alternative for controlling root-knot disease of chili caused by *M. incognita.*

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S. Singh, R.K. Singh. Разработка интегрированного подхода к контролю галлообразующих нематод на перце (*Capsicum annum* L.) в полевых условиях.

Резюме. В течение двух лет отрабатывался экологически безопасный метод контроля галлообразующих нематод на перце. Эксперименты проводили в Индийском Институте исследований овощей в Варанаси, шт. Уттар Прадеш, на сильно зараженных галловой нематодой *Meloidogyne incognita* полях (до 5,2 личинок на грамм почвы). В качестве предобработки использовали трехкратную запашку плугом с интервалом в 15 дней, что приводило к сокращению на 53,8% начального уровня зараженности почв *M. incognita*. Использовали также введение почвенных добавок (масса листьев дерева «ним» - *Azadirachta indica* - в количестве 20 тонн на гектар), обработка семян (6 г на кг семян) и/или обработка почвы смесью талька и спор грибов *Pochonia chlamydosporia* (2×10^7 образующих колоний единиц в грамме) - 12 кг на га и *Trichoderma viride* (2.8×10^6 образующих колоний единиц в грамме) - 12 кг на га. Внесение массы листьев дерева ним проводили за 10 дней до посева в рассадные грядки и/или проведения обработки основных полей биоагентами. Внесение всех перечисленных компонентов существенно снижало галлообразование по сравнению с контролем в каждом из двух годов эксперимента. Совместное применение каждого из двух компонентов давало лучшие показатели восстановления растений по сравнению с примененеим только одной обрабоки или с контролем. Наилучшие результаты в смысле снижения числа галлов (58%) и репродуктивного фактора P_f/P_i (R) = 0.3) были достигнуты при совмещении применения массы листев дерева ним, грибов *P. chlamydosporia* и *T. viride* (с использованием сокращенной наполовину дозы). Такая обработка повышала коммерческий выход с поля до 54%. Повторное выделение из почвы двух использованных видов грибов показало, что они успешно обосновались в ризосфере растений перца по завершении эксперимента.