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Combined Efficacy of *Moringa oleifera* Leaves and a Fungus, *Trichoderma harzianum* Against *Meloidogyne javanica* on Eggplant

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Abstract.- The efficacy of *Trichoderma harzianum* and *Moringa oleifera* at various concentrations (S, S/2, S/4) and in combinations was tested on the reproduction, egg hatching and juvenile mortality of *Meloidogyne javanica* on eggplant. The evaluation was done *in vitro* as well as *in vivo* in green house. Both alone and combined application of *T. harzianum* and *M. oleifera* caused significant (P = 0.05) reduction in egg hatching and juvenile mortality at all time intervals (24, 48 and 72 h). Reproduction of *M. javanica* was evaluated on eggplant in green house. Most effective concentration from *in vitro* experiment was used in further protective and curative experiment. There were three treatments and five replications. The plant growth parameters including shoot length, shoot weight, root length, root weight and number of leaves and nematode parameters including number of galls, egg masses, number of females, number of juveniles per root system and no. of juveniles/100ml^3 soil were recorded after 60 days of growth. Nematode population was significantly reduced when biocontrol agent was applied before the inoculation of nematodes as protective application. So, *T. harzianum* and *M. oleifera* might be successfully used in field conditions against *M. javanica*.

Keywords: *Trichoderma harzianum*, protective, combine, *Moringa oleifera, Meloidogyne javanica,* biocontrol agent.

INTRODUCTION

Tomato, okra, cucumber, eggplant, bitter gourd, sponge gourd and pumpkin are the major summer vegetables grown in country. Eggplant (*Solanum melongena* L.) also known as brinjal is a vital cheap summer vegetable. It contains 92.7% water, 1.4% protein vitamin A in small quantity (Tindall, 1978). In Pakistan, it is cultivated on an area of 8490 ha having annual production of 84707 tonnes (FAO, 2011). The average harvested yield is very low because they are invaded by various pests such as insects, fungi, bacteria, viruses and nematodes. The biological entities, including nematodes mainly root knot nematodes are wide spread and minimize the yield of vegetable crops in Pakistan (Anwar *et al*., 2009; Kamran *et al*., 2012). Losses due to root knot nematodes on various vegetables were 10 to 100% in Pakistan (Shahid *et al*., 2007). Eggplant is severely harmed by *Meloidogyne* spp. (Dhawan and Sethi, 1976; Netscher and Sikora, 1990). *Meloidogyne javanica* is one of main plant parasitic nematodes in tropical and subtropical areas of world including Pakistan (Anwar *et al.,* 2007). Root knot nematodes are categorized as important pest of vegetables in Pakistan and their host range comprising more than 3000 plant species (Reddy, 1983).

Trichoderma harzianum has been extensively studied as a biological control organism against a wide range of soil-borne pathogens including root-knot nematodes and having plant growth-promoting capacity (Saifullah and Thomas, 1996; Sharon *et al.*, 2001). Sharma and Pandey (2009) observed the potential of fungi *T. harzianum*, *Paecilomyces lilacinus* and *Arthrobotrys oligospora* along with natural organic compound (Neem compound mix) to control *M. incognita*.

Prasad *et al*. (1993) have shown that soil amendment with neem cake, green manure, or other plant materials may improve the effectiveness of biocontrol agents. The incorporation of chopped green leaves of *Crotolaria* spp., *Sesbenia* spp., and *Tagetes* spp. facilitated the colonization of *Trichoderma* spp. and *P. lilacinus*. *Moringa oleifera* Lam (Moringaceae) native to western and sub–Himalayan region, India, Pakistan, Africa and Arabia is now cultivated in the Philippines, Cambodia, Central, North and South America and the Caribbean Islands (Morton, 1991). Devi (2002) evaluated the effect of *Ocimum sanctum*,

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Azadirachta indica, Myristica malabarica, M. oleifera, Mangifera indica, and *Acacia Arabica* on mung bean, cowpea, and urdbean against nematode population and observed that they not only reduced nematode population but also enhanced plant yield. Claudius-Cole *et al.* (2010) observed water extracts of *Ocimum gratissimum Azadirachta indica, Vernonia amygdalina* and *M. oleifera* for their effect on pathogenicity of *M. incognita* race 2 and on the growth and yield of cowpea. The extracts inhibited the egg hatchings 40-63.7% increased the juvenile mortality from 82-93.8%. Keeping in view the biological management, present study was designed to assess the effectiveness of *M. oleifera* leaves and *T. harzianum* on reproduction of *M. javanica*.

MATERIALS AND METHODS

Nematode inoculum

In order to make pure culture of sampled population, single egg mass inoculation of *M. incognita* was done. Single mature egg mass was inoculated in pots around the root of young eggplant seedling. Sub – culturing was done by inoculating new eggplant seedling with at least 15 egg masses, each obtained from pure culture in order to maintain sufficient inoculum for further studies.

Fungal culture

Pure culture of *Trichoderma harzianum* was obtained from the culture collection of Arid Agriculture University, Rawalpindi. One week old cultures of bioagent maintained on PDA slants at 28 ± 1 ^{O}C were used for the present study. Culture filtrates of *T. harzianum* were obtained by growing one week old culture of *T. harzianum* on Potato Dextrose Broth (in 250 ml flasks containing 100 ml broth media). Flasks were autoclaved at 121°C and 15 psi for 15 minutes. Each flask was inoculated with three scoops of 5mm diameter from pure culture containing Petri dishes and incubated at 25 ^{O}C for 15 days. After incubation the cultures were filtered through Whatman filter paper No.1 to remove the mycelial mats. These filtrate thus obtained were considered as standard ("S" concentration) and it contains 10^7 spores/ml.

Preparation of aqueous plant extract

Leaves of the tested plants Moringa (*M. oleifera*) were taken to prepare the aqueous extracts. Ethanol was not used because it may itself affect the nematodes. These were kept in sun at 45- 50 ^{0}C and dried for 24 hrs. Before drying these were washed to remove any dust. Water extracts of tested plant were prepared by grinding 25g of each in 250ml of sterilized water (Adegbite and Adesiyan, 2005) and filtered through muslin cloth, then it was filtered through the filter paper to get the clear extract and it served as standard "S", S/2 and S/4 concentrations ware also be made.

Effect of T. harzianum + *aqueous extract of* M. oleifera *juveniles mortality leaves on* M. javanica

Different concentration of culture filtrate *T. harzianum* S, S/2, S/4 and aqueous extract S, S/2, and S/4 were prepared by adding sufficient amount of sterilized water. The population of *M. javanica* was maintained on the roots of eggplant (var. Dilnasheen) in the glass house. Egg masses were isolated and nematodes were extracted (Hussy and Barker, 1973). To investigate the effects of *M. oleifera* (Moringa) leaves extract and culture filtrate of *T. harzianum* on second stage juveniles' mortality, 5ml from both concentration of *M. oleifera* (Moringa) and *T. harzianum* were poured into each Petri plate. At the same time fifty freshly hatched juveniles of *M .javanica* in 1ml distilled water were added to each Petri plate. Each treatment was replicated three times and kept in incubator at 28^{0}C. The experiment was conducted with three replications using completely randomized design. Fresh hatching juveniles placed in distilled water served as control. Data on juvenile mortality was recorded after 24, 48, 72 h. Dead and immobilized J2 were counted under microscope after 24, 48, 72 h. To confirm the death of juveniles, ten to fifteen juveniles were transferred to distilled water. If they did not move they were considered dead (Mehmood *et al*., 1979).

Percent juveniles' mortality was calculated.

Egg hatching

For hatching test, all the experimental protocol and conditions were similar to the mortality experiment except for fifty eggs of *M. javanica* in 1ml distilled water were added to each Petri plate. Number of hatched juveniles were counted under

microscope after 24, 48, 72 h.

Percent egg hatching was calculated.

Management by curative and protective application

Egg plants were cultivated in plastic pots to study the management of root knot nematode by protective and curative application of *T. harzianum* and *M. oleifera*. Most effective concentration of aqueous extract of *M. oleifera* and culture filtrate of *T. harzianum* determined by previous experiments was used to check their effect as protective and curative. One month old eggplant nursery was transplanted into these pots. The freshly hatched 1000 J_2 per plant used as inoculums. There were three treatments and five replications. Combine application of *T. harzianum* and *M. oleifera* was done before inoculation as protective and after inoculation as curative effect. One treatment without application was kept as control. The effect of *T. harzianum* and *M. oleifera* as protective and curative application against *M. javanica* on egg plant was evaluated after 60 days of inoculation. The roots of plants were washed carefully to remove soil from the roots and stained with Phloxine B solution for 5 minutes to facilitate counting of egg masses. Nematode reproduction parameters includes number of galls per root system, egg masses, number of females, no. of juveniles per root system and no. of juveniles per $100ml^3$ soil and plant growth parameters shoot length (cm), shoot weight (gm), root length (cm) and root weight (gm) was recorded, experiment was repeated to confirm the results. The data was subjected to two-way ANOVA and significant difference among the treatment means were separated by Duncan's multiple range test (DMR) at 0.05% level of significance (Steel *et al*., 1997).

RESULTS

Effect of Trichoderma harzianum + *aqueous extract of* Moringa *leaves*

Juveniles mortality of M. javanica

Mortality of juveniles of *M. javanica* was affected by concentrations and exposure time in all treatments (Table I). The J2s treated with *T. harzianum* and combine application of *T. harzianum* and *M. oleifera* had significant more mortality at all time intervals (30.00, 40.00 and 46.00) "S" concentration respectively. *Trichoderma harzianum* at "S" concentration had significant (P = 0.05) more effect on mortality (38.66) after 72 h from its S/2 (22.00) and S/4 (10.66) concentration. *Moringa oleifera* at "S" concentration had significant (P = 0.05) more effect on mortality (30.00) after 72 h from its S/2 (24.00) and S/4 (16.00) concentration.

Egg hatching

Egg hatching of *M. javanica* was inversely proportional to the concentration of *M. oleifera* leaf extracts and culture filtrate of *T. harzianum*. Egg hatching was significantly decreased with the duration of exposure (Table II). Combine application of *T. harzianum* and *M. oleifera* exhibited the maximum reduction in egg hatching of *M. javanica* after all time intervals at "S" concentration, respectively. Hatching of *M. javanica* was significantly (P=0.05) reduced in "S" and S/4 concentrations of *T. harzianum* after 7 days of exposure as compared to control. At "S" concentration of *M. oleifera* caused significant reduction (40.00) in hatching after 7 days from its S/2 and S/4.

Management by protective and curative application of T. harzianum *and* Moringa *leaves extractt*

Plant growth parameters

Combined effect of *T. harzianum* and *M. oleifera* was observed after application in green house. The results of all the treatments were statistically significant (P = 0.05). Maximum root length was observed after combined application of *T. harzianum* and *M. oleifera*. Minimum root length was observed in check plants which were treated with root knot nematodes only (Table II). Effect of *T. harzianum* and *M. oleifera* on the root weight of egg plant after combined application in the green house was tested. The results were statistically significant (P = 0.05). Due to attack of root knot nematode maximum root weight was observed in control plants. Minimum root weight was observed in the plants having combined application of *T. harzianum* and *M. oleifera*. The results was noted that root length varied significantly (P = 0.05) in all treatments. Maximum shoot length was observed in

Table I.- Efficacy of *T. harzianum* and *M. oleifera* on juvenile mortality and egg hatching of *M. javanica*.

Treatments	Concentrations	Mortality after (hours)			Egg hatching after (Days)		
		24	48	72	1 day	3 day	7 day
T. harzianum	S	23.3d	30.0e	38.6b	6st	12pq	20lm
T. harzianum	S/2	12.0ghi	17.3gh	22.0d	14op	18mn	28hi
T. harzianum	S/4	2.6lm	6.6jk	10.6hi	18mn	26ij	34f
M. oleifera	S	14.0fg	18.0c	30.0c	8rs	24jk	40e
M. oleifera	S/2	9.3ij	12.6e	24.0d	14op	30gh	46d
M. oleifera	S/4	4.6kl	6.6jk	16.0ef	22kl	34f	54c
T. harzianum+ *M. oleifera*	S	30.0c	40.0b	46.0a	4t	10qr	16no
T. harzianum+ *M. oleifera*	S/2	16.0ef	22.0d	28.0c	10qr	16no	22kl
T. harzianum+ *M. oleifera*	S/4	6.0k	10.0hi	16.0ef	17mno	24jk	32fg
Control		0.0m	0.6m	2.0lm	22kl	58b	64a

Data are mean of three replications. Means within a column sharing the same letter are not significantly different from each other at P = 0.05 according to Duncan Multiple Range Test. For juvenile mortality LSD (T) = 0.9875, LSD (I) = 1.8089, LSD (TXI) = 3.1227. for Egg hatching LSD (T) = 1.1828, LSD (I) = 2.1594, LSD (TXI) = 3.7402

Table II.- Growth responses of Egg plant against *M. javanica* treated with combine application of *T. harzianum* and *M. oleifera*.

T. harzianum + *M. oleifera*	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)	No. of leaves
Protective	16.2a	2.2a	26.4a	9.5a	11.4a
Curative	13.8b	2.9ab	23.8b	6.7b	7.5b
Control	10.7c	4.7a	12.5c	4.9b	4.5c
LSD value	2.6	2.1	3.8	3.6	3.6

*Means followed by the same letter are not significant from each other at P = 0.05 according to Duncan's Multiple Range Test.

Table III.- Effect on reproduction parameters of *M. javanica* treated with combine application of *T. harzianum* and *M. oleifera*.

T. harzianum + *M. oleifera*	No. of galls	No. of females	No. of egg masses	J2/root system	J2/100 ml^3 soil
Protective	67.5c	57.2c	40.2c	286.4c	910.4c
Curative	82.4b	72.8b	48.6b	345.5b	1124.2b
Control	290.0a	315.5a	255.5a	1720.2a	1580.8a
LSD value	2.1	4.2	3.0	4.2	5.0

*Means followed by the same letter are not significant from each other at P = 0.05 according to Duncan's Multiple Range Test.

combined application. Minimum shoot length was observed in control plants due to the attack of root knot nematodes. The maximum shoot weight was recorded in combined application. The minimum shoot weight was observed in control plants due to the attack of root knot nematode. All the treatments were statistically significant (P = 0.05). The data was recorded that maximum number of leaves was in combined application. The minimum number of leaves was recorded in control plants. All the treatments were statistically significant (P = 0.05).

Nematode reproduction parameter

Effect of *T. harzianum* and *M. oleifera* on reproduction of nematode *M. javanica* on eggplant was evaluated. Number of egg mass, number of females per root system, J2/100ml^3 of soil , J2 per root system and number of galls per root system were recorded. Number of galls was more significant (P = 0.05) on control plant. Protective application of *T. harzianum* and *M. oleifera* was significant as compared to other treatment. Comparison of treatment means indicated that

protective application was more effective as compared to other treatment (Table III). Due to nematodes, control treatment showed maximum number of females and the protective application of *T. harzianum* and *M. oleifera* showed lowest number of females as compared to other treatment. Significant number of females was developed in control plants. In Curative application more number of egg masses (48.6) was observed as compared to protective application of *T. harzianum* and *M. oleifera* (40.2). In control plants maximum number of J2 was observed. In protective application less number of J2 (284.4) was observed. Protective application of *T. harzianum* and *M. oleifera* showed lowest number of J2 (910.4) after 60 days. Control showed more number of J2 due to pathogenic effect of nematodes. As compared to all other treatment control was highly significant as compared to all other treatments.

DISCUSSION

Root knot nematodes are most important and very imperative group of plant parasitic nematodes occurring all over the world but found more in areas having warm climate. Biological management of nematodes has become one of the most feasible alternatives to the nematicide. Different practices are used in then integrated pest management (IPM) but the biological control would be the most enviable, because most of the successful nematicides have been banned in agriculture because of high risk to human health and environment (Veremis and Roberts, 1996). The biological management of nematodes has become reviewed (Kerry, 1987; Stirling, 1991).

The results of present investigation proved that *T. harzianum* and *M. oleifera* suppressed nematode population under green house conditions, which are in conformity with other investigators (Kerry and De-Leij, 1992; Parveen *et al*., 1993; Hafeez *et al*., 2000; Meyer *et al.*, 2000; Sharon *et al*., 2001; Claudius-Cole *et al*., 2010). Filtrates from various strains of fungi shown nematicidal activity (Hallman and Sikora, 1996; Abbas *et al*., 2011). In our study it was observed that *T. harzianum* and Moringa leaves extract have the potential of egg hatching inhibition. Egg hatching inhibition of *M. javanica* increased with the concentration and duration of exposure. Maximum egg hatching inhibition was assessed after 72 h. Most of the antagonistic fungi are egg parasitizing but few culture filtrates of these fungi produced the antibiotics, glioviren and gliotoxin (Lumsden *et al*., 1992). Antagonistic fungi possessed larvicidal and oviposidal properties against root knot nematodes. It was assessed from the present investigation that not only that plant growth parameters significantly enhanced but also the reproduction of *M. javanica* was reduced. It was observed that root weight was directly proportional to the no. of galls which were highest in check plants. The growth parameters were improved by biological control agent *T. harzianum* which is inconformity with the results of previous studies (Sharma and Pandey, 2009; Mahfouz *et al*., 2010). Devi (2002) evaluated the effect of *Ocimum sanctum*, *Azadirachta indica, Myristica malabarica, M. oleifera, Mangifera indica*, and *Acacia Arabica* on mung bean, cowpea, and urdbean against nematode population and observed that they not only reduced nematode population but also enhanced plant yield. From the present findings it has been concluded that combine application of *T. harzianum* and *M. oleifera* proved to be very effective in the management of *M. javanica* both in vitro and under field conditions.

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