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Bio-management of root-knot disease of Chili (*Capsicum annum*) caused by *Meloidogyne incognita*

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Abstract A field survey was conducted during 2008-09 to observe nematode infestation at IIVR research Farm and found that brinjal crop was heavily infested with root-knot nematode, *Meloidogyne incognita* (5 J_2/g soil). Keeping this fact in view a trial under field condition was laid out the for the management of root-knot disease of chili caused by *M. incognita* with integration of summer ploughing, biological control agents (*Pochonia chlamydosporia* and *Trichoderma viride*) and organic amendment (Neem cake). Three summers (May to June) ploughing at the interval of two weeks caused 48% reduction in *M. incognita* population density and dropped the initial inoculums level to 2.4 J_2/g soil. The best protection of root-knot disease of chili was achieved through combined use of neem cake, *P. chlamydosporia* and *T. viride* that gave statistically significant ($p<0.05$) increase in plant growth, Biomass, and fruit yield (69%, 61% and 80% respectively) compared to control. A significant reduction (at $p<0.05$) in *M. incognita* multiplication and development in terms of root galling, egg masses and soil population (58%, 46 and 85% respectively) was also achieved in the treatment where all the three components were applied compared to control. Both the fungal biocontrol agents were successfully established in the rhizosphere of chili plants up to the termination of experiment. It can be concluded that application of summer ploughing followed by combined application of fungal biocontrol agents + neem cake increased not only plant health and yield but also reduced nematode multiplication and development on chili cv. Pusa Sadabahar.

Keywords: Biocontrol, chili, neem, *Pochonia chlamydosporia* and *Trichoderma viride.*

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Chili pepper (*Capsicum annum*) is being grown worldwide as one of the most important vegetable and spice crop for its multipurpose uses (consumed as fresh or processed) so most popular among vegetable crop. Its high demand makes it a commercial commodity which providing a boost to the chili industry. Its climatic requirements (tropical and subtropical region) very much suited to many plant pathogens. The production of chili is increasingly hampered by pest and diseases including plant parasitic nematodes. On world wide basis a 12.2 % loss was recorded on chili crop by plant parasitic nematodes (Sasser and Freckman 1987). Root-knot disease caused by *Meloidogyne incognita* has been found as the most frequently encountered disease and is one of the limiting factors affecting the production of chili in India (Nagnathan 1984); Jain (1992). A national loss due to this nematode pest in chili was worked out 12.85% and in monetary term has been worked out to the tune of 210 million rupees (Jain *et al.* 2007).

No doubt, chemical control of root-knot nematode is most efficient method but very expensive, not sustainable and has adverse effects on human health, ground water and environment. In view of the uneconomical and hazardous effects of chemical nematicides, researchers have focused their attention to adopt biological control of *Meloidogyne* spp. (Singh and Mathur 2010); Ahmed and Khan 2004). However, biocontrol agents often are not thought of as acceptable alternatives for pesticides. Reasons for this include lack of broad spectrum activity, inconsistent performance in field and slower in action by the biocontrol agents 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 to manage the *M. incognita* which have different mode of action on various life stages of nematodes during completion of life cycle. Such combinations have potential for more extensive colonization of the rhizosphere, more consistent expression of beneficial traits under a broad range of soil conditions than strains applied individually

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(Pierson and Weller 1994); Susan *et al.* (2002). Biocontrol agents could be an effective part of eco-friendly integrated nematode management. Literature on this aspect is vital for the selection of appropriate strategy to control the root-knot disease caused by *M. incognita* infesting chili. The huge loss caused by *M. incognita* needs due attention so as to work out effective nematode management technology for reducing the losses due to *M. incognita*. Hence, a field experiment at IIVR research farm was undertaken to expose the possibility of integrating the use of summer ploughing, biocontrol agents, (*T. viride* and *P. chlamydosporia*) and neem cake for the management of root-knot disease caused by *M. incognita* infesting chili (*Capsicum annum* var. Pusa Sadabahar).

Materials and Methods

A field survey was conducted during 2008-09 to observe nematode infestation at IIVR research Farm and found that brinjal crop was heavily infested (5 J_2/g soil) with root-knot nematode, *Meloidogyne incognita* (Singh *et al.,* 2009). The present field trial was laid out in two sets separately to manage the root-knot disease caused by *M. incognita* on chili crop. The experimental field was irrigated and given three deep ploughing between May to June with the help of a tractor pulled cultivator at a interval of two weeks period. Samples were taken after every ploughing and data on *M. incognita* population was recorded. The field was than prepared by applying standard agronomic practices and divided into equal size micro plots (4 m × 2.5 m). Talc based formulation was used to introduce fungal biocontrol agents in the soil. The experiment comprises of eight treatments 1. Control (untreated), 2. Formulation of *Trichoderma viride* alone at 30g/plot (2.6×10^6 cfu/g), 3. Formulation of *Pochonia chlamydosporia* alone at 20g/plot (3.2×10^6 cfu/g), 4. Neem seed cake alone at 0.15 kg/plot, 5. Formulation of both *T. viride* + *P. chlamydosporia*, 6. Formulation of *T. viride* + neem cake, 7. Formulation of *P. chlamydosporia* + neem cake, 8. Formulations of both *T. viride* + *P. chlamydosporia*, + Neem cake. Each treatment was replicated four times and randomized.

Data on plant health, yield/plot (one picking) and nematode multiplication and development were recorded after 80 days of transplanting. Data of both the trials was pooled before statistical analysis. For interpretation of results, the increase and/or decrease in plant growth parameters and nematode multiplication was worked out in terms of percentages. Nematode population from soil was assessed on number of *M. incognita* juveniles per 200ml soil basis by using Cobb's sieving and gravity method followed by Baerman's funnel technique (Southy 1986). The reproduction factor (R) was calculated by dividing final population (Pf) by the initial population (Pi).

The number of galls and egg masses per root system (average of 10 plants) of each treatment were counted. The number of eggs per egg mass in each treatment was calculated by taking an average of five egg masses of similar size. Re-isolation of the fungi from surface sterilized roots was done to re-examine the antagonistic activity of the fungi by adopting the method used by Monfort *et al.* (2005). Both the fungi were also re-isolated from treated soil using soil dilution plating technique.

Preparation of talc based formulation

Potato dextrose (PD) broth medium was used for mass multiplication of both the tested fungi. PD broth (300 ml) contained in the 500 ml capacity conical flasks were autoclaved at 15 lbs for 20 minutes. After cooling at room temperature each flask was inoculated with the tested fungal isolates separately for mass multiply. Each flask was then incubated at 25±2ºC in an incubator for 21 days. Thereafter both, broth and fungal mat were mixed thoroughly with an electric mixer and grinder. This fungal biomass was then mixed with 1 kg of talc as carrier material to bulk up material. To this, fine powder of chickpea pod waste (10% w/w) and carboxymethyle cellulose (1% w/w) were added to the carrier material as 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 to observe shelf life and further biological control trials. The spore loads of each tested fungi was estimated in diluted samples using a haemocytometer. The spore load thus was maintained at 2×10^6 cfu/g. This formulation was used to introduce both antagonistic fungi to soil.

Data analysis

Both the sets of experimental design were a randomized complete block design. Each treatment of both trials was replicated four times. The pooled data were analyzed by ANOVA using SPSS (Ver-9.01). Differences between treatments were determined by Duncan's Multiple Range Test at 5% significance level.

Results and Discussion

Summer ploughing exposes nematodes to solar heat and desiccation and kills at least a good part of the population. It fits well in agricultural practices adopted by farmers. Summer ploughing reduces nematode population to a great extent and including up to 42.4%

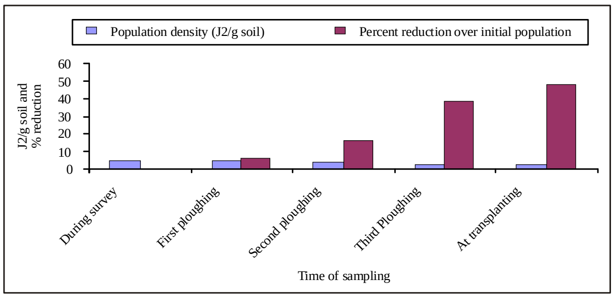

Fig. 1: Effect of summer ploughing on the population of *M. incognita*

reduction in the population of *Heterodera avenae* and consequent wheat yield increase up to 97.5% (Mathur *et al.*, 1987). In the present investigation three deep summer ploughing were applied at an interval of two weeks period in May to June which reduced *M. incognita* population significantly after every ploughing (Fig.1) up to 48%. Due to which the initial inoculums level of *M. incognita* dropped down at 2.4 J_2/g soil at the time of transplanting of chili crop. This reduction in population due to the fact that *M. incognita* had no host plant to feed and no reproduction and died after reserve food in the nematode body depleted. The average temperature during the study period was around 43ºC. The present findings are in confirmation with the findings of Vedhara *et al.* (2002) on tomato under naturally infested field. Summer ploughing helps to reduce the initial economic injury level of *M. incognita*, which is in cases very important as damage to crop yield is depend upon the initial nematode population. It is notable in the present investigation that still it was more than the economic threshold level (2 J_2/g soil) at the time of transplanting.

Individually *T. viride*, *P. chlamydosporia* and Neem cake significantly increased plant growth, biomass and yield of chili cv Pusa sadabahar compared to untreated plants at $p<0.05$. However, the magnitude of the enhancement in each growth character varied with the combination of *T. viride* and *P. chlamydosporia* with or without neem cake (Table 1). Combined use of neem cake and bioagents caused greater plant growth with a significant reduction in nematode multiplication in terms of number of galls, egg masses and soil population. Similar results were recorded by (Sharma *et al*. 2007); Kumar and Khanna, (2006) during their *in-vivo* studies. Interactions between biocontrol agents and neem cake affected plant growth and nematode multiplication and combination of these components produced an additive effect of the individual agents. In the present field investigation the better performance of fungal biocontrol agents may be due to distinct mode of action of *T. viride* which produce toxin fatal to *M. incognita* juveniles and also parasitized eggs together with *P. chlamydosporia*, a well known egg parasite. Both has taken care of remaining juveniles those who escaped from the toxin produced by *T. viride* and penetrate roots, complete their life cycle (Singh and Mathur, 2010).

All the treatments significantly increased the yield of chili over control. The maximum recovery in yield (80%) in terms of green mature fruits was also recorded with the treatment that received *T. viride* + *P. chlamydosporia*

Table 1: Effect of integration of biological 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)
	Shoot	Root	Shoot	Root	
Control untreated	*25.50±1.29^{f}	7.35±0.30^{e}	19.55±0.94^{e}	6.80±0.23^{c}	0.43±0.01^{e}
Trichoderma viride alone	63.35±1.72^{c} ****(+59.75)**	13.73±0.40^{c} **(+46.47)**	35.95±1.14^{cd} **(+45.62)**	9.75±0.33^{b} **(+30.26)**	1.17±0.89^{d} **(+63.25)**
Pochonia chlamydosporia alone	57.03±1.55^{d} **(+55.29)**	13.05±0.64^{c} **(+43.68)**	34.45±0.98^{d} **(+43.25)**	9.23±0.51^{b} **(+26.33)**	1.02±0.09^{d} **(+57.84)**
Neem cake alone	49.58±1.23^{e} **(+48.57)**	8.80±0.25^{d} **(+16.48)**	33.05±0.91^{d} **(+40.86)**	8.65±0.25^{bc} **(+21.39)**	1.13±0.13^{d} **(+61.95)**
T. viride+ *P. chlamydosporia*	72.85±2.48^{b} **(+65.00)**	15.95±0.56^{b} **(+53.93)**	43.30±0.51^{b} **(+54.85)**	11.98±0.35^{a} **(+43.21)**	1.65±0.05^{b} **(+73.94)**
T. viride + neem cake	66.78±1.89^{c} **(+61.81)**	16.60±0.46^{ab} **(+55.72)**	38.05±0.62^{c} **(+48.62)**	8.93±2.41^{bc} **(+23.85)**	1.41±0.06^{c} **(+69.50)**
P. chlamydosporia +neem cake	62.68±1.71^{cd} **(+59.32)**	16.35±0.38^{ab} **(+55.05)**	33.15±0.83^{d} **(+41.03)**	10.03±0.57^{ab} **(+32.20)**	1.42±0.15^{c} **(+69.72)**
T. viride + *P. chlamydosporia* +Neem cake	82.13±1.39^{a} **(+68.95)**	17.25±0.23^{a} **(+57.39)**	50.86±1.81^{a} **(+61.56)**	12.33±0.21^{a} **(+44.85)**	2.20±0.11^{a} **(+80.45)**
CD at 0.05	4.98	0.92	3.20	2.42	0.22
SEm	1.68	0.31	1.08	0.82	0.07

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.

+ neem cake followed by *T. viride* + *P. chlamydosporia* (74%).

Viable formulation of *T. viride* and *P. chlamydosporia* significantly suppressed number of *M. incognita* in soil as well as on roots of chili roots. Additionally neem cake increased the efficiency of both the biocontrol agents. This might have partly due to the reduction in nematode population by the action of toxic and egg parasitic action of fungal bioagents and partly to the fact that neem cake additive also served as manure. The ability of *T. viride* and *P. chlamydosporia* to manage the root-knot disease seems to be increased in integration with neem cake. The integration of all the components caused minimum reproductive index (0.25) as compared to control which have greater reproductive index (1.7) as presented in Table 2. Verma *et al*. (2005) also recorded that *P.lilacinus* + *T. harzianum* + neem cake significantly reduced the nematode population resulted in greatest shoot length in pointed gourd. It is also notable in the present investigation that re-isolation of bioagents at the time of harvesting from treated soil and roots proved that both the bioagents established better in neem cake amended plots. The colony forming units were much more in the treatment where neem cake was applied as compared to the treatments where neem cake was not applied with biocontrol agents. This was therefore an indication that the applied agents were proliferating well in the rhizosphere of chili plants in presence of neem cake. No tested fungus was found in the control treatment. Similar results were recorded by Nagesh and Janakiram (2004) who recorded that *P. chlamydosporia* established better in neem cake amended beds.

Chemical nematicides are overpriced, unfriendly and responsible for causing ground water contamination, it is high time to amend the nematode management options like biocontrol agents and organic amendment which are ecofriendly and cost effective. Past and present studies indicate that combinations of biocontrol agents having different mode of action may have a future for management of plant parasitic nematodes. The talc formulations of *T. viride* and *P. chlamydosporium* in addition of neem cake can be effectively used to control root-knot disease of chili caused by *M. incognita*. Additional studies should be continued to elucidate details of possible mechanism(s) and commercialization under field conditions. Accordingly this package of practice could be safety and eco-friendly alternative used in controlling root-knot disease of chili caused by *M. incognita*.

सारांश

2008–2009 के दौरान प्रक्षेत्र सर्वेक्षण भारतीय सब्जी अनुसंधान संस्थान, प्रक्षेत्र पर गोलकृम पर्याक्रमण अवलोकन आयोजित किया गया। और पाया गया कि भिण्डी फसल जड़ गाँठ गोलकृमि, मिलाडोगाइनी इनकोगिनिटा (5J_2/ भूमि) के साथ अधिकतम् सवंमित था। मिलाइडोमाइनी इनकोगिनिटा के साथ ग्रीष्म कालीन जुताई की एकीकरण जैविक नियन्त्रण कर्ता और कार्वनिक सुधार (नीम खली) के कारण मिर्च के जड़ गाँठ रोग के प्रबन्धन के लिए प्रक्षेत्र दशा में

Table 2: Effect of integration of biological agents and neem cake on multiplication and development of root-knot nematode, *Meloidogyne incognita* infesting chili

Treatment	Number of galls/ root system±S.E	Number of egg mass/rott system±S.E	Number of eggs/egg mass	Soil population /200 ml soil	$R=P^f/P^i$
Control untreated	62.75±2.78^{a}	41.00±4.34^{a}	237±22.32^{a}	882.75±42.02^{a}	1.70^{a}
Trichoderma viride alone	43.30±4.35^{b}	26.75±2.06^{bcde}	182.50±9.37^{b}	522.50±17.50^{bc}	1.00^{b}
	(-31.00)	**(-22.71)**	**(-23.00)**	**(-40.81)**	
Pochonia chlamydosporia alone	49.50±2.50^{c}	23.00±2.08^{cdef}	89.75±7.26^{d}	528.00±20.99^{bc}	1.02^{b}
	(-21.12)	**(-28.69)**	**(-62.13)**	**(-40.19)**	
T. viride+ P. chlamydosporia	33.25±2.87^{bc}	19.00±2.08^{ef}	70.75±3.35^{d}	407.50±26.89^{d}	0.78^{d}
	(-47.01)	**(-35.06)**	**(-70.15)**	**(-53.84)**	
Neem cake alone	40.25±3.97^{c}	31.00±4.20^{b}	198.00±8.62^{b}	494.50±44.62^{c}	0.95^{c}
	(-35.86)	**(-15.94)**	**(-16.46)**	**(-43.98)**	
T. viride + neem cake	31.00±1.68^{d}	30.50±3.30^{bc}	155.50±6.65^{c}	320.00±25.17^{d}	0.62^{f}
	(-50.60)	**(-16.73)**	**(-34.39)**	**(-63.75)**	
P. chlamydosporia +neem cake	32.00±2.83^{d}	28.00±4.02^{bcd}	77.50±4.57^{d}	370.50±21.28^{d}	0.71^{e}
	(-49.00)	**(-20.72)**	**(-67.30)**	**(-58.03)**	
T. viride + *P. chlamydosporia* + Neem cake	26.50±2.72^{e}	12.25±1.32^{f}	54.25±7.71^{e}	131.00±20.09^{e}	0.25^{g}
	(-57.77)	**(-45.82)**	**(-77.11)**	**(-85.16)**	
CD at 0.05	6.72	7.82	27.23	49.22	0.21
SEm	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.

एक प्रयोग इस तथ्य के लिए रखा गया। तीन ग्रीष्म कालीन तुताई (मई से जून) दो सप्ताह के अन्तराल के आधार पर मिलाइटोगाइनी इनकोगिनिटा जनसंख्या घनत्व में 48 प्रतिशत की कमी और 2ण्4 / ग्राम मिट्टी के प्रारम्भिक इनाकुलम स्तर झुक गया। सबसे उत्तम मिर्च के जड़गाँठ के संरक्षण पोचोनिया चेलासाइडोस्पोरिया और ट्राइकोडरनामा विरिडी जैसे नीम खली के मिश्रिणद्वारा पाया गया कि नियन्त्रण के अपेक्षा पौध में वृद्धि ($P<0.05$), जैसे भार और फल उपज (69 प्रतिशत, 61 प्रतिशत और 80 प्रतिशत क्रमशः) सार्थक साख्यिकी पाया गया। मिलाइडोगाइनी इनकोगिनिटा में सार्थक ह्रास बहुलीकरण एवं विकास जड़ कष्ट, अण्ड समूह, और मिट्टी जनसंख्या (58 प्रतिशत, 46 प्रतिशत और 85 प्रतिशत क्रमशः) पंक्ति में भी पाया गया जहा नियन्त्रण की अपेक्षा सभी तीनो में प्रयोग किया। दोनों फफूंद जैव नियन्त्रण कर्ता प्रयोग की अवधि के पहले मिर्च पौधे के साइजोस्पियर में सफल रूप से स्थापित हो गया। यह सम्पन्न कर सकते है कि ग्रीष्म जुताई की संप्रयोग की फफूंद जैव नियन्त्रण कर्ता नीम खली के मिश्रित प्रयोग अनुकरण करता है, यह के पौध स्वास्थ्य और उपज को ही नहीं बढ़ाता बल्कि मिर्च किस्म पौध सदाबहार पर गोल कृमि बहुलीकरण और विकास का ह्रास करता है।

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