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Biological Control of *Meloidogyne javanica* (Treub) Chitwood, Root Knot Nematodes of Okra (*Abelmoschus esculentus* (L.) Moench

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Abstract: In a greenhouse study *P. lilacinus*, *T. harzianum* and *T. flavus* were used as seed treatment and soil drench. Seed treatment by *P. lilacinus* reduced gall formation, egg mass production, nematode soil and root densities as compared to control and other treatments. *P. lilacinus* improved plant growth followed by *T. flavus* and *T. harzianum* comparing with control. In another experiment, where soil was treated with conidial suspension, maximum plant height and shoot weight was achieved by *P. lilacinus* > *T. harzianum* compared to control, whereas *T. flavus* was found less effective in the enhancement of plant tops. Maximum suppression in gall formation (at $p < 0.01$) and egg mass production (at $p < 0.001$) was obtained in okra plants treated with *P. lilacinus* whereas *T. flavus* and *T. harzianum* were almost equally effective. Conidial suspension of microbial antagonists used as soil amendment also reduced nematode root invasion (at $p < 0.001$) as well as soil densities.

Key words: *Meloidogyne javanica*, biological control, *Paecilomyces lilacinus*, okra

Introduction

Meloidogyne javanica (Treub) Chitwood is found worldwide in tropical and temperate climates. This root knot nematode cause extensive damages to a wide range of crops, including many vegetables (Sasser, 1989). The yield of okra, tomato and brinjal suffered 90.9, 46.2 and 27.3 % loss, respectively due to *M. incognita* infestation @ 3-4 larvae per gram soil under field conditions in India (Bhatti, 1994). Losses caused by the root-knot nematode on chickpea are 31-37% in Pakistan (Anwar *et al.*, 1995).

However chemicals provide immediate and effective control against soilborne plant diseases. Nematicides, have been too expensive for use in developing countries, where their application has been limited to few cash crops (Hague and Gowen, 1987). Concern over these chemicals has led to an increased interest in biological control in its widest sense, in order to achieve environmentally safe methods of reducing the nematode damage (Davise *et al.*, 1991)

The fungal antagonists of the nematodes consists of a great variety of organisms which include the nematode trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs, parasites of cyst nematodes, and fungi which produced metabolic toxins to nematodes. Biological control has been defined as "any condition under which, or practice whereby, survival or activity of the pathogen is reduced through the agency of any living organism except man himself with the result that there is a reduction in incidence of the disease caused by the pathogen" (Garrett, 1965). The fungus *Paecilomyces lilacinus* (Thorn) Samson has been reported to reduce nematode population densities and is considered as one of the most promising and practicable biological control agent for the management of plant parasitic nematodes (Morgan-Jones *et al.*, 1984; Jatala, 1986). Similarly *Trichoderma harzianum* Rifai and *Talaromyces flavus* have been reported to provide significant control of root-knot nematodes and other soilborne pathogens (Fahima and Henis, 1990; Amer-Zareen and Zaki, 1999). The purpose of this study was to evaluate such fungal strains, which could provide better check against root knot problems of vegetables.

Materials and Methods

Fungal Cultures: Fungal antagonists used in this study viz. *Paecilomyces lilacinus* (Thorn) Samson (KUCC-244), *Trichoderma harzianum* Rifai (KUCC-801) and *Talaromyces flavus* (Klocher) Stock & Samson were obtained from the Karachi University Culture Collection (KUCC). The cultures were grown on PDA supplemented with antibiotics (Penicillin @100,000 units/L and Streptomycin sulphate @ 0.2 g/L). Plates were incubated for 7 days at $30 \pm 2°C$. Fungal suspension was prepared by adding 15 ml of sterilized water in each culture plate, scrapped the surface of culture with sterilized glass rod and conidial suspension was pooled in 200 ml glass beakers separately for further use.

Nematode Culture: Root-knot population for artificial soil infestation was originated from vegetable fields around the Karachi, and identified as *Meloidogyne javanica* with the help of perennial patterns as described by Tayler and Nester (1974). Roots were cut into small pieces and eggs were extracted by the method of Hussey and Barker (1973). Eggs were put in sieves lined with tissue paper in order to get freshly hatched juveniles after 48 hours. Nematode population was maintained on egg-plant cv. Black beauty, in 25-cm diam. clay pots containing steam-sterilized soil.

Exp. 1) Seed Treatment: For seed dressing, conidial suspension was prepared using 2% gum arabic solution as sticker. Ten ml of conidial suspension in gum arabic was used to coat okra seeds. Surface dis-infected seeds of okra were treated with culture of *P. lilacinus*, *T. harzianum* and *T. flavus*. The spore load per seed was determined by preparing serial dilutions using 5 seeds per 9 ml of sterilized water as stock solution (*P. lilacinus* 2.5×10^6, *T. harzianum* 1.6×10^6 and *T. flavus* 2.3×10^6 cfu/ml). Okra seeds (cv. *Pussa sawni*) coated with fungal suspension + sticker, were sown in plastic pots (d. 8 cm) containing 300 cc sandy loam soil @ 10 seeds in each. There were 3 replicates for each treatment. Seeds treated with sticker only served as control.

Exp. 2) Soil Treatment: For cfu/ml of each test, fungal suspension was estimated by serial dilution method (*P. lilacinus* 3.2×10^6, *T. harzianum* 2.8×10^6 and *T. flavus* 3.1×10^6 cfu/ml). Twenty-five ml of conidial suspension (10^6 cfu/ml) of each test fungus was drenched in plastic pot (d. 8 cm) containing 300 cc sandy loam soil in each. Ten okra seeds (cv. *Pussa sawni*) per pot were sown.

As the seedlings emerged, 4 seedlings were maintained per pot. Thinning was followed by root-knot nematode *M. javanica* inoculation @ 2500 J_2 in the holes made around the roots of seedlings. Each treatment was replicated three times. Pots receiving 25 ml sterilized water only, served as control. Plants were up-rooted after 50 days of nematode inoculation. Roots were gently washed in running tap water and gall counting was performed under binocular stereoscope microscope (x 6). Nematode soil population in 200 cc soil/replicate for each treatment was assessed using modified Baermann funnel technique (Schindler, 1961). Nematode root density for each experiment was determined per g root sample per replicate (Franklin and Goody, 1949). Data was statistically analyzed using analysis of variance (ANOVA). Treatment means were compared following Duncan's multiple range test (Gomez & Gomez, 1984).

Results and Discussion

Exp. 1) Seed Treatment

Effect of Fungal antagonists on Plant growth: All the test fungi used as seed treatments significantly (at $p<0.001$) enhanced the tops of okra plants. Maximum plant height was achieved where *P. lilacinus* was used, followed by pots treated with *T. flavus* and *T. harzianum* as compared to control. Significant difference in root length ($p<0.05$) and root weight ($p<0.001$) was observed among the treatments. *P. lilacinus* was found most effective to increase root length of okra plants as compared to control and other treatments (Fig. 1).

Effect of Fungal antagonists on the disease potential of root knot nematode *M. javanica:* *P. lilacinus* used as seed dressing found effective in reducing gall formation, egg mass production, nematode soil and root densities compared to control and other treatments. Significant difference in gall formation (at $p<0.05$) and egg mass production (at $p<0.01$) was observed among the treatments. Test microbial antagonists i.e. *T. harzianum* and *T. flavus* also provided effective check against nematode development after *P. lilacinus* on okra plants compared to untreated control (Fig. 2).

Soil Treatment

Effect of Fungal antagonists on Plant growth: Results presented in Fig. 3 show that set of pots treated with conidial suspension of microbial antagonists i.e. *P. lilacinus*, *T. harzianum* and *T. flavus* influenced plant growth and fresh shoot weight to varying degree. There was a significant difference in plant height (at $p<0.01$) and fresh shoot weight (at $p<0.001$) among the treatments. Maximum plant height and shoot weight was achieved by *P. lilacinus* > *T. harzianum* as compared to control. Whereas *T. flavus* was found less effective to enhance the plant tops compared to other test antagonist treatments. There was no significant difference in root length among the treatments. Maximum root length was observed in set of pots receiving *P. lilacinus*. A significant difference (at $p<0.01$) in root weight was observed among treatments. Maximum root weight was observed in control plants.

Effect of Fungal antagonists on the disease potential of root knot nematode *M. javanica:* Data presented in Fig. 4 described that significant difference in gall formation (at $p<0.01$) and egg mass production (at $p<0.001$) was found among the treatments. Highest degree suppression in gall formation and egg mass production was observed in plants treated with *P. lilacinus* whereas *T. flavus* and *T. harzianum* which were found almost equally effective. Treatments showed non-significant differences in nematode soil densities among themselves. Less root knot juveniles were recovered from pots, which were treated with *P. lilacinus*, compared to other treatments and control. Conidial suspension of microbial

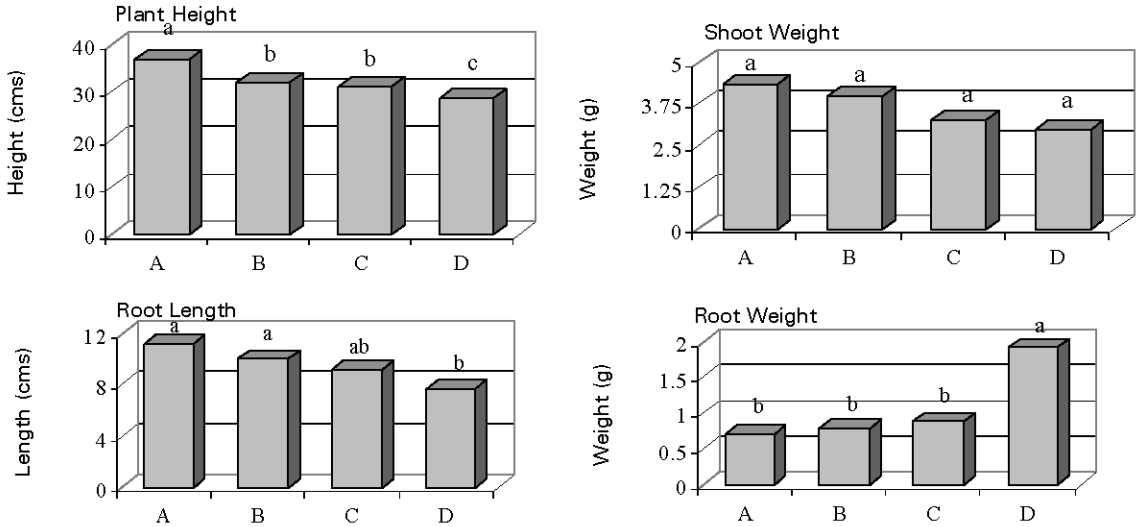

Fig. 1: Effect of Microbial antagonists (used as seed dressing) on the growth of okra
A = *Paecilomyces lilacinus*, B = *Trichoderma harzianum*, C = *Talromyces flavus*, D = Control
Bars sharing the same letters are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.
SED (at $p<0.05$)
Plant height = 0.87(***), Shoot weight = 0.67 (NS), Root length = 0.91 (*), Root weight = 0.15 (***)
* = 0.05; *** = 0.001; NS = Non- significant

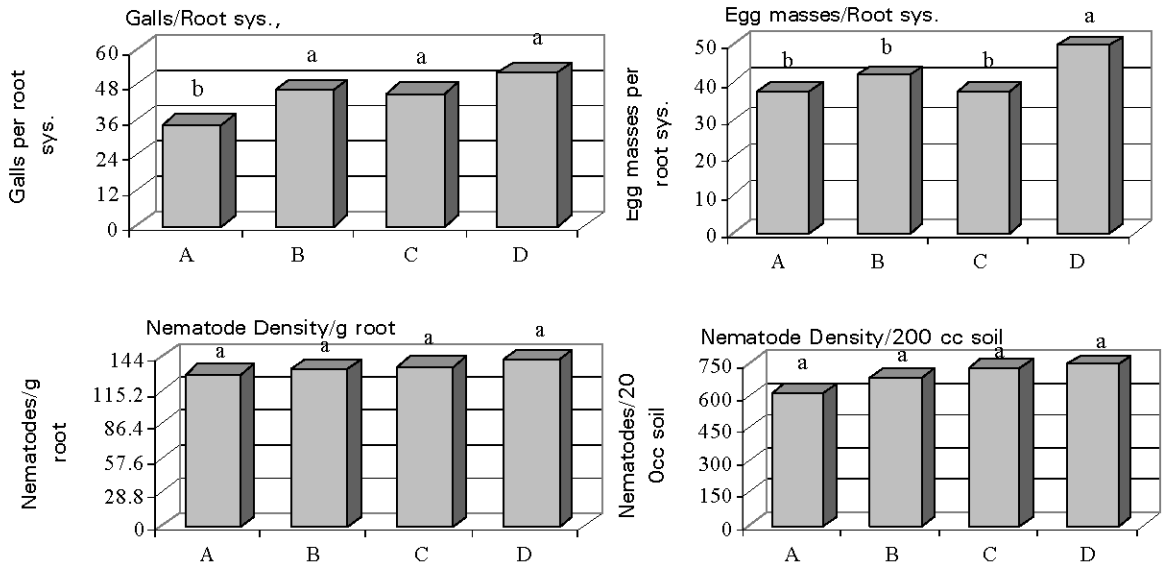

Fig. 2: Effect of fungal antagonists (used as seed dressing) on development of root knot *Meloidogyne javanica*.
A = *Paecilomyces lilacinus*, B = *Trichoderma harzianum*, C = *Talromyces flavus*, D = Control
Bars sharing the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.
SED (at $p < 0.05$)
Galls = 3.46(*), Egg masses = 1.9 (**), Nematode/g root = 4.99 (NS), Nematodes/200 cc soil = 51.76 (NS)
* = 0.05; ** = 0.01; NS = Non- significant

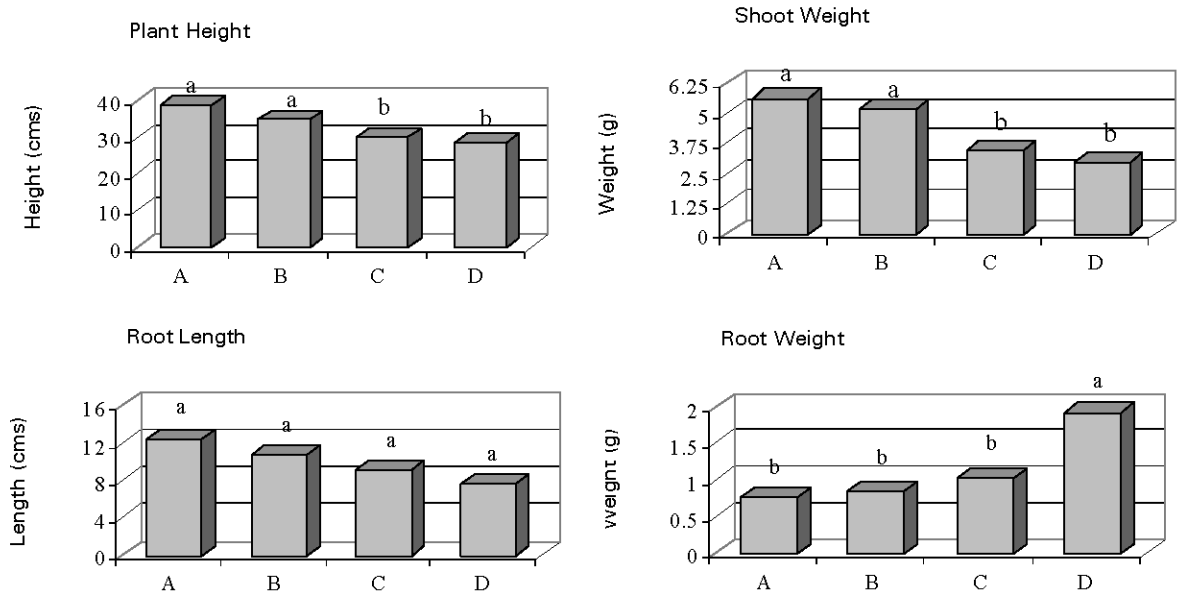

Fig. 3: Effect of Microbial antagonists (used as soil drench) on the growth of okra.
A = *Paecilomyces lilacinus*, B = *Trichoderma harzianum*, C = *Talromyces flavus*, D = Control
Bars sharing the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.
SED (at $p < 0.05$)
Plant height = 1.87(**), Shoot weight = 0.25 (***), Root length = 2.25 (NS), Root weight = 0.18 (**)
** = 0.01; *** = 0.001; NS = Non- significant

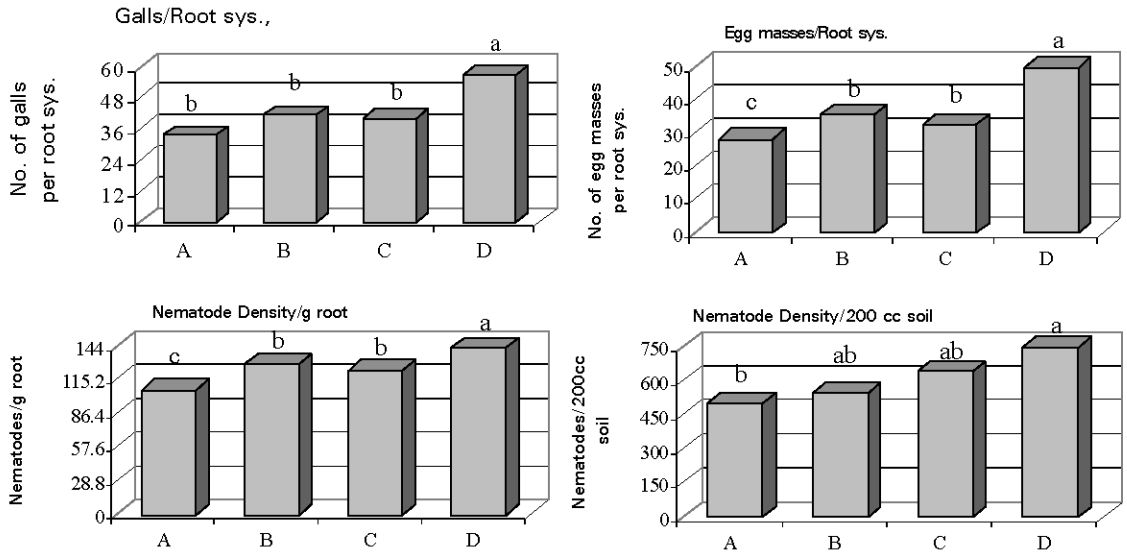

Fig. 4: Effect of Microbial antagonists (used as soil drench) on development of root knot *Meloidogyne javanica*.
A = *Paecilomyces lilacinus*, B = *Trichoderma harzianum*, C = *Talromyces flavus*, D = Control
Bars sharing the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.
SED (at $p < 0.05$)
Galls = 3.25(**), Egg masses = 1.67 (***), Nematode/g root = 3.99 (***), Nematodes/200 cc soil = 8.08 (NS)
** = 0.01; *** = 0.001; NS = Non- significant

antagonists used as soil amendment reduced the nematode root invasion (at $p < 0.001$). In *P. lilacinus* treated pots maximum reduction in root invasion was observed as compared to other treatments and control (Fig. 4).

Discussion

Maximum disease suppression was induced by *P. lilacinus* and is attributed to its parasitic nature and production of nematicidal serine proteases which degrades egg shell and check the egg hatching. The partial disintegration of the vitelline layer of egg in the presence of proximal fungal hyphae may be involved. This disruption pre-disposes the eggs to fungal infection by physical weakening of the shell and increases permeability, thus facilitating inward passage of fungal metabolites, both toxic and enzymatic (Bonants *et al.*, 1994).

Windham *et al.* (1989) reported that presence of *T. harzianum* and *T. koningii* suppressed the reproduction of *M. arenaria* on maize roots, when used as seed dressing. Harmen *et al.* (1993) documented lytic enzyme production by *Trichoderma* spp. Similarly, *T. harzianum* was found as egg parasite of *M. incognita* race-3 and killed 53% of eggs *In vitro* (Dos Santos *et al.*, 1992). Once a fugal hyphae enters an egg, presumably involving localized enzymatic dissolution of the chitin layer, mycelial proliferation ensues resulting in probable biosynthesis of destructive metabolites endogenously. Endogenous mycelial proliferation, supported in part nutritionally by the lysis of egg shell materials, hyphae are able to penetrate the larval cuticle with apparent ease. Saifullah (1996) has also reported the potential of *Trichoderma* sp. to infect the immature potato cyst nematode female. In the present study suppression of root knot disease may be due to the involvement of one or more mechanisms. *T. flavus* is known as parasitic of micro sclerotia of *Verticillium dahliae* and sclertia of *Sclertinia sclerotiorum* (Fahima and Henis, 1990). Under similar culture conditions, *T. flavus* isolate Tf1 produced an extracellular metabolite that had strong antimicrobial effects against fungi, bacteria and protozoa (Kim *et al.*, 1986). The satisfactory results obtained compared to control in the present study may attribute to above mentioned metabolites produced by *T. flavus* strain used. There is no such report concerning the nematode parasitism by *T. flavus*, therefore it is hypothesized that *T. flavus* suppress the growth of such microorganisms which enhance the nematode reproduction in rhizosphere of okra plants and fungal metabolites may be responsible for the suppression of root-knot nematodes in soil.

For avoiding chemical use in sustainable agriculture, interest in the use of microbial antagonists will increase in future in order to achieve environmentally benign disease control principle (Davise *et al.*, 1991). It is also necessary to develop techniques to monitor their incidence in soil for the promising control of nematode and other soilborne diseases which needs further investigation in this perspective.

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