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INTEGRATED MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON TOMATO

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Summary. *Paecilomyces lilacinus*, *Bacillus subtilis* and green manuring with *Eicchornia crassipes* were used alone and in combination for the management of *Meloidogyne incognita* on tomato. Treatment of *P. lilacinus* to *M. incognita* inoculated plants caused greater plant growth than *B. subtilis* or *E. crassipes*. Use of *B. subtilis* plus *P. lilacinus* caused a similar increase in plant growth of *M. incognita* inoculated plants as treatment of *P. lilacinus* plus *E. crassipes*. The use of both biocontrol agents along with a green manure of *E. crassipes* resulted in greatest plant growth of *M. incognita* inoculated plants. Treatment of *B. subtilis* plus *E. crassipes* resulted in less growth of *M. incognita* inoculated plants compared to any other combined treatment of management agents.

An attempt was made to use the fungus *Paecilomyces lilacinus* (Thom.) Samson, the bacterium *Bacillus subtilis* Cohn emend. Prazmowski and *Eicchornia crassipes* Solms as green manure for the management of the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. on tomato (*Lycopersicon esculentum* Mill.).

Materials and methods

Twenty grams of fresh chopped leaves of *E. crassipes* were incorporated into 1 kg steam-sterilized soil contained in 15 cm diameter earthen pots. Pots were watered daily and after one month one week old seedlings of tomato cv. Pusa ruby were transplanted. To obtain one week old seedlings, seeds of tomato were surface sterilized with 0.1% mercuric chloride for two minutes, washed three times in sterilized water and then sown in 25 cm diameter pots.

Paecilomyces lilacinus was cultured in Richards liquid medium (Riker and Riker, 1936) for 15 days at 25 °C. The mycelium in 10 g quantities was macerated in 100 ml distilled water and 10 ml suspension was inoculated at the base of the one week old seedlings to provide 1 g mycelium per pot. *Bacillus subtilis* was cultured on nutrient agar medium (Riker and Riker, 1936). Plates were incubated at 37 °C for 24 hrs and the bacteria were scraped from the plates and suspension prepared to contain $10x10^8$ bacterial cells/ml as determined by serial dilution plating procedure (Cappucinno and Sherman, 1983). Ten ml of this suspension was poured around the roots of each plant.

M. incognita was collected from a tomato field and multiplied on egg plant (*Solanum melongena* L.) from a single egg mass. For the inoculum, egg-masses were hand picked with sterilized forceps and placed on layers of tissue paper in 8 cm diameter sieves immersed in water. A suspension of 2000 freshly hatched second stage juveniles was inoculated in the root zone of one week old seedlings.

Each treatment (Table I) was replicated five

TABLE I - *Effects of treatments on tomatoes in soil infested with* Meloidogyne incognita.

Treatment	Plant height (cm)	Plant fresh wt. (g)	Shoot dry wt. (g)	No. of galls per root system	Nematode population in root and soil		Percent Reisolation of *P. lilacinus* from		Percent Reisolation of *B. subtilis* from	
					Females per root system	Eggs+Juveniles per Kg soil plus per root system	Females	Eggs	Females	Eggs
Control	54.6	15.3	2.76	–	–	–	–	–	–	–
B. subtilis (BS)	56.7	16.7	2.94	–	–	–	–	–	–	–
P. lilacinus (PL)	55.1	15.9	2.81	–	–	–	–	–	–	–
E. crassipes (EC)	59.7	17.8	3.02	–	–	–	–	–	–	–
Nematode alone (N)	28.2	6.7	1.17	196	289	11160	–	–	–	–
N+BS	42.8	10.2	1.93	112	180	7940	–	–	4	3
N+PL	46.5	12.4	2.24	84	141	6720	41	73	–	–
N+EC	43.5	11.0	2.01	130	207	8760	–	–	–	–
N+BS+PL	50.8	13.9	2.58	32	62	3940	38	69	3	5
N+BS+EC	45.7	11.9	2.22	68	88	4920	–	–	5	3
N+PL+EC	48.2	13.1	2.44	51	69	4560	45	63	–	–
N+BS+PL+EC	53.8	15.4	2.80	19	27	1980	39	74	2	4
C.D. P≤0.05	2.4	1.3	0.21	8	15	142	4	9	4	3

times and plants were watered periodically as required. Sixty days after inoculation data on plant height, plant fresh weight, shoot dry weight, number of galls per root system and nematode density were recorded. Nematodes in the soil were extracted by Cobb's sieving and decanting technique followed by Baermann funnel. Number of juveniles, eggs and females were estimated in the roots stained with cotton blue by taking a 1 g root sample from a homogenous mixture and macerated for 45 seconds in a Waring blender. Nematode density in the roots was calculated by multiplying the number of nematodes in 1 g root with total weight of root.

Bacillus subtilis and *P. lilacinus* were re-isolated from eggs and females of *M. incognita* to determine the infection of these biocontrol agents on nematode populations. For re-isolation of bacterium and fungus 20 eggs and similar no. of females were collected from treatments in which they were inoculated and were surface sterilized with 0.1% mercuric chloride for 2 minutes, washed three times in sterilized water and placed in nutrient agar and potato dextrose agar medium for bacterial and fungal growth respectively. The plates were incubated as described earlier. Any bacteria and fungi if found were identified. Data obtained were analysed statistically and critical differences were calculated at P≤0.05.

Results

Green manuring of *E. crassipes* against uninoculated plants resulted in a significant increase in plant growth (plant height, plant fresh weight and shoot dry weight) compared to uninoculated plants (Table I). Treatment of *P. lilacinus* to *M. incognita* inoculated plants induced greater plant growth than *B. subtilis* or *E. crassipes*. In

combined treatments, use of *B. subtilis* plus *P. lilacinus* caused similar increase in plant growth of *M. incognita* inoculated plants as the treatment of *P. lilacinus* plus *E. crassipes*. The use of both biocontrol agents alongwith green manure of *E. crassipes* resulted in a greatest increase in plant growth of *M. incognita* inoculated plants. Treatment of *B. subtilis* plus *E. crassipes* against *M. incognita* inoculated plants caused lesser increase in plant growth compared to *P. lilacinus* plus *B. subtilis* or *P. lilacinus* plus *E. crassipes* treatment (Table I). In individual treatment, *P. lilacinus* caused higher reduction in nematode multiplication and galling followed by *B. subtilis* and *E. crassipes* (Table I). Reduction in nematode multiplication and galling was higher when *B. subtilis* plus *P. lilacinus* were used compared to treatment of *P. lilacinus* plus *E. crassipes*. Highest reduction in nematode multiplication and galling was observed when both biocontrol agents alongwith green manure of *E. crassipes* were used. Approximately 40% of the females and 70% of the eggs of *M. incognita* were found to be infected with *P. lilacinus* when reisolation of *P. lilacinus* was made from the final nematode population. *P. lilacinus* infected eggs of *M. incognita* more frequently and destroyed the embryo while females were parasitized through anus. When reisolation of *B. subtilis* from females and eggs was made the results were statistically irrilevant (Table I).

Discussion

Paecilomyces lilacinus parasitized eggs and females of *M. incognita*. This parasitism of *P. lilacinus* caused reduction in nematode multiplication resulted in an improved growth of nematode infected plants. The parasitism of *P. lilacinus* was found to begin with the growth of fungus hyphae in the gelatinous matrix.

The use of *B. subtilis* may also improve plant growth by suppressing non-parasitic root pathogen or by the production of biologically active substances or by unavailable mineral and organic compounds in the forms available to plants (Broadbent *et al.*, 1977). Green manuring of *E. crassipes* suppress nematode multiplication by increasing organic matter and natural enemies as well as improving crop tolerance. *E. crassipes* contains high potash and chlorine contents.

Combined use of these two biocontrol agents along with green manuring of *E. crassipes* appears to be an interesting approach for the management of *M. incognita* on tomato. Green manuring with *E. crassipes* provides a substrate for the multiplication of *P. lilacinus* in the rhizosphere as it is reported to multiply on leaf residues (Siddiqui and Mahmood, 1994). Multiplication of *P. lilacinus* on leaf residues in the rhizosphere provides an additional increase in *P. lilacinus* inoculum. Inoculum of *B. subtilis* may also increase as green manuring favours the growth of natural enemies like fungi and bacteria. This integrated approach for the management of *M. incognita* appears promising on tomato.

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