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SEED DRESSING WITH BIOCONTROL AGENTS AND NEMATICIDES FOR THE CONTROL OF ROOT KNOT NEMATODE ON SUNFLOWER AND OKRA

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Abstract

Application of biocontrol agents viz., *Rhizobium meliloti*, *Bacillus thuringiensis*, *Aspergillus niger*, *Trichoderma harzianum* and nematicides viz., fertinemakil, vermox as seed dressing for the reduction of *Meloidogyne javanica* infection on sunflower and okra was examined. *In vitro* experiments, maximum reduction in hatching of *M. javanica* egg was observed in *R. meliloti* aqueous extract whereas *T. harzianum* showed significant mortality of second stage juveniles of *M. javanica*. *In vivo* experiment the biocontrol agents and nematicides coated with sugar, mollasses, glucose and gum arabic used @ 1 and 2% significantly reduced the infection of root knot nematode on okra and sunflower roots. An increase in concentration of coating materials significantly enhanced the germination and growth parameters in terms of shoot length, shoot weight, root length and root weight on both okra and sunflower plants. Of the different microbial antagonists and nematicides used, *T. harzianum* and fertinemakil were found more effective followed by *R. meliloti, B. thuringiensis,* vermox and *A. niger* in the control of root knot nematode.

Introduction

The diseases by plant parasitic nematodes reduce the yield of the world's 40 major food and cash crop by an average of 12.3% for the 20 life sustending crops that serves as men primary food source with an estimated annual yield loss of 10.7% for the 20 crops not considering to be life sustending (Sasser & Freekman, 1987). Root knot nematodes are plant parasitic nematodes from the genus *Meloidogyne*. The members of the genus have a wide host range of plant species (Goodey *et al.*, 1965). Upto 70 *Meloidogyne* species have been identified so far (Luc *et al*, 1988). *M. javanica* (Treub) Chitwood is the most common in subtropical and tropical regions (Schneider, 1991). About 2000 plants are susceptible to infection by root knot nematodes and they cause approximately 5% of global crop looses. Root knot nematode larvae infect plant root causing the development of root knot galls that drains the plant's photosynthetic and nutrient. In addition these nematodes have the ability to interact synergistically with other plant pathogen and cause up to 5-34% yield losses in vegetables in tropical climates (Eisenback & Triantaphyllous, 1991).

The biology, ecology and potential of biological control agents for the control of nematodes have been extensively reviewed in recent years (Kerry, 1987; Stirling, 1991; Sayre & Walter, 1991; Sikora, 1992; Baoyn Tian *et al.*, 2007). Nematologists have identified natural enemies with a range of modes of action similar to those currently studied by plant pathologists for the control of soil-borne diseases. *Rhizobium* spp., have a beneficial effect on plants including biological control of soil borne pathogens, induce systematic resistance to plant pathogen and improvement of nutrient uptake of plant (Seuk Bae *et al*., 2000). The damage caused by nematode was more in unbacterized plants than in bacterized ones. Nematode multiplication reduced in the presence of *Rhizobium* (Siddiqui & Mahmood, 1995). *B. thuringiensis* toxins have also been shown to be somewhat active towards species of *Meloidogyne* (Devidas & Rehberger, 1992). There are reports where use of *T. harzianum* significantly suppressed root knot diseases in maize (Windhum *et al*., 1989). *T. harzianum* has also been found as an egg parasite of *M. javanica* race-3 killing 53% of eggs *In vitro* (Santos *et al*., 1992). Present research was undertaken to control root knot nematode on okra (*Abelmoschus esculentus* L.) and sunflower (*Helianthus annus* L.) by using different biological antagonists and nematicides.

Materials and Methods

Microbial antagonists viz., *Bacillus thuringiensis* (Bt 10), *Rhizobium meliloti* (R5), *Aspergillus niger* (An 20) and *Trichoderma harzianum* (KUCC 65) obtained from Karachi University culture collection (KUCC) and nematicides fertinemakil and vermox obtained from local market were used.

***In vitro* studies:** For hatching test, eggs of *M. javanica* were obtained from the roots of egg plant (*Solanum melongena* L.) collected by the method of Hussey & Barker (1973). Eggs suspension was prepared in distilled water and 2 ml suspension containing 20-40 eggs were poured in each cavity block with microbial antagonists and nematicides and kept at room temperature (34-38˚C). Cavity blocks without suspension served as control. Each treatment was replicated three times. The numbers of juveniles were counted at 24, 48 and 72 hrs intervals.

For mortality test, freshly hatched second stage juveniles of *M. javanica* were suspended in sterile distilled water and 2 ml of this suspension containing 15-20 larvae/ml was placed in each cavity block. Cavity blocks without extract of microbial antagonists and nematicides served as control. There were three replicates of each treatment. The number of juveniles that were killed at 24, 48 and 72 h intervals was recorded using a stereoscope.

***In vivo* experiments:** The roots infested with *M. javanica* root knot nematode were collected from the experimental plot of Department of Botany, University of Karachi. The roots were washed under running tap water and cut into small pieces then dipped in 100 ml of 1% $Ca\ (OCl)_2$ in a bottle and the mouth was tightly closed then vigorously shake by hands for 5 min and content was poured on to a 100 or 200 mesh sieve fitted over a 400 mesh sieve, and the roots were washed under running tap water for 1 min. The residues from 400 mesh sieve were transferred into 250 ml beaker. Number of eggs and larvae/ml of suspension were determined with the help of counting dish (Hussey & Barker, 1973).

Five seeds each of okra (*Abelmoschus esculentus* L.) and sunflower (*Helianthus annus* L.) were surface sterilized with 1% $Ca(OCl)_2$ for three minutes, rinsed thoroughly in running water and dried aseptically. The seeds were treated with microbial antagonists viz, *B. thuringiensis, R. meliloti, A. niger, T. harzianum* separately by using 1% and 2% sugar, mollases, glucose and gum arabic solution as a sticker. Ten seeds after treatment with suspension of microbial antagonists were transferred in test tube containing 9ml sterilized distilled water. The test tube was shaken and dilution series was made. One ml suspension was poured on PDA and cells/seed of bacteria and number of conidia/seed of fungi was calculated by using the formula:

$$\frac{\text{Number of cells}}{\text{Seed and conidia}} \text{ X dilution factor}$$

Table 1. Population of bacteria and fungi on seeds of okra and sunflower after seed treatment.

Treatments	Cfu/Seed			
	Okra		Sunflower	
	1%	2%	1%	2%
	A. niger			
Sugar	$16x10^5$	$13 x10^5$	$30 x10^5$	$24 x10^5$
Mollases	$14 x10^5$	$14 x10^5$	$16 x10^5$	$21 x10^5$
Glucose	$19 x10^5$	$12 x10^5$	$31 x10^5$	$34 x10^5$
Gum arabic	$13 x10^5$	$15 x10^5$	$15 x10^5$	$15 x10^5$
	T. harzianum			
Sugar	$11 x10^5$	$10 x10^5$	$15 x10^5$	$13 x10^5$
Mollases	$18 x10^5$	$17 x10^5$	$17 x10^5$	$14 x10^5$
Glucose	$12 x10^5$	$8 x10^5$	$22 x10^5$	$20 x10^5$
Gum arabic	$10 x10^5$	$5 x10^5$	$10 x10^5$	$7 x10^5$
	B. thuringiensis			
Sugar	$10 x10^5$	$8 x10^5$	$9 x10^5$	$16 x10^5$
Mollases	$38 x10^5$	$7 x10^5$	$9 x10^5$	$12 x10^5$
Glucose	$18 x10^5$	$10 x10^5$	$10 x10^5$	$12 x10^5$
Gum arabic	$14 x10^5$	$5 x10^5$	$12 x10^5$	$6 x10^5$
	R. meliloti			
Sugar	$53 x10^5$	$76 x10^5$	$60 x10^5$	$30 x10^5$
Mollases	$83 x10^5$	$40 x10^5$	$72 x10^5$	$48 x10^5$
Glucose	$35 x10^5$	$41 x10^5$	$35 x10^5$	$29 x10^5$
Gum arabic	$40 x10^5$	$70 x10^5$	$45 x10^5$	$32 x10^5$

In seed dressing, seeds of okra and sunflower coated with 48 hrs old cultures of *B. thuringiensis, R. meliloti, A. niger, T. harzianum,* fertinemakil and vermox using 1 and 2% gum arabic, sugar, mollasses and glucose solution as sticker were sown in 8cm, diam., plastic pots, each pot containing 300gm soil. Pots were kept randomized on screen house bench at the Department of Botany, University of Karachi, where soil was kept at 40% MHC (Keen & Raczkowski, 1922). After two weeks of plant growth, the plants were inoculated with 2000 freshly hatched second stage juveniles by introducing holes around each plant. Pots without microbial antagonists served as control. Treatments and control were replicated thrice. After 60 days of growth, plants were uprooted and number of root knots was determined.

Data were analyzed and subjected to analysis of variance (ANOVA) using procedure given by Sokal & Rohlf (1995).

Results and Discussion

Population of bacteria and fungi after seed treatment was counted by serial dilution technique (Table 1). *In vitro* studies, aqueous extract of all biocontrol agents and nematicides showed reduction in *M. javanica* egg hatching. *R. meliloti* was found more effective in reducing egg hatching followed by *B. thuringiensis*, *A. niger*, *T. harzianum*, vermox and fertinemakil. Hatching of eggs was reduced considerably with the increase in time period (Table 2).

Table 2. Effect of aqueous extract of microbial antagonists and nematicides on hatching and mortality % of *Meloidogyne javanica.*

Treatments	Time (hrs)					
	Hatching %			Mortality %		
	24	**48**	**72**	**24**	**48**	**72**
Control	5	21	35	2	5	7
A. niger	3	5	8	10	17	34
B. thuringiensis	3	5	7	7	13	20
Fertinemakil	8	15	21	8	11	22
R. meliloti	2	2	3	4	12	24
T. harzianum	0	3	6	19	26	52
Vermox	3	7	15	3	9	17
	$LSD0.05_{treatment} = 2.76$, $LSD0.05_{time} = 1.81$			$LSD0.05_{treatment} = 4.37$, $LSD0.05_{time} = 2.86$		

Results showed that aqueous extract of biocontrol agents and nematicides caused appreciable mortality of *M. javanica* juveniles due to the reasons that they contain some compounds which are toxic to *M. javanica* and produce lethal effect. *T. harzianum* was found to be more effective as compared to other biocontrol agents and nematicides for mortality of *M. javanica* (Table 2).

An increase in germination of okra seeds and significant increase in shoot length, shoot weight, root length and root weight were observed. Significant reduction of number of knots ($p<0.001$) was observed in okra plant when seeds were treated with microbial antagonists viz., *B. thuringiensis, R. meliloti, Aspergilus niger, T. harzianum* using 1 and 2% of sugar, mollases, glucose and gum arabic as stickers. There was significant increase in shoot length, shoot weight, root length and root weight ($p<0.001$) and significant reduction in number of knots ($p<0.001$) on sunflower plant was observed. Results showed that use of nematicides viz, fertinemakil and vermox significantly increased the germination and growth parameters in terms of shoot length, shoot weight, root length and root weight and reduction in number of knots was observed on sunflower and okra plants. Among the different microbial antagonists used *B. thuringiensis* and *T. harzianum* showed significant results for growth of plant and reduction in number of knots on both sunflower and okra plants. Of the different coating materials used with different concentration, 2% concentration showed significant reduction in number of knots on both sunflower and okra plants (Table 3).

Present result showed that aqueous extract of microbial antagonists exhibited nematicidal activity which reduced egg hatching of *M. javanica* and increased mortality of larvae with the increase in exposure time. Presumably the production of antibiotics (Dennis & Webster, 1971) and extracellular enzymes (Elad *et al.*, 1982) are involved in antagonisms. Dawar *et al.*, (2008) observed that *Bacillus* species viz., *B. subtilis, B. thuringiensis* and *B. cereus* significantly reduced hatching of larvae of *M. javanica* root knot whereas mortality of larvae was significantly increased with an increase in time. Seed treatment is an attractive method for introducing biocontrol agents into a soil root environment since it protects the seed from seed-borne and soil-borne pathogens and enable the seed to germinate and become established as a healthy seedling (Chang & Kommedahl, 1968). *T. harzianum* has also been found as egg parasite of *M. javanica* race-3 killing 53% of eggs *In vitro* (Santos *et al*., 1992). Besides parasitisms of the root knot nematode, it is also hypothesized that the production of nematicidal compounds by *Trichoderma* spp., directly affect the nematode or made root less attractive which might

have resulted in the reduction in nematode population. In the present study, maximum disease suppression induced by *T. harzianum* followed by *A. niger* is attributed to its parasitic nature and production of nematicidal serine proteases which degrades egg shell and check egg hatching (Bonants *et al*., 1994). *A. niger* applied alone or in combination with the bacterial inoculants inhibited root-knot nematode galling in tomato (Siddiqui *et al.*, 2003).

In the present study, shoot length, shoot weight, root length and root weight were significantly increased in sunflower and okra when seeds were coated with *R. meliloti* and *B. thuringiensis*. Similar report was made by (Siddique *et al*., 2000) in okra where *Rhizobia* used as seed dressing and soil drenching significantly increased growth parameters and number of nodules. In the present investigation, *Rhizobium* used either as seed dressing significantly improved plant growth and reduced disease intensity of plants due to initial colonizers of rhizospheres of test plants. It is interesting to note that *Rhizobia* not only showed significant control of root pathogens on leguminous plants like chickpea, mungbean as well as non leguminous plants like okra and sunflower but also increased plant height and fresh shoot weight (Zaki, 2000). Present results showed that seed coated with *B. thuringiensis* (Bt) strains exhibited nematicidal activity on okra and sunflower. *B. thuringiensis* toxins have also been shown to be somewhat active towards species of *Meloidogyne* (Devidas & Rehberger, 1992).

The results of the present study indicates the potentialities of seed treatment with fungal and bacterial antagonists viz., *B. thuringiensis, R. meliloti, A. niger, T. harzianum* and nematicides in the suppression of root knot nematode on okra and sunflower. There is therefore need to characterize nematicidal compound produced by biological antagonists resulting in control of root knot nematode instead of use of pesticides, which are costly and hazardous.

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