COMPARATIVE EFFICACY OF BIOAGENTS AS SEED TREATMENT FOR MANAGEMENT OF *MELOIDOGYNE INCOGNITA* INFECTING OKRA

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Summary. A pot experiment was undertaken to evaluate different fungal and bacterial antagonists as seed coating treatments against the root-knot nematode, *Meloidogyne incognita*, infecting okra. The seeds of okra cv. A-4 were treated with *Trichoderma harzianum, Trichoderma viride, Pochonia chlamydosporia, Paecilomyces lilacinus* and *Pseudomonas fluorescens* at 20 g/kg seed. Carbosulfan 25 (DS) at 3% (w/w) was used as a treated control. The treated seeds were sown in soil infested with two second stage juveniles of root-knot nematode/g soil. Sixty days after sowing, the growth of okra plants was greater and the root-knot nematode population was reduced in all treatments compared to the untreated control.

Key words: *Abelmoschus esculentus*, biological control, root-knot nematode.

Okra, *Abelmoschus esculentus* (L.) Moench. is an important vegetable crop grown extensively under various agro-climatic zones of India and is adversely affected by the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. Yield losses of up to 90% have been estimated under field conditions, depending upon initial soil nematode population densities (Bhatti and Jain, 1977; Jain and Gupta, 1986). In India, losses in okra due to *M. incognita* have been reported, in monetary terms, as US $ 8.7 million (Jain *et al*., 2007). Application of synthetic nematicides for the control of root-knot nematodes has been found effective and is recommended. The use of bioagents is an economical and eco-friendly management option that can constitute an important component of the integrated management of root-knot nematodes. Hence, research was undertaken to compare and evaluate the bio-efficacy of different bioagents used as seed treatment for the management of an Indian population of *M. incognita* infecting okra.

Five bioagents were tested. They were the four fungi *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers., *Pochonia chlamydosporia* (Goddard) Zare *et* Gams and *Paecilomyces lilacinus* (Thom.) Samson, at spore concentrations of 2×10^6 cfu/g, and the bacterium *Pseudomonas fluorescens* Migula with a spore load 1×10^8 cfu/g. The colony forming units of *P. chlamydosporia* are from chlamydospores and those of the other fungi from conidia. The local isolates (so far with no code number) were obtained from the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, and National Bureau of Agriculturally Important Insects, Bangalore. All the isolates were obtained from the rhizosphere and rhizoplane of vegetable crops. The efficacy of these isolates was compared with that of untreated seeds and seeds treated with carbosulfan 25(DS) at 3.0% a.i. This is the first time these local isolates are tested against *M. incognita* under New Delhi conditions. After proper mixing of the agents with the seeds of okra (cv. A-4), the treated seeds were air dried in the shade before sowing. The seeds, treated with the respective bioagents (at 20 g/kg seed) or the synthetic nematicide, were sown in clay pots (20 cm diameter) containing 2 kg of sandy loam soil having an initial nematode population of two second stage juveniles of *M. incognita* per g of soil. Observations on plant growth (root and shoot length, fresh shoot and root weight and dry shoot weight) and nematode variables (galls/root system, egg masses/root system, eggs/egg mass, soil population/200 cm^3 soil) were recorded 60 days after sowing. During the study, the temperature in the greenhouse ranged between 20 and 30 °C.

To determine the number of eggs per egg mass, ten egg masses were randomly picked and placed in a small glass vial to which fifteen ml of 0.53% sodium hypochlorite were then added and the vials vigorously shaken for 30 sec. The egg suspension was quickly sieved through a 200 mesh sieve nested over a 500 mesh sieve. The eggs retained on the 500 mesh sieve were rinsed with water to remove the sodium hypochlorite and collected in beakers. Then three aliquots, each of 5 ml, of the nematode suspension, were transferred to counting dishes, the nematodes counted twice and the total nematodes in the suspension calculated.

A combination of the modified Cobb's sieving and Baermann's funnel techniques (Christie and Perry, 1951) was used to extract nematodes from the soil. The soil of each pot was thoroughly mixed and a 200 cm^3 sub-sample processed. Individual soil samples were placed in a plastic pan containing water and stirred. The soil suspension was then sieved through a graded series of sieves (20, 60 and 325 mesh). The nematodes re-

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Table I. Effects of five bioagents and carbosulfan on growth (root and shoot lengths, fresh and dry shoot weights, root fresh weight) of okra infected with the root-knot nematode, *Meloidogyne incognita*. (Each figure is the average of four replicates).

Treatment	Root length (cm)	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root fresh weight (g)
Trichoderma harzianum (T_1)	34.5 a	36.6 b	35.2 cd	5.2 b	11.0 a
Trichoderma viride (T_2)	39.5 a	40.4 b	39.1 bc	6.3 ab	8.4 a
Pochonia chlamydosporia (T_3)	36.0 a	37.1 b	34.0 cd	5.1 b	9.9 a
Paecilomyces lilacinus (T_4)	41.0 a	39.6 b	38.7 bc	6.2 ab	8.5 a
Pseudomononas fluorescens (T_5)	36.4 a	37.3 b	34.7 cd	5.0 b	8.7 a
Carbosulfan at 3% w/w (T_6)	42.0 a	46.0 a	42.8 ab	6.8 a	7.1 a
Untreated control (T_7) (Infected)	32.0 a	32.0 c	30.9 d	4.8 b	13.4 a
Non-inoculated control (T_8) (Healthy)	40.1 a	49.0 a	44.3 a	7.0 a	11.5 a
CD (P = 0.05)	NS	4.2	5.1	1.5	NS

Table II. Effects of five bioagents and carbosulfan on galling and reproduction of *M. incognita* infecting okra. (Each figure is the average of four replicates).

Treatment	Galls/root system	Egg masses/root system	Eggs/egg mass	Soil population/200 cm^3
Trichoderma harzianum (T_1)	103.7 b	88.0 b	263.7 a	778.7 a
Trichoderma viride (T_2)	69.5 c	50.7 de	243.2 a	562.5 d
Pochonia chlamydosporia (T_3)	98.2 b	70.5 bc	262.5 a	730.0 bc
Paecilomyces lilacinus (T_4)	71.7 c	48.0 de	243.3 a	560.0 d
Pseudomononas fluorescens (T_5)	93.2 b	66.7 cd	254.8 a	700.0 c
Carbosulfan 3% w/w (T_6)	57.7 c	37.2 e	231.5 a	455.0 e
Untreated control (T_7) (Infected)	188.0 a	141.0 a	274.5 a	1134.3 a
CD (P = 0.05)	15.26	19.24	NS	73.38

tained on the 325 mesh sieve were poured gently over a double layer of tissue paper supported on coarse wire gauze which, in turn, was put in a Petri dish (Schindler, 1961) for extraction of the nematodes. After 24 hours, the water nematode suspension in the Petri dish was drawn out into a beaker and made up to a fixed volume. The suspension was then thoroughly agitated by blowing air through a pipette and the numbers of nematode juveniles were estimated by counting three times those in two ml of the suspension in a counting dish.

Each treatment was replicated four times. All data were subjected to analysis of variance and CD at P = 0.05 calculated.

No significant differences in root length and weight of okra in the different treatments were observed (Table I). However, shoot length in pots with seed treatments (T_1, T_2, T_3, T_4, T_5 and T_6) with all the bioagents was significantly greater than in the untreated control (T_7), with the non-inoculated healthy control (T_8) having the greatest shoot length (49.0 cm) of all. Among the treatments, the greatest shoot length of 46.0 cm was observed with carbosulfan, followed by 40.4 cm with *Trichoderma viride*. The result with carbofuran was similar to that in the non-inoculated and untreated control but significantly greater than that in all other treatments. The bioagents significantly increased shoot length over the untreated inoculated control but the increase was significantly less than that in the carbosulfan and non-inoculated treatments (Table I). A similar, trend was observed with root length although none of the differences were significant. Fresh shoot weights were significantly greater in the treatments with *T. viride*, *Paecilomyces lilacinus* and carbosulfan than that in the untreated control. Dry shoot weights were increased in all treatments but were statistically at par with the untreated control for all treatments except carbosulfan, in which dry shoot weight was significantly greater than in the untreated control.

The numbers of galls/root system 60 days after sowing were significantly less than in the untreated control in all treatments (Table II) and carbosulfan was the best. A similar trend was observed for egg masses/root sys-

tem. The efficacy of carbosulfan was followed by that of *P. lilacinus*, *T. viride*, *P. fluorescens*, *P. chlamydosporia* and then *T. harzianum*. The fewest egg masses/root system (37.2) was obtained by treating the seeds with carbosulfan and the greatest number (141) in the untreated control. The maximum number of eggs/egg mass was observed in the untreated control and the least in pots whose seeds had been treated with carbosulfan. All treatments significantly reduced the soil population density of the nematode, although the reduction with *T. harzianum* was not significant. Seed coatings with *T. viride* and *P. lilacinus* were equally effective in reducing the nematode soil population.

Seed treatment with carbosulfan was the most effective treatment in increasing growth of okra and reducing galling and reproduction of the root-knot nematode. Application of fungal and bacterial antagonists also reduced the damage caused by *M. incognita* and improved the growth of okra in soil infested with the nematode but to a lesser extent and never completely when compared with the non-inoculated control.

Several authors have proved the efficacy of bacterial and fungal bioagents and nematicides used as seed treatment in reducing *M. incognita* populations. Goswami and Mittal (2002) reported greatest egg parasitizing efficiency of *Paecilomyces lilacinus* (80%) as compared to other fungi. Few fungi (toxic) viz. *Aspergillus niger* Van Tieghem, *Aspergillus terreus* Thom., and egg parasitic viz. *Cladosporium oxysporum* Berk *et* Curtis, *P. lilacinus*, etc. have been reported to perform well in reducing the population of *M. incognita* (Mittal, 2006). In several reports, the combination of two fungal bioagents (*Aspergillus* spp., toxic, and *Paecilomyces* spp., egg parasitic) was found more effective than a single bioagent against *M. incognita*, resulting in better plant growth (Verma *et al*., 2009). Both types of fungal bioagents (toxic and egg-parasitic), used in a talc-based formulation, were successful in the management of diseases under field conditions (Goswami and Sharma,1999; Kumar and Jain, 2010). Therefore, this formulation could be proposed as an ideal component of an integrated pest management package (Mittal, 2006; Verma, 2009). However, more research is suggested to confirm the efficacy of the tested bioagents under Indian soil conditions.

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