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Biocontrol of *Meloidogyne incognita* in *Lycopersicon esculentum* with AM Fungi and Oil Cakes

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Abstract: Currently chemical pesticides are the major means to control nematode-induced diseases but they are neither economical nor ecofriendly. Alternative methods are being sought to reduce the amount of chemical pesticides required. Arbuscular mycorrhizal fungi have been reported to reduce the infestation of root-knot nematodes on vegetable crops. Amendment of soil with some oil cakes has been found effective. A study was conducted on exploitation of the combined effect of AM with three different oil cakes *Azadirachta indica*, *Brassica campestris* and *Ricinus communis* oil cakes in controlling the root knot nematode *Meloidogyne incognita*. Combined use of AMF and cakes resulted in reducing the galling and nematode multiplication thus improving the plant growth and yield. The best results pertaining to AM root infection, nematode reproduction and plant growth and yield were obtained with the combination of AMF and *R. communis* oil cake.

Key words: AM fungi, biocontrol, biopesticides, *Meloidogyne incognita*, oil cakes, root-knot nematode, tomato

INTRODUCTION

Root knot nematode *Meloidogyne incognita* is one of the important constraints in the successful cultivation of the tomato which is highly susceptible to *M. incognita* (Kalloo *et al.*, 2001). The yield loss due to *M. incognita* has been reported to be 28.0-47.0% in tomato (Patel *et al.*, 1999). Currently chemical pesticides are the major means to control nematodes. But the excessive and injudicious use of chemical pesticides has resulted in several ill effects such as health hazards, ecological imbalances, development of resistance in pests, resurgence of pests, emergence of newer pests and environmental pollution. All these factors led to search for safer and more compatible alternatives among which natural products are of first importance.

Arbuscular Mycorrhizal Fungi (AMF) provide nutrition to the plant by sequestering the nutrients and water from the soil and translocating them to the plant and in return get carbon from the plants. They have also been reported to reduce the infestation of root-knot nematodes on vegetable crops (Hussey and Roncadori, 1982; Vaast, 1997). Organic matter plays pivotal role that affects the crop growth and yield either directly by supplying nutrients or indirectly by modifying soil physical properties that can improve the root environment and

stimulate plant growth (Bridge, 1996). Also plant parasitic nematodes are effectively controlled by the application of soil organic amendments in various parts of the world (Khan, 1976; Muller and Gouch, 1982; Rodriguez-Kabana and Morgan-Jones, 1987). Organic matter in soil appears to be an important factor for the development of arbuscular mycorrhizae (Joner and Jakobsen, 1992) and seems to lead to better mycorrhizal development (Hayman, 1982) thus reducing disease incidence (Baby and Manibhushanrao, 1996). Addition of nitrogenous organic manure to soil has often been reported to reduce population densities of plant parasitic nematodes. The decomposition of nitrogenous organic materials by microorganisms result in increased enzymatic activity of amended soil and accumulation of specific end products like ammonia which have nematicidal properties (Rodriguez-Kabana, 1986).

Therefore, a study was conducted to evaluate potential of AMF along with oil cakes in reducing the *M. incognita* infestation in tomato (*Lycopersicon esculentum* var. Pusa Ruby).

MATERIALS AND METHODS

To study the efficacy of mycorrhiza along with selected oil-cakes as biocontrol agent for root-knot

nematode *M. incognita* in tomato (*Lycopersicon esculentum* var. Pusa Ruby) a study was conducted in June 2003 at Indian Institute of Technology (IIT), Delhi, India.

Procurement and Multiplication of culture of root knot nematode: The pure culture of *M. incognita* race one was procured from Nematology Division, Indian Agricultural Research Institute, New Delhi. The culture was multiplied and maintained on *Solanum melongena* var. Pusa Purple Long plants grown in IIT, Delhi.

Inocula production of AM fungi: The AM fungal spores were isolated from the rhizosphere of maize, *Zea mays* by sieving and decanting technique (Gerdemann and Nicolson, 1963) and then surface-sterilized the spores (Mosse and Phillips, 1971). The surface sterilized spores were then used to infect seedlings of castor, *Ricinus communis*, grown in soil sterilized by formalin (0.1%). Well infected (90-100%) roots, together with adhering soil, were chopped and used as starter inoculum to scale up the production of inoculum in bulk by infecting fresh seedlings of castor grown in sterilized soil.

Soil analysis: The soil which was used in the experiment was analyzed for its physico-chemical properties i.e., pH (1:2 soil: water ratio) by pH meter, Electrical Conductivity (EC) (1:2 soil: water ratio) by EC meter (Singh *et al.*, 1999a), organic carbon (C) by Walkley and Black method (Singh *et al.*, 1999a), organic nitrogen (N) by Kjeldahl method (Rowell, 1994a), available (extractable) phosphorus (P) by spectrophotometer (Rowell, 1994b) and available (exchangeable) potassium (K) by flame photometer (Singh *et al.*, 1999a).

Filling of pots: Three parts field soil and one part Farmyard Manure (FYM) thoroughly mixed was filled in earthen pots (22.9 cm height with 21.6 cm mouth diameter) at the rate of 4.5 kg/pot on wet basis for experimentation.

Cultivation of tomato and Inoculation of AM fungi: The seeds of tomato (*L. esculentum* var. Pusa Ruby) were sown in nursery at IIT Delhi. Three-week-old seedlings were uprooted gently and transplanted in earthen pots (1 seedling/pot). Hundred gram root based soil inoculum with approximately 200 spores/100 g soil, infected root bits and mycelial fragments of the AM fungi were placed as a thin layer of about 4 cm below the surface of the soil in the pots before transplantation of seedlings. The control set was left without AM inoculum.

Selection and collection of oil cakes: Three oil cakes selected on the basis of their potential as biopesticides as well as on the local availability were *Azadirachta indica*

(neem) oil cake, *Brassica campestris* (mustard) and *R. communis* (castor) oil cake. The oil cakes of *A. indica* and *B. campestris* were collected from a horticultural shop near IIT, Delhi. The *R. communis* cake was prepared in the laboratory by using castor seeds. The castor oil was extracted from the seed kernal using Soxhlet apparatus and hexane as the solvent.

Addition of oil cakes: Twenty five gram each of *A. indica*, *B. campestris* and *R. communis* oil cake were used. All these cakes were added to the pots 1 week before transplantation of seedlings. One set (control set) without any mycorrhiza and cakes was also set up simultaneously. Each treatment was replicated five times. The following five combinations were tested for nematode control:

- T₁: Control i.e., without Mycorrhiza inoculation (M) and only Nematode (N)
- T₂: Mycorrhiza + Nematode (M+N)
- T₃: Mycorhhiza + *Azadirachta indica* + Nematode (M+Ac+N)
- T₄: Mycorhhiza + Brassica campestris + Nematode (M+Bc+N)
- T₅: Mycorhhiza + *Ricinus communis* + Nematode (M+Rc+N)

Infestation of root-knot nematodes in tomato: After 1 week of transplanting of seedlings, roots of *S. melongena* plants maintained in culture plot were uprooted and washed gently under running tap water. Fresh and uniform egg masses of *M. incognita* were detached from the roots gently with the help of a pair of forceps and placed in a layer of thin water in petri plate. Three egg masses (each containing about 300 eggs) of *M. incognita* were inoculated into holes made with the help of needle around the roots of seedlings in each pot. The holes were immediately plugged with the soil and pots were watered gently.

The study was continued for 4 months. After 4 months the data related to plant growth parameters i.e., fresh and dry shoot and root weight, shoot and root length, fruit yield and total biomass yields were recorded. Dry shoot and root weights were taken after oven drying (70°C until the stable weight was achieved). Nematode population/100 g soil was estimated by sieving and decanting technique (Cobb, 1918). Number of galls/root system and egg masses/root system were determined. The number of eggs and larvae per egg mass were counted after treating the egg masses with 5.25% NaOCl and staining with acid fuchsin. Final nematode population was calculated by adding root population and soil population. Nematode multiplication rate was calculated by dividing nematode population by initial nematode population. Mycorrhizal Colonization Percentage (MCP)

was determined as per the procedures outlined by Philips and Hayman (1970). The number of spores/100 g soil was estimated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Mycorrhizal Inoculation Effect (MIE) was calculated by the formula given by Bagyaraj et al. (1988). The post experimental chemical analysis (pH, EC, C, N, P and K) of the soil used in all the treatments was also conducted by the standard methods. The plant samples of all the treatments were estimated for major nutrients content after drying completely at 70°C. C and N content were determined by the dichromate and Kjeldahl methods (Rowell, 1994b), respectively. P estimation was done by spectrophotometer (Singh et al., 1999b) and K by flame photometer (Singh et al., 1999b) after the digestion of plant samples in a tri-acid mixture (HNO₃, H₂SO₄, HClO₄ in 10:1:4 v/v). The data were analyzed statistically by one-way analysis of variance (ANOVA) and critical difference (CD) was calculated using MSTATE software programme.

RESULTS

The mycorrhizal response in *L. esculentum* var. Pusa Ruby infested with *M. incognita*, inoculated with AM fungi and oil cakes (Table 1) showed that mycorrhizal colonization was increased significantly by the addition of oil cakes. Amongst the treatments of AM fungi with three oil cakes viz., cakes of *A. indica*, *B. campestris* and *R. communis*, the highest root colonization percentage (76.7%) was recorded with M+Rc+N (T₅). It was followed by T₄-M+Bc+N (75.3%), T₃-M+Ac+N (71.3%), T₂-M+N (65.3%) and T₁-N (30.0%). Number of spores and mycorrhizal inoculation effect also followed the same trend.

The results of effect of AM fungi along with different oil-cakes on nematode infection in *L. esculentum* var. Pusa Ruby infested with *M. incognita* (Table 2) showed that among all the cakes used in the present experiment, *R. communis* oil cake was highly effective as plants amended with the same supported lowest number of galls (30.8) and egg masses/plant (40.5) as compared to other

Table 1: Mycorrhizal response in *L. esculentum* var. Pusa Ruby infested with *M. incognita*, inoculated with AM fungi and oil cakes

	Mycorrhizal response				
Treatment	No. of spores/100 g of soil	% infection in roots	Mycorrhizal inoculation effect		
$\overline{T_1}$	41.8±1.74	30.0±1.63	-		
T_2	79.5±3.05	65.3±2.63	31.5±1.29		
T_3	103.0±4.04	71.3±2.49	59.7±2.51		
T_4	118.2±4.71	75.3 ± 2.67	69.1±2.61		
T_5	121.2±3.98	76.7 ± 3.40	73.3±2.82		
CD at 5%	5.31	4.82	2.67		

T₁- Control i.e., without arbuscular mycorrhizal fungi (M) inoculation and only nematode Meloidogyne incognita (N),

oil cakes and control set. It was followed by M+Bc+N with 34.7 and M+Ac+N with 66.5 number of galls. The reduction in the infection by nematodes was clearly observed with the ultimate reduction in the nematode multiplication rate (Table 2). The lowest multiplication rate (12.0) was recorded with T₅ followed by T₄, T₃, T₂ and T₁.

Addition of oil cakes increased nutrient level in plants infested with nematodes significantly as compared to only nematode infected (Table 3). The best results were obtained with *R. communis* cake wherein maximum level of nutrient content was observed. The C, N, P and K contents in M+Rc+N (T₅) were increased upto 41.94%, 3.16%, 0.30% and 3.60% from 39.99%, 2.18%, 0.19% and 2.53%, respectively in control (only N) i.e. T₁. A direct relationship between increased mycorrhizal colonization (Table 1), reduced nematode infection (Table 2) and better nutrition uptake (Table 3) was observed.

The data pertaining to the effect of AM fungi along with oil cakes on the chemical characteristics of the rhizospheric soil after cultivation of L. esculentum var. Pusa Ruby and infested with M. incognita are presented in Table 4. A slight decrease in pH was observed in all the treatments. An increase in available C was observed in all the treatments. It was observed that the availability of the nutrient contents in the amended soil was more than that of the T_1 treatments (only nematode). The cake of R. communis was found with maximum amount of available nutrients in rhizospheric soil of L. esculentum (Table 4).

The results pertaining to synergistic effect of AM fungi with oil cakes on plant growth parameters and yield of *L. esculentum* var. Pusa Ruby infested with *M. incognita* are presented in Table 5. All cakes increased growth and yield of plants significantly. The increase in shoot weight due to AM was significantly higher when compared to plants inoculated with *M. incognita* alone. The best results were obtained with M+Rc+N (T₅) in terms of all the growth parameters. The maximum fruit yield/plant (1805.1 g) in T₅ was followed by T₄ (1738.4 g), T₃ (1338.3 g), T₂ (821.4 g) and T₁ (629.0 g). Similar trend was observed with the other parameters. The efficacy of oil cakes with AM fungi was found in the order of *R. communis* > *B. campestris* > *A. indica*.

DISCUSSION

The present study indicated the enhanced performance of AM fungi in plants infested with M. incognita, in the presence of oil cakes. The direct effect of mycorrhizal response on nematode infection was observed as T_5 treatment had the maximum root colonization percentage with highest number of spores/100 g soil (Table 1) and least number of galls (30.8) (Table 2). This observation was consistent with other treatments also. Present results pertaining to effect of

T₂- M+N, T₃- Azadirachta indica cake+M+N,

T₄- Brassica campestris cake+M+N,

T₅- Ricinus communis cake+M+N

Table 2: Nematode (M. incognita) reproduction in L. esculentum var. Pusa Ruby treated with AM fungi and oil cakes

Nematode reproduction

Treatment	No. of galls/plant	Egg masses/plant	Eggs and larvae/egg mass	Soil population/ 100 g	Final population	Nematode multiplication rate
T_1	132.1 ± 5.2	211.2 ± 8.7	293.0±13.9	569.6±27.0	62451.2±2956.9	69.4±4.0
T_2	121.4 ± 6.5	169.4±10.3	290.7±15.7	560.0 ± 35.1	49804.6±2071.0	55.3±2.2
T_3	66.5±2.6	92.2 ± 3.7	272.5±12.9	480.9±22.8	25605.4±1212.3	28.3±1.6
T_4	34.7 ± 1.9	46.6±2.8	258.8±14.0	425.1±26.6	12485.2±519.1	13.9±0.6
T_5	30.8 ± 1.2	40.5±1.6	256.5±12.2	416.6±19.7	10804.9±511.6	12.0±0.7
CD at 5%	6.1	9.5	20.7	40.5	2657.9	3.3

T₁- Control i.e., without arbuscular mycorrhizal fungi (M) inoculation and only nematode Meloidogyne incognita (N), T₂- M+N,

T₃- Azadirachta indica cake+M+N, T₄- Brassica campestris cake+M+N, T₅- Ricinus communis cake+M+N

Table 3: Nutrient content of L. esculentum var. Pusa Ruby infested with M. incognita, treated with AMF and oil cakes

Treatment	C (%)	N (%)	P (%)	K (%)
T ₁	39.99±1.32	2.18±0.077	0.19±0.008	2.53±0.100
T_2	40.31±1.09	2.35±0.077	0.21±0.007	2.71±0.087
T_3	41.16±1.36	3.00±0.106	0.26±0.010	3.18 ± 0.126
T_4	41.83±1.13	3.11 ± 0.103	0.29±0.009	3.54 ± 0.113
T_5	41.94±1.38	3.16±0.111	0.30±0.012	3.60±0.142
CD at 5%	1.99	1.151	0.015	0.182

T₁- Control i.e., without arbuscular mycorrhizal fungi (M) inoculation and only nematode Meloidogyne incognita (N), T₂- M+N,

cakes in increasing AM spore population and mycorrhizal colonization are also supported by St. John *et al.* (1983) and Sankaranarayanan and Sundarababu (1997). The addition of oil cakes might have had some influence on the physiology of the plants and thus some alterations in the nature of root exudates which in turn must have influenced mycorrhizal colonization. Organic matter influences soil structure, pH, nutrient and water holding capacity all of which alone or in combination influence mycorrhizal colonization and efficiency (Srivastava *et al.*, 1996).

The use of AM in combination with oil cakes in transplantable crops was found to be highly beneficial in terms of reduced nematode infection and increased yields (Sankaranarayanan and Sundarababu, 1997; Rao et al., 1995; Rao et al., 1997; Parvatha Reddy et al., 1997). It was found that desirable rhizospheric changes, by addition of castor cake to the soil, facilitated effective utilization of G. fasciculatum for the management of M. incognita in tomato (Rao et al., 1997a). Nagesh et al. (1999) observed that mycorrhiza in combination with neem cake recorded higher plant growth parameters compared to carbofuran-treated plants indicating that the application of these combinations was superior to that of carbofuran. It has been noted that the organic amendments tend to alter the host-parasite relationships in favour of the crop (Jothi et al., 2003). Nematicidal effect of oil cakes observed in the present study confers to the earlier studies (Akhtar and Alam, 1991; Khan and Saxena, 1997; Patel et al., 1998). The glucosinolates present in significant quantity in the tissues, on hydrolysis in soil release compounds like thiocyanates, isothiocyanates, nitrites or oxazoli denethiones which are highly biocidal to wide range of organisms including nematodes,

bacteria, fungi and insects (Brown and Morra, 1997; Rosa et al., 1997). Addition of organic substrates stimulates the activity of predaceous fungi and suppresses the activity of plant parasitic nematodes (Gray, 1987). Ammonia, nitrites, hydrogen sulphide, organic acids and other chemicals that are produced form organic matter may be directly nematicidal or affect egg-hatch or the mobility of juveniles (Badra and Eligindi, 1979; Rodriguez-Kabana, 1986). There is a direct relation between the amount of nitrogen in organic amendments and their effectiveness as nematode population suppressors (Mian and Rodriguez-Kabana, 1982). Population of Catenaria anguillulae (an endoparasite of nematodes) in response to soil amendments with oil cakes (mustard, linseed, sesamum, neem and mahua) around the root region of citrus mango and chilli was proliferated (Singh et al., 2002). The magnitude of microbial stimulation and the qualitative nature of the responding microflora and fuana depend on the nature of the organic matter added. Since organic amendments take a long time to decompose, the nematicidal properties also persist for a long period, sometimes more than six months (Alam et al., 1977). Significant reduction in the number of galls and egg masses/root system in the plants amended with AMF and oil cakes, as compared to that of plants without amendments, could be attributed to the low C:N ratio of these cakes (Sarwar and Kikegaard, 1998). Lear (1959) also reported castor cake to be effective in nematode control. The ricin, a known nematotoxic compound, might have reduced the nematode population.

The increase in N content in plants might be due to the presence of high N content in the oil cakes. Our results of increased content of nutrients in plants by AM fungi have been supported by Krishna and Bagyaraj,

 T_3 - Azadirachta indica cake+M+N, T_4 - Brassica campestris cake+M+N, T_5 - Ricinus communis cake+M+N

Table 4: Interaction effect of oil cakes and AM fungi on the chemical characteristics of the rhizosphere soil of L. esculentum var. Pusa Ruby

Treatment	pН	EC (mmho cm ⁻¹)	C (%)	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)
T_1	8.95±0.354	0.384 ± 0.015	0.54 ± 0.021	400.5±13.33	5.43±0.257	227.3±10.72
T_2	8.90±0.419	0.392 ± 0.018	0.56 ± 0.026	402.1 ± 18.95	5.62 ± 0.265	228.0 ± 8.98
T_3	8.83±0.349	0.416±0.016	0.61 ± 0.024	406.6±13.53	6.23 ± 0.295	228.5±10.77
T_4	8.75±0.412	0.440 ± 0.021	0.64 ± 0.030	407.2±19.19	6.68 ± 0.315	230.3 ± 9.07
T_5	8.74±0.346	0.444 ± 0.018	0.65 ± 0.026	407.5±13.56	6.80 ± 0.322	230.6±10.87
CD at 5%	0.595	0.028	0.041	25.13	0.460	15.94

T₁- Control i.e., without arbuscular my corrhizal fungi (M) inoculation and only nematode Meloidogyne incognita (N), T₂-M+N,

Table 5: Combined effect of AM fungi and oil cakes on growth and yield of L. esculentum var. Pusa Ruby infested with M incognita

	Plant growth parameters							
	Shoot			Root				
Treatment	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)	Fruit yield/plant (g)	Total biomass yield/plant (g)
$\overline{T_1}$	44.1±1.640	29.8±0.992	4.0±0.132	34.2±1.127	6.4±0.253	1.0±0.040	629.0±24.89	665.2±21.93
T_2	51.6±1.832	45.3±1.84	6.1±0.240	43.1±1.932	7.7 ± 0.261	1.2 ± 0.041	821.4±27.10	874.4±35.55
T_3	59.2±1.998	71.0±2.363	10.5±0.347	50.3±1.658	11.5±0.455	1.9 ± 0.075	1338.3±52.96	1420.8±46.84
T_4	67.4±2.222	97.4±3.971	13.9±0.547	61.9±2.775	14.7 ± 0.499	2.3 ± 0.078	1738.4±57.34	1850.5±75.23
T_5	73.4±2.656	114.9±3.824	16.3±0.538	67.4±2.222	15.2 ± 0.601	2.4 ± 0.095	1805.1±71.43	1935.2±63.79
CD at 5%	3.981	4.476	0.624	3.181	0.687	0.109	78.92	82.37
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T₁- Control i.e., without arbuscular my corrhizal fungi (M) inoculation and only nematode Meloidogyne incognita (N), T₂- M+N,

(1984), Manjunath and Habte, (1988), Kucey and Janzen (1987). Mycorrhizal fungi are reported to contain enzymes, which break down the organic N and contain N reductase for altering the forms of N in soil. Tilak and Dwivedi (1990) found that all the arbuscular mycorrhizal spores exhibited the property of nitrate reducing ability, which varied from 1.5 μmoles to 3.8 μmoles/tube 24 h. With a capacity for reducing nitrate it is likely that the symbiotic effectiveness of the arbuscular mycorrhizal fungi is enhanced in terms of N assimilation and translocation to the host plant. Also hyphae may increase the availability of nutrients like N or P from locked sources by decomposing large organic molecules (George *et al.*, 1995).

The post experiment soil analysis revealed a slight increase in pH, which might have been due to production of organic acids by AM fungi (Graham *et al.*, 1981). The increased C content may be due to the improved root system along with aerial part as compared to non-mycorrhizal one. The presence of mycorrhizal hyphae in soils adds carbon to the system and also affects the decomposition of organic matter (Raman and Mahadevan, 1996).

Thus a remarkable increase in plant growth and yield (Table 5) was achieved with a concomitant increase in colonization by AM fungi (Table 1), reduction in the nematode population (Table 2) and better uptake of nutrients (Table 3).

Oil cakes and AM fungi in present study had synergistic interaction with each other in controlling the nematodes with consequential improvement on growth.

Dual inoculation of AM fungi and oil cakes should be integrated in the management strategy of root-knot nematode *M. incognita*. This would reduce the dependence on synthetic pesticides used for nematode control and thus provides an alternative environmentally safe and economical method for control of nematodes.

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