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Effect of Fungal Filtrates of *Aspergillus* Species on Development of Root-knot Nematodes and Growth of Tomato (*Lycopersicon esculentum* Mill.)

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Abstract: Seven species of *Aspergillus* were screened for their biocontrol potential against *Meloidogyne javanica* root-knot nematodes on tomato under greenhouse conditions. Culture filtrates (CF) of *Aspergillus niger*, *A. terreus* and *A. tamarii* improved plant height, root length and fresh shoot weight when used as soil drench or bare root dipped seedlings. Besides, maximum reduction in gall formation and egg mass production was obtained by CF of *A. niger*, *A. fumigatus* and *A. terreus* in both soil drench and bare root dip seedlings compared to control. *A. niger* was found most successful in minimizing root and soil densities of root-knot nematodes in soil drench treatments compared to other test CF and control. Bare root dipped seedlings by *A. nidulans* CF reduced the root-knot development in tomato roots and in rhizosphere.

Key words: *Aspergilli*, biocontrol potential, *Meloidogyne javanica*, culture filtrate, soil density, root invasion

Introduction

Root-knot nematodes damage plants by devastating root tips and causing formation of swellings on the roots. This group of nematodes is distributed throughout the world but they are frequent in temperate and hot climatic agricultural zones. More than 2000 plant species have been reported to be attacked by this group of nematodes including almost all of the cultivated plants and reduce the world crop production by about 5 %, but losses in individual fields may be much higher (Sasser, 1980).

Tomato is cultivated over 288.8 thousand hec., with an average yield of 3,13072 tones in Pakistan (Anon., 1997). Root-knot disease of tomato caused by *Meloidogyne* species is wide spread in Pakistan and other tropical countries.

Restriction of pesticides due to their harmful and hazardous impacts on global ecosystem has increased the necessity of safe, environmentally acceptable controlling alternatives, such as biological control. The biological approach involves the introduction of specific microbial antagonists into the soil ecosystem (Cook and Baker, 1983), which have the ability to interfere the growth and survival of plant pathogens. Seed or seedling treatment with antagonistic fungi for the control of diseases caused by soil-borne pathogens i.e. fungi, bacterial, nematodes etc., is an attractive method for the application of biocontrol agents (Lifshitz *et al.*, 1986). Previous work, concerning biocontrol of root-knot nematodes (*Meloidogyne* spp.) by rhizosphere fungi (Zaki, 1999; Hussain *et al.*, 2001) has provided encouraging results both *In vitro* and under greenhouse conditions. *Aspergilli* which is one of the most frequent group of fungi found in rhizosphere of crop plants and are reported to produce antagonistic metabolites that are lethal to nematodes (Zuckerman *et al.*, 1994). Some of the *Aspergillus* spp. have also been found to parasitized nematode forms (Ayoub *et al.*, 2000). The objectives of the present study were, to determine the influence of cell free culture filtrates of *Aspergillus* spp. used as soil drench and bare root dipped seedling on tomato under greenhouse conditions.

Materials and Methods

Fungal Cultures: Cultures of test fungi i.e. *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. nidulans* (Eidam) Wint, *A. niger* Van Tegham, *A. terreus* Thom, *A. tamarii* Kint and *Aspergillus* sp. were obtained from Karachi University Culture Collection (KUCC). Cultures were maintained on potato dextrose agar (PDA-Oxoid), amended with antibiotics i.e. Penicillin and Streptomycin sulphate at 28 ± 2 °C for 6-7 days.

Broth Culture: Liquid culture of test fungi were prepared by inoculating 100 ml of sterilized Czapek's Dox Broth (CDB-Oxoid) in 250 ml Erlenmeyer flask with 2-3 discs (d.5 mm) of actively growing test fungi. Flasks receiving only PDA discs served as control. The flasks were incubated at 28 ± 2 °C for 15 days. Each liquid culture was filtered through Whattman No. 1 filter paper in a Buchner funnel. Filtrates obtained were designated as standard (S), which were used further.

Nematode Cultures: Roots of tomato cv. Roma VF infested with root-knot nematodes were collected from culture pots of Soilborne Diseases Lab., Dept., of Botany, Univ., Karachi. Nematode eggs were extracted by the method of Hussey and Barker (1973). Egg suspension were put on sieve, lined with tissue paper in order to gain freshly hatched root-knot juveniles after 24-48 hours.

Soil: Sandy loam soil obtained from Karachi University cultivated field was used in these experiments. Soil was sieved through 2 mm sieve to remove non-soil particles. Soil pH was recorded to be 8.1 and maximum water holding capacity was 34.1%.

Greenhouse Experiments

Soil Amendment (Soil Drench): Twenty-five ml of stock filtrate of each test species was drenched in plastic pot (d. 8 cm), containing 350 g soil. Pots receiving broth culture only served as control. Three weeks old tomato cv. Roma VF seedlings raised on steam sterilized soil in greenhouse were transplanted @ 2-3 plants per pot. After one week each pot was inoculated with freshly hatched juveniles' suspension @ 2500 J_2 per pot. Each treatment was replicated three times. Pots were arranged on greenhouse bench in randomized complete block design. Pots were irrigated daily up to harvest.

Bare Root Dipped Seedlings: Three weeks old tomato cv. Roma VF seedlings were treated with each test filtrate for 15 minutes and were transplanted in plastic pots (d. 8 cm) containing 350 cc soil. Roots of tomato seedlings were treated with broth CF only served as control. After one week of transplantation each pot was inoculated with freshly hatched juvenile suspension @ 2500 J_2 per pot. Pots were arranged in

randomized complete block design on green house bench and watered daily till termination of experiment.

Each experiment was terminated after 45 days of nematode inoculation. Fresh shoot and root weights, plant height and root length were recorded. Galls per root system, egg mass per root system were counted under stereoscope zoom microscope. Soil population of root-knot nematodes per 250 cc soil sample for each replicate was determined by a modified Baermann funnel technique (Schindler, 1961). Root densities of root-knot nematode were assessed per g root sample using acid fuchsin technique (Southy, 1986). Experimental data were subjected to analysis of variance (ANOVA) followed by LSD and comparison of means by Duncan's multiple range test (Gomez and Gomez, 1984).

Results

Exp 1) Effect of Fungal filtrates on plant growth: All the test culture filtrates (CF) of *Aspergillus* species were found to enhance the plant growth parameters. Significant difference (at $P<0.01$) was observed among the treatments in case of plant height and fresh shoot weight (at $P<0.05$). Maximum plant height was obtained by *A. niger* filtrate followed by *A. terreus*, *A. tamarii*, *A. flavus*, *A. fumigatus*, *A. nidulans* and *Aspergillus* sp. Shoot weight was enhanced by the treatments in the order *A. flavus* > *A. fumigatus* > *A. niger* = *A. terreus* > *A. nidulans* > *A. tamarii* and *A. flavus* filtrates over the control. On the other hand root weight in all the treatments, was significantly lower (at $p< 0.01$) than that of control (Fig. 1).

Effect of Fungal filtrates on the reproductive potential of *M. javanica*: Use of culture filtrates of *A. terreus*, *A. fumigatus*, *A. niger*, *A. nidulans* and *A. tamarii* significantly (at $P<0.001$) hampered gall formation and egg mass production per root system as compared to control plants. CFs of *A. niger*, *A. flavus* and *A. fumigatus* significantly reduced the nematode population in soil ($P<0.05$) and roots ($P<0.001$) at the termination of experiment (Fig. 2).

Exp. 2) Effect of Fungal filtrates on plant growth: Fungal filtrates of *A. flavus*, *A. niger*, *A. terreus* and *A. tamarii* significantly increased plant height (at $P<0.05$) while fresh shoot weight was significantly (at $P<0.001$) elevated in following order: *A. terreus* > *A. tamarii* > *A. flavus* and *A. fumigatus* as compared to control. There was no significant difference in root length among the treatments. Maximum root length was observed in set of pots receiving *A. flavus* > *A. nidulans* > *A. niger* = *A. terreus* > *Aspergillus* sp. and *A. tamarii* in comparison with control. A significant difference (at $p< 0.001$) in root weight was observed among the treatments. Maximum increase in root weight was observed in set of nematode controls whereas, no considerable increase in root weight was observed in treated pots (Fig. 3).

Effect of Fungal filtrates on the reproductive potential of *M. javanica*: Bare root dipped of tomato seedlings by *A. terreus*, *A. tamarii*, *A. niger* and *A. fumigatus*, *A. flavus*, *Aspergillus sp*

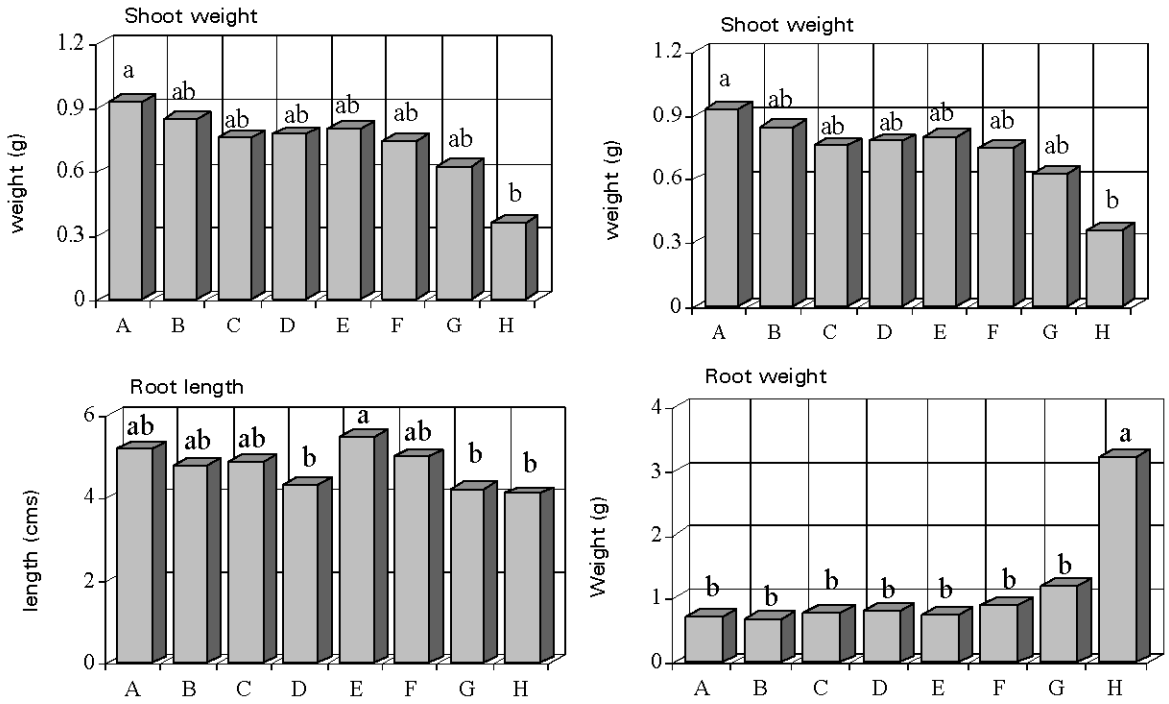

Fig. 1: Effect of filtrates of Aspergillus species on tomato growth used as soil drench.
A = *Aspergillus flavus*, B = *A. fumigatus*, C = *A. nidulans*, D = *A. niger*, E = *A. tereus*,
F = *A. tamarii*, G = *Aspergillus* sp., H = Broth culture
Bars sharing the same letters are not significantly different at $P<0.05$ according to Duncan's Multiple Range Test.
Significance level ($p<$)
Plant height,** ; Shoot weight, ***; Root length,***; Root weight,** **=0.01; ***=0.001

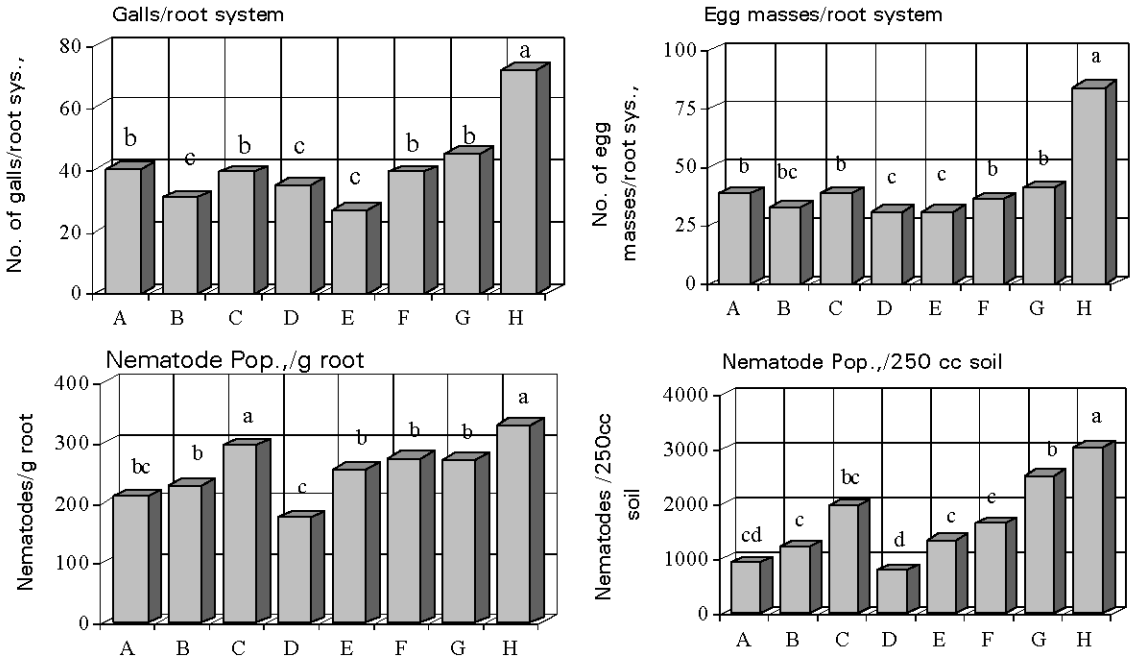

Fig. 2: Effect of filtrates of *Aspergillus* species on the development of *Meloidogyne javanica* on tomato used as soil drench.
A = *Aspergillus flavus*, B = *A. fumigatus*, C = *A. nidulans*, D = *A. niger*, E = *A. tereus*,
F = *A. tamarii*, G = *Aspergillus* sp., H = Broth culture
Bars sharing the same letters are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.
Significance level($p <$) Galls, ***; Egg masses, ***; Nematode/g root, *; Nematodes/250 cc soil, **
*, 0.05; **, 0.01; *** =0.001

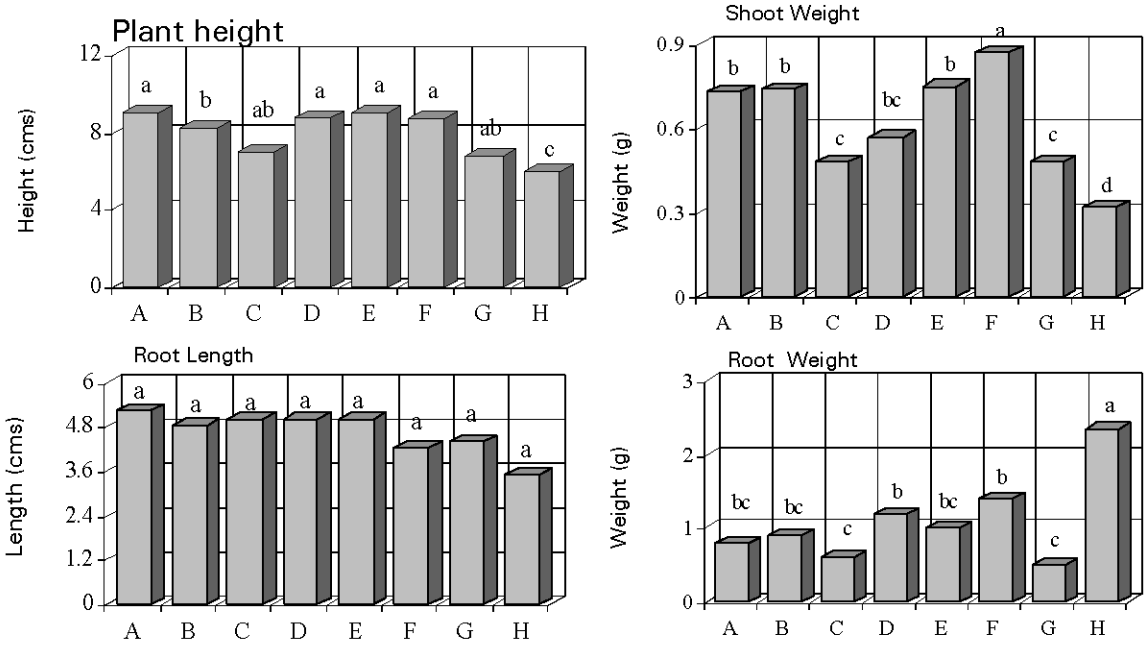

Fig. 3: Effect of filtrates of *Aspergillus* species on growth of tomato, used as bare root dipped seedlings.
A = *Aspergillus flavus*, B = *A. fumigatus*, C = *A. nidulans*, D = *A. niger*, E = *A. tereus*,
F = *A. tamarii*, G = *Aspergillus* sp., H = Broth culture
Bars sharing the same letters are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.
Significance level ($p <$)
Plant height, * ; Shoot weight, ***; Root length, NS; Root weight, *** * =0.05; *** =0.001; NS, Non-significant

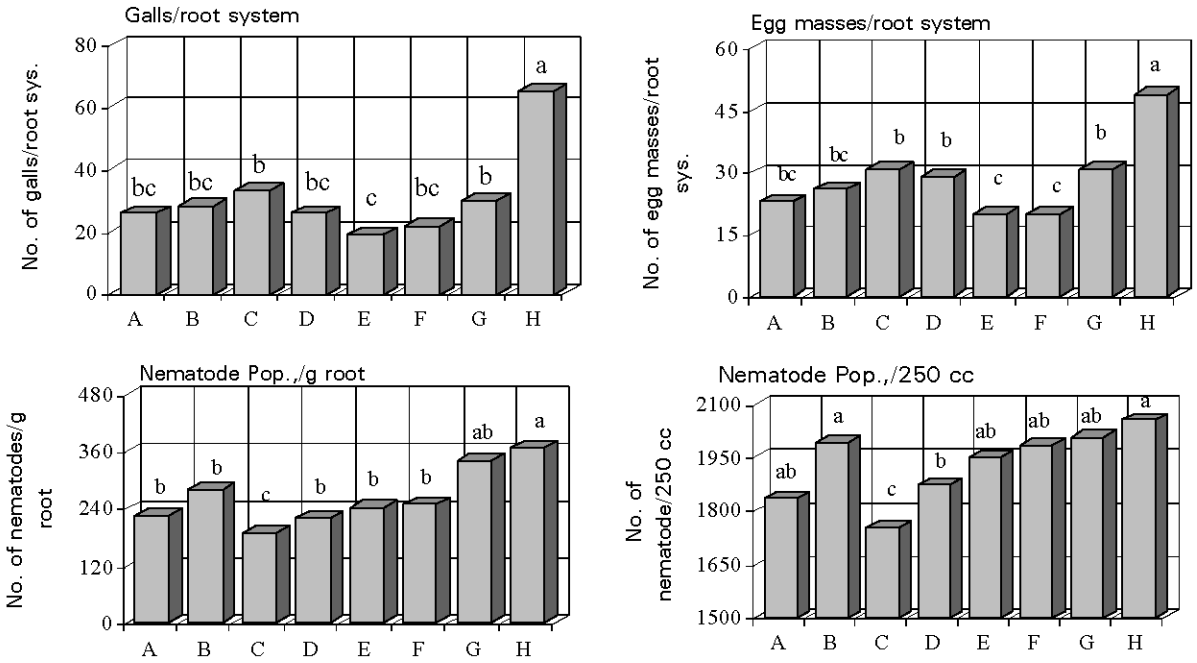

Fig. 4: Effect of filtrates of *Aspergillus* species on the development of *Meloidogyne javanica* on tomato used as bare root dipped seedlings.
A = *Aspergillus flavus*, B = *A. fumigatus*, C = *A. nidulans*, D = *A. niger*, E = *A. tereus*,
F = *A. tamarii*, G = *Aspergillus* sp., H = Broth culture
Bars sharing the same letters are not significantly different at $P<0.05$ according to Duncan's Multiple Range Test.
Significance level($p<$)Galls, ***; Egg masses, ***; Nematode/g root, ***; Nematodes/250 cc soil, *** ***=0.001

and *A. nidulans* significantly reduced (at $P<0.001$) gall formation and egg mass production as compared to control plants. Root invasion and nematode soil densities were suppressed significantly (at $p< 0.001$) in set of pots receiving CF of *A. nidulans* and *A. niger* as bare root dipped seedling. All other test CF of *Aspergillus* species provided varying degree of reduction in both soil and root densities compared to untreated control (Fig. 4).

Discussion

Microorganisms that can grow in rhizosphere are ideal for use as biocontrol agents since rhizosphere provides front line defense for roots against attack by soilborne pathogens (Weller, 1988). In the present study root-knot disease was suppressed while the growth of tomato plants was enhanced by filtrates (CF) of *Aspergillus* species either used as soil drench or bare root dipped seedlings. *Aspergilli* are major group of fungi found in soil ecosystem. Filtrates of nematophagous fungi have found to be active against plant parasitic and free living nematodes (Krizkova *et al.*, 1976; Giuma *et al.*, 1973; Amer-Zareen and Zaki, 1999a, b). Filtrates produced by non-specialized fungi are reported to be lethal to different plant parasitic species (Alam *et al.*, 1973; Mani and Sethi, 1984; Cayrol *et al.*, 1989). In both greenhouse experiments all test species of *Aspergillus* showed variable effects in control of root-knot disease. This variable behaviour against nematode pest may be attributed to their genetic make up and the nature of secondary metabolites, produced. Different species of *Aspergillus* produce different toxins such as *Aspergillus terreus* produce toxin like citrinin, *A. flavus*, *A. parasiticus* produce aflatoxin B_1, B_2 G_1, and G_2 (4 metabolites of Aflatoxin), *A. fumigatus* produces kojic acid. Culture filtrates of *Aspergillus* spp, were found to have some nematicidal properties (Mankau, 1969; Khan *et al.*, 1984). Microorganisms present in the rhizosphere adversely influence the intimate relationship between the plant parasitic nematodes and their host by production and degradation of specific root exudates that control nematode behaviour (Sikora and Hoffmann-Hargaten, 1993). Nematicidal activity of filtrates of some *Aspergillus* species and other soil fungi has been documented earlier (Shukla and Swarup, 1971; Krizkova *et al.*, 1979; Singh *et al.*, 1983). Culture filtrate of *A. niger* provided better results in the present study which may be attributed to it's toxic principle (s). and nematicidal action (Mankau, 1969). Zuckerman *et al.* (1994) reported a nematicidal compound (oxalic acid) which was isolated from the *Aspergillus niger* strain. *Aspergilli* are reported as pathogenic to humans (Wyllie and Morehouse, 1978). Siddiqui *et al.* (2000) demonstrated that *A. terreus* possess thermostable nematicidal compound(s) and has the potential to control nematode population densities in soil and subsequent root-knot disease severity in the economically important crop. In the present effort, suppression of nematode disease and plant growth enhancement by fungal filtrates of *Aspergilli* either applied as soil drench or bare root dipped seedlings leads towards the possible effects occurring during plant parasitism and their potential exploitation as pesticides of natural origin.

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