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Response of Ten Chickpea (*Cicer arietinum* L.) Cultivars Against *Meloidogyne javanica* (Treub) Chitwood and Disease Control by Fungal Filtrates

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Abstract: All test cultivars of chickpea responded to root knot nematode *M. javanica*. Plant tops in nematode inoculated pots were significantly hampered (at $p<0.01$) compared to nematode free control. Maximum suppression in plant height by nematode was observed in cv. Nes 95 0174 followed by cv. 91 A039, whereas maximum suppression in shoot weight was observed in cv. Nes 95 0012 & Nes 95 0174 in response to nematode infection. Lower degree of gall formation per root system was observed in cv. Nes 95 004. Whereas cv. 90 122, 93 127 & Nes 950174 limited nematode densities in soil and root compared to rest of cultivars. According to the host response index (0- >10), all test cultivars were moderately resistant. Reproduction factor (r) was higher in cv. Nes 96 003 ($r = 1.88$) compared to rest of cultivars. In biocontrol experiment CF of *A. niger* was effectively increased plant height, fresh shoot weight and root length followed by *A. flavus* and *A. temarii* compared to other treatments and control. Maximum reduction in nematode soil density was achieved by *A. flavus* (>17%) followed by *A. temarii* (134%) compared to other treated pots. Gall formation per root system was significantly hampered (at $p<0.001$) where *A. niger* was applied followed by *A. nidulans* > *A. flavus* > *A. terreus* > *A. fumigatus* compared to control.

Key words: Cultivars, chickpea, *M. javanica*, host response index (0- >10), *A. niger*.

Introduction

Besides traditional management tactics including cultural and physical control, commercially available chemicals are used for reliable and immediate control. But this tactic is becoming more critical and chemical nematicides are continue to be removed form the market because of environmental and health concerns. In alternative control strategy, biocontrol is a promising (Stirling, 1991). The plant host can have a drastic effect on nematode population dynamics and host plant resistance is a very important method for managing plant parasitic nematode. However, resistance is usually not an "all or nothing " phenomenon. There are well-documented reports that hosts with some degree of resistance may allow low or even moderate levels of reproduction (Gallher & McSorley, 1993). The biological response of plant parasitic nematodes to secondary metabolites produced by soil fungi is of interest because of the possible effects occurring during plant parasitism and their potential exploitation as pesticides of natural origin (Zaki, 1999). Filtrates produced by non-specialized soil fungi were also reported as toxic or lethal to different plant parasitic nematode species (Cayrol *et al.*, 1989). *Fusarium* spp. Filtrates were toxic to free-living and plant parasitic nematode *in vitro*. The occurrence and activity of mycotoxins can be responsible for the different synergistic or antagonistic effect observed among fungi and nematodes (Nordmyer & Sikora, 1983).

The objectives of the present studies are to screen out some nematode resistant line of chickpea (*Cicer arietinum* L) cultivars and fungal filtrates of species of *Aspergillus* and were used for the control of root knot nematode *M. javanica* on chickpea, under greenhouse conditions.

Materials and Methods

Chickpea lines: Ten chickpea lines i.e. 90122, 93127, 91 A039, 91 A 001, Nes 950174, Nes 950193, Nes 95004, Nes 950012, Nes 96002 and Nes 96003 provided by Pulses Program, National Agriculture Research Council, Islamabad, were screened out against root knot nematodes.

Nematodes: Root knot population used in present study for artificial soil infestation was originated from eggplant beds at Karachi University Garden, and identified as *Meloidogyne javanica* with the help of perennial pattern as described by Tayler & Nester (1974). Nematode population was maintained on eggplant cv. Black beauty, in 25-cm diam. clay pots containing steam-sterilized soil.

Fungal Cultures: The test *Aspergillus* species namely, *A. terreus, A. temarii, A. niger, A. nidulans, A. flavus, A. fumigatus* and *Aspergillus* sp. used in present study, were obtained from Karachi University Culture Collection (KUCC), Department of Botany, University of Karachi. All test fungal cultures were maintained on PDA (2%) amended with antibiotics. Culture filtrates were prepared by growing these fungi on Czapek's liquid medium in 250ml Erlenmeyer flasks, 100-ml medium in each. Flasks were inoculated with 2-3 plugs (diam. 0.5 mm) of actively growing mycelium of test fungal cultures. Each fungus was replicated thrice. Flasks receiving plan PDA plugs served as control. All flasks were incubated at 28 ± 2°C. Culture filtrates were harvested after two weeks of fungal growth. Mycelial mats were removed and the media was filtered through Whatman No. 1 filter paper twice. The filtrate obtained was designated as standard (S).

Experiment 1.

Screening of chickpea line against root knot nematodes: Four seeds of each test cultivar of chickpea were sown in 8-cm diam., plastic pot, containing 350-g sandy loam soil, pH 8.1, obtained from Karachi University Experimental Field. Of seedlings with 2 seedlings/pot were maintained. About 2-3 days old hatched juveniles of root knot nematode *M. javanica* were inoculated @2000 J2/pot in root zone of chickpea plants. Each test cultivar has four replicates. Set of pots free of nematode inoculum served as control. Pots were randomized on greenhouse bench. Pots were watered whenever needed. Insecticidal sprays were also used twice during the course of experiment. Plants were harvested after two months and growth parameters such as plant height, fresh shoot & root weight and length were recorded. Root knot damage was estimated by counting number of galls per root system under binocular microscope. Nematode densities per 250cc soil and per g of root was also estimated

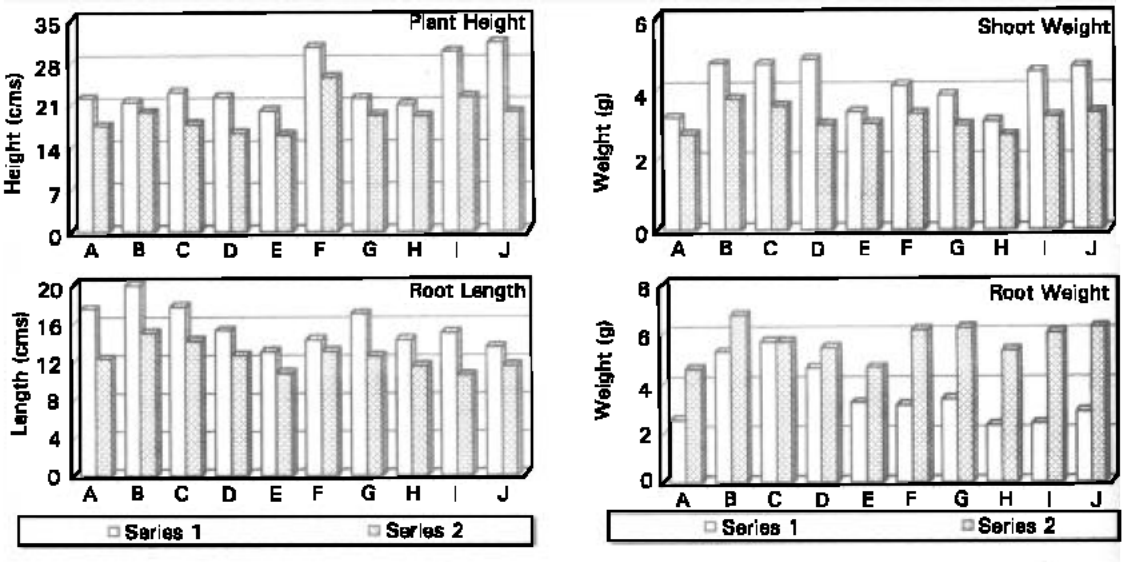

Fig. 1: Effect of root knot nematodes *Meloidogyne javanica* on the growth of chickpea cultivars.
Series 1, Nematode free; Series 2, Nematode inoculated
A= 90 122, B=93 127, C= 91 A001, D=A039, E=Nes. 95 0174,
F= Nes. 95 0193, G=Nes. 95 004, H=Nes. 95 0012, I=Nes. 96 002,
J=Nes. 96 003 SED($p<0.05$) for,
Plant height, 4.12^{NS} (-N) and 1.94** (+N) Shoot weight, 0.48** (-N) and 0.65^{NS} (+N)
Root length, 3.45^{NS} (-N) and 1.77^{NS} (+N) Root weight, 062*** (-N) and 1.29^{NS} (+N)
**,$p<0.01$, ***,$p<0.001$; NS, Non-significant

Table 1: Resistance ranking (0->10) of ten chickpea varieties according to the reproduction factor (r)

Varieties	r=Pf/Pi	Resistance rating
90122	1.3	Moderately resistant
93127	1.49	Moderately resistant
91 A001	1.7	Moderately resistant
91 A039	1.5	Moderately resistant
Nes. 95 0174	1.2	Moderately resistant
Nes. 95 0193	1.52	Moderately resistant
Nes. 95 004	1.77	Moderately resistant
Nes. 95 0012	1.85	Moderately resistant
Nes. 96 002	1.75	Moderately resistant
Nes. 96 033	1.88	Moderately resistant

(Southey, 1986). The chickpea reaction of each cultivar was rated according to the reproduction factor, r=Pf /Pi, as highly resistant 0, resistant <1, moderately resistant 1-2, moderately susceptible 2.1 – 5, susceptible 5.1 – 10 and highly susceptible >10 (Di Vito *et al.*, 1996).

Experiment 2.

Culture filtrate of species of *Aspergillus in* the control of root knot nematode *M. javanica* used as seed treatment Sandy loam soil, pH 8.1 obtained from experimental field of Botany Dept., University of Karachi was transferred in 8 cm diam., plastic pots, each containing 350 cc. The seeds of chickpea line were surface disinfected with 2% sodium hypochlorite solution and 5% ethanol, air dried and treated with test culture filtrate (100% conc.) of each test *Aspergillus* species for 15-20 minutes. After seed treatment, seeds were air dried and sown in pots @4 seeds/pot. Seeds soaked in broth filtrate and sterile distilled water served as control. After emergence of seedlings thinning was performed and kept 2 plants/pot. Each pot was inoculated with freshly hatched juveniles @ 2000 J2/pot. Each treatment was replicated

Table 2: Effect of culture filtrates of *Aspergillus* spp. on growth of chickpea varieties

Treatment	Plant height(cms)	Shoot weight(g)	Root lenght(cms)	Root weight(g)
Aspergillus terrarii	18.43	2.92	5.25	5.70
A. terreus	16.50	2.27	7.26	4.54
A. niger	19.33	4.03	6.75	4.63
A. nidulans	18.00	3.13	6.44	4.66
A. flavus	18.66	3.18	6.55	5.29
A. fumigatus	17.50	2.93	6.14	5.82
Aspergillus sp.	16.55	2.30	5.85	6.12
Broth culture control	14.85	1.00	3.86	6.75
Control	16.05	1.05	4.66	6.60
SED $p<0.05$	1	0.25	0.49	0.69
Significant level ($p<$)	**	***	***	*

Seed soak *,0.05; **,0.01; ***,0.001;
NS, Non-significant

thrice. Pots were randomized and watered when needed. Experiment was terminated after 60 days of nematode treatment. Growth parameters such as plant height, fresh shoot & root weight and length, nematode development as number of galls, egg masses, nematode density per 250cc soil and per g root was estimated. Data was statistically analyzed using analysis of variance (ANOVA). Treatment means were compared following Duncan's multiple range test (Gomez & Gomez, 1984).

Results

Experiment 1.

Variable effects of nematode population on plant growth parameters were observed in all chickpea cultivars. Almost all cultivars responded to root knot nematode. Plant tops in nematode inoculated pots, were significantly suppressed compared to nematode free control. There was no significant

Table 3: Effect of culture filtrates of Aspergillus spp. On development of Meloidogyne javanica on chickpea varieties.

Treatment	No. of galls/ root sys	No. of egg masses/root sys	Population		
			250 cc soil	% Reduction in soil	g^{-1} root
Aspergillus temarii	68.35	13.82	2840	13.4	286
A. terreus	62.66	14.75	2900	11.58	264
A. niger	42.28	11.79	2640	10.36	249
A. nidulance	58.33	12.58	2980	9.14	275
A. flavus	59.45	12.01	2700	17.68	260
A. fumigatus	65.72	12.54	2860	12.8	268
Aspergillus sp.	79.52	12.25	3000	8.99	310
Broth culture control	78.89	17.26	3320	-	231
Control	80.48	15.78	3280	-	217
SED $p<0.05$	3.59	0.78	98.17	-	12.94
Significant level	***	NS	***	-	***

difference in weight of shoot & root and root length among the cultivars receiving nematode inoculum. Maximum suppression in plant height by nematode was observed in cv. Nes 95 0174 followed by 91 A 039 whereas maximum shoot weight was reduced in cv. Nes 950012 & Nes 95 0174 compared to other treatments. Similarly nematode population reduced root length in cv. Nes 96 002. Increased root weight in test cultivars compared to untreated set of pots, was attributed to *M. javanica*. Set of pots free from nematode inoculum were found healthier compared to treated set of pots.

All chickpea cultivars screened for the resistance against root knot nematode, favored nematode development in terms of gall formation per root system and population densities in soil and root. Lower degree of gall formation per root system was observed in cv. Nes 95 004 followed by Nes 95 0012, Nes 95 0193 compared to other cultivars. Chickpea lines 90 122, 93 127 & Nes 95 0174 limited nematode population density in soil and root.

According to the rating used for host response, nematode reproduction factor was lowest in cv. Nes 95 0174 followed by cv. 90 122 compared to rest of test cultivars. All cultivars of chickpea were found "moderately resistant". Highest reproduction factor (r) was observed in cv. Nes 96 003 followed by Nes 95 0012 compared to other cultivars tested.

Experiment 2.

Culture filtrates of species of *Aspergillus* induced varying degree of influence on chickpea growth. Among the all test filtrate treatments, significant increase in plant height, fresh shoot & root weight and root length was observed compared to untreated control. *A. niger* was found most effective with maximum plant tops, fresh shoot weight and root length followed by *A. flavus* and *A. temarii* compared to remaining set of treatments. In treated set of pots lesser root weight compared to untreated control was observed (Table 2).

Aspergillus species significantly reduced nematode densities both in soil and root of chickpea plant. Maximum reduction in soil density and root knot invasion was observed in *A. niger* followed by *A. flavus*> *A. temarii* and *A. terreus* compared to untreated control. Percentage (%) reduction in nematode soil recovery was observed where *A. flavus* was used as seed treatment followed by *A. temarii* > *A. fumigatus* > *A. terreus* > *A. niger* > *A. nidulans* and *Aspergillus* sp. compared to untreated control. Number of egg mass per root system was not significantly different among the treatments. Gall formation per root system was significantly suppressed in set of pots where *A. niger* was used to treat seeds before sowing followed by *A. nidulans* > *A. flavus* > *A. terreus* > *A. fumigatus* compared to rest of treatments and untreated control (Table 3).

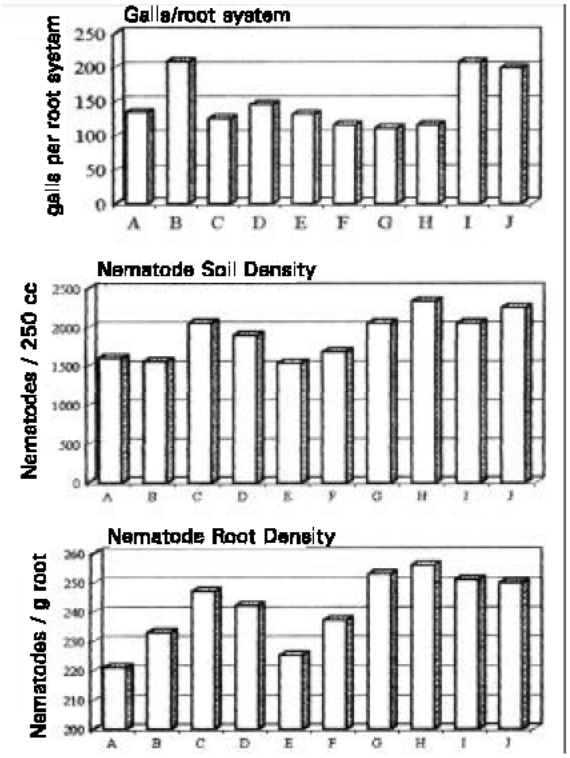

Fig. 2: Root knot nematodes Meloidogyne javanica development on chickpea cultivars.
A = 90 122, B = 93 127 C = 91 A001
D = A 039 E = Nes. 95 0174
F = Nes.95 0193 G = Nes. 95 004,
H = Nes. 95 0012 I = Nes. 96 002
J = Nes. 96 003 SED ($p<0.05$) for,
Galls, 27.73**,
Nematode soil density/250cc, 60.1***
Nematode root density/g root, 18.75^{NS}
, <0.01; *, <0.001; NS, Non significant

Discussion

Use of resistant cultivars for the management of nematode population is expected to be a vital management component in the future. In the present study plant response to *M. javanica* was measured by amount of galling and estimating soil and root densities. Results described that there was

variation among the different cultivars of chickpea, screened out against root knot nematodes. Nematode population used for the disease induction, suppressed maximum plant height, fresh shoot & root weight and root length (Fig 1) in cv. Nes 95 0012 & Nes 95 0174. Whereas if observe from an aspect of disease control, lower degree of gall induction per root system was achieved by cv. Nes 95 004. Resistance describes the amount of plant damage in response to nematode population and other soilborne pathogens. Presence of nematode resistance genes make the plant root less attractive (Table 1) for attacking nematodes. There may be possibility of interception of signal transduction in recognition event. Further, results demonstrate that cv. 90 122, 93 127 & Nes 95 0174 limited nematode densities in soil and root compared to remaining cultivars (Fig 2). As the plant parasitic nematodes are obligate parasites, planting highly resistant cultivars place selective pressure on the nematode population for biotypes that can reproduce on the resistant cultivar (Young, 1998). The results of the screening experiment helped us to understand the behavior and response of different cultivar of chickpea against *M. javanica*. Planting resistant cultivars and using other controlling measures are the primary practical methods of suppressing nematode damages to crop of low economic value. Seed treatment with biocontrol agent for control of soilborne disease is an alternative method for the application of biocontrol agents (Barker, 1983). Seed treatment by filtrates of *Aspergillus* species provided variable results in present study (Table 2 & 3). Compared to other test filtrates *A. niger* was found most effective as seed treatment against root juvenile's penetration and further development. In previous studies *Aspergillus* species filtrates have been provided better results when used as soil drench (Amer-Zareen & Zaki, 1999) or seed treatment (Zuckerman *et al.*, 1994). Variable effectiveness of test fungal filtrates is attributed to their genetic make up, source, concentration, exposure time, active principle (s) and susceptibility of nematodes. *Aspergillus* spp. have been provided better results against root knot and other plant parasitic nematodes *in vitro, in vivo* and under filed conditions (Zaki, 1999). Zuckerman *et al.* (1994) has reported the presence of oxalic acid and citric acid, which found to be partial determinant of the nematicidal activity. Further these two acids had synergistic action upon each other which help to understand the mode of action of *A. niger's* nematoxicity. Fungal filtrates of *A. niger* and *Rhizoctonia solani* moderately improved plant growth, hampered juvenile's penetration and root knot reproduction on tomato, when applied as bare rot dip (Khan *et al.*, 1984). Ali (1990) studied the effect of fungal filtrates of some *Aspergillus* species and other soil fungi against root knot nematodes and *Rotylenchulus reniformis*. The culture filtrates are considered to be lethal against nematode, and /or effected the movement and penetration of host roots, which improved growth of the host plant. Siddiqui and Husain (1991) studied the effect of filtrates of *Paecilomyces lilacinus, A. flavus, A. fumigatus* and other antagonistic fungi against root knot, *M. javanica* and *Macrophomina phaseolina* on chickpea. *P. lilacinus* was found most effective against root knot nematodes followed by *A. niger* > *A. flavus* and other test fungal filtrates. Variation in results of the present study might be due to organic or inorganic substances contained by fungal filtrates. Results also suggest that effective filtrates may induce physiological changes in the root, which affect nematode development and maturation. On the basis of results presented and discussed here, it is to be concluded that commonly occurring *Aspergillus* strains could be exploited for non-hazardous, non-chemical and cheap tactic for control of phytoparasitic nematodes. Specificity of mycotoxins to suppress nematode populations is a real advantages, which could limit only pest densities without creating imbalance in soil microfauna, as commercially available pesticides are inducing.

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