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APPLICATION OF *BACILLUS* SPECIES IN CONTROL OF *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD ON COWPEA AND MASH BEAN

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Abstract

Application of *Bacillus* spp., significantly reduced hatching of larvae of *Meloidogyne javanica* root knot whereas mortality of larvae was significantly increased with the increase in time. Germination of seeds of cow pea and mash bean and growth parameters in terms of shoot length, root length, shoot weight and root weight significantly increased in treated seed and soil with all the species of *Bacillus* as compared to control. Maximum inhibition of knots was observed in cowpea as compared to mung bean. Of the different species of *Bacillus* used, *B. subtilis* showed maximum inhibition of knots.

Introduction

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops including green gram (Sikora & Greco, 1993). The infection starts with root penetration of second stage juveniles hatched in soil from eggs encapsulated in egg masses laid by the females on the infected roots (Barker *et al*., 1985). The interaction between the root infecting fungus and the nematode results in the reduction of seed emergence and increase in both galling and nematode fecundity, with the results that the population of *M. javanica* increased in the presence of *Rhizoctonia solani* (Kassab & Ali, 1996). Simultaneously, the disease development caused by soil-borne fungal pathogens was also stimulated by nematode on soy bean (Shawadfy & Mousa, 1997).

Chemical control is becoming more and more expensive because of increased costs in the synthesis of new compounds and their use is increasingly undesirable because of environmental hazards associated with their application. Now some attention has been given to biological control of plant-parasitic nematodes with the use of natural enemies as a safe and cheap alternative method to chemical control (Gowen & Ahmad, 1990). Encouraging results were obtained with the use of *Pasteuria penetrans* and *Paecilomyces lilacinus* as biological control agents of nematodes on different crops (Zaki & Maqbool, 1991a, b).

Bacillus sp., produces large, spreading, gray-white colonies with irregular margins. A unique characteristic of this bacterium is its ability to produce endospores when environmental conditions are stressful. Although most species of *Bacillus* are harmless saprophytes, two species viz., *B. thuringiensis* and *B. cereus* are considered medically significant. *B. thuringiensis* is a plant growth promoting bacterium which produces bacteriocin compounds (Gray *et al*., 2006). Bankole & Adebanjo (1998) reported that soils inoculated with *B. subtilis, B. cereus and Trichoderma* spp., reduced seedling infection and that the efficacy of antagonists increased with increase in dose. Lytic enzymes are known to be produced by *B. cereus* (Csuzi, 1978), these enzymes and other

antibiotics produced by *B. cereus* have been reported to have antagonistic effects on some microorganisms (Dorherty & Preece, 1988). *B. thuringiensis* (commonly known as 'Bt') is an insecticidal bacterium, marketed worldwide for control of many important plant pests, mainly caterpillars of Lepidoptera, mosquito larvae and black flies etc. Bt products represent about 1% of the total "agrochemical" market (fungicides, herbicides and insecticides) across the world (Knowles, 1994). The commercial Bt products are powders containing a mixture of dried spores and toxin crystals. They are applied to leaves or other environments where the insect larvae feed. The toxin genes have also been genetically engineered into several crops. Application of bacteria either as seed dressing or as soil drenching has shown a significant suppression of root infecting pathogens on leguminous and non- leguminous plants (Shahzad & Ghaffar, 1992). Experiments were therefore carried out to examined the efficacy of *Bacillus* spp., in the control of root knot nematode on cow pea (*Vigna unguiculata* L.) and mash bean (*Vigna mungo* L.).

Materials and Methods

Cultures of different species of *Bacillus* viz., *B. subtilis* (Bs-12), *B. thuringiensis* (Bt-10), and *B. cereus* (Bc-20) were obtained from Department of Microbiology, University of Karachi.

***In vitro* experiments:** beef extract 1.5 gm, peptone 2.5 gm and glucose 1.25gm were added in 500ml of distilled water to prepare nutrient broth.

For hatching test, eggs of *M. javanica* was 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 or without *Bacillus* spp., 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 suspension of *Bacillus* spp., served as control. There were three replicates of each treatment. The number of juveniles that were killed at the 24, 48 and 72 h intervals was recorded using a stereoscope.

***In vivo* experiment:** The roots infested with root knot nematode *M. javanica* 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 100ml of 1% Ca (OCl)2 in a bottle and 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).

In seed dressing, seeds of cow pea and mash bean coated with 48 hrs old cultures of *B. subtilis*, *B. thuringiensis* and *B. cereus* using with 2% gum arabic solution as sticker were sown in 8cm, diam., plastic pots, each pot containing 300gm soil. Pots were kept

Table 1. Effect of *B. subtilis, B. thuringiensis* and *B. cereus* on hatching and mortality of *Meloidogyne javanica* at different time intervals.

Treatments	Time (hrs)			
	0	**24**	**48**	**72**
	Hatching			
Control	20	43	23	17
B. subtilis	22	18	8	7
B. thuringensis	21	22	13	22
B. cereus	14	29	12	5
	Mortality			
Control	12	6	12	26
B. subtilis	17	12	14	34
B. thuringensis	13	8	16	42
B. cereus	14	21	21	40

randomized on screen house bench at the Department of Botany, University of Karachi, where soil was kept @ 40% MHC (Keen & Raczkowski, 1922). In soil drenching with *Bacillus* spp., a 20 ml cell suspension of *Bacillus* spp., viz; *B. subtilis* ($8.83x10^9$ cells/ml), *B. thuringiensis* ($2.1x10^9$ cells/ml), *and B. cereus* ($9.3x10^9$ cells/ml) were drenched in 8cm diam., plastic pots each containing 300 gm soil. Seeds of cowpea and mash bean were sown @ 5 seeds / pot. Pots were kept randomized on a screen house bench at the Department of Botany, University of Karachi, where soil was kept @ 40% M.H.C (Keen & Raczkowski, 1922). Pots without bacterial suspension and juveniles served as control. The pots were arranged in randomized complete block design. After two weeks of plant growth, the plants were inoculated with 1000 larvae/pot. 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 Gomez & Gomez (1984).

Results and Discussion

***In vitro*:** The present study showed that *Bacillus* spp., viz., *B. subtilis, B. thuringiensis* and *B. cereus* reduced the hatching of eggs of *M. javanica* to varying degree. *B. cereus* exerted maximum lethal effect, only few juveniles were hatched (Table 1). The mortality of *Meloidogyne* spp., increased as the exposure period increased, *Bacillus subtilis, B. thuringiensis* and *B. cereus* caused 50% mortality (Table 1). Siddiqui *et al*., (2000) obtained the same result by using ethylacetate and hexane fraction at different concentration in the mortality of *M. javanica*.

***In vivo* effect:** To study the effect of *Bacillus* spp., in the control of root knot nematode seeds of cow pea and mash bean plants were coated with the *Bacillus* spp., viz., *B. subtilis* @ (89.33×10^6 cells /seed), *B. thuringiensis* @ (67.66×10^6 cells /seed) and *B. cereus* @ (58×10^6 cells /seed) in cow pea where as in mash bean *B. subtilis* @ (133.33×10^6 cells /seed), *B. thuringiensis* @ (70×10^6 cells /seed) and *B. cereus* @ (74.66×10^6 cells /seed). *Bacillus* species showed 100 % germination of mung bean seeds (Table 2). Growth parameters as shoot length, root length ($p<0.05$), shoot weight and root weight of cow pea and mash bean significantly increased in seed treatment with all the species of *Bacillus* as compared to control (Table 2). Maximum increase in growth parameters in mung bean was

observed. Sheikh *et al*., (2006) reported that *B. thuringiensis* applied as seed dressing and soil drenching showed a significant increase in seed germination, shoot length, shoot weight, root length and root weight Present result showed a significant reduction ($p<0.01$) in the number of knots in cow pea and mash bean plants. Mehdi *et al*., (2001) reported that *A. marina* and *R. mucronata* with or without *Pseudomonas aeruginosa* significantly reduced the root knot infection in tomato. Maximum inhibition of knots was observed in cowpea. Of the different species of *Bacillus* used, *B. cereus* showed maximum inhibition of knots (Table 2).

A 20 ml cell suspension of *Bacillus* spp., viz., *B. subtilis* ($8.83x10^9$ cells/ml), *B. thuringiensis* ($2.1x10^9$ cells/ml) and *B. cereus* ($9.3x10^9$ cells/ml) were drenched in soil to study the effect of *Bacillus* spp., in the control of root knot nematode on cow pea and mash bean plants. Plant growth parameters in term of shoot length, shoot weight, root length ($p<0.05$) and root weight were significantly increased (Table 2). Knots /plant were significantly reduced in both cow pea and mash bean plants ($p<0.05$). The present result showed that of the three *Bacillus* spp., used, *B. subtilis* showed significant suppression of knots in both cow pea and mash bean plants. *B. thuringiensis* and *B. cereus* also showed more inhibition of knots in mash bean than in cow pea plants. In case of low dosage of all *Bacillus* spp., less inhibition was observed. The application of *B. thuringiensis* reduced root length, root biomass and reduced multiplication of nematodes by 1.5 to 3 times as compared to control on barley plant (Irina *et al.*, 2002). Present results indicates the abilities of *Bacillus* species viz., *B. cereus*, *B. subtilis* and *B. thuringiensis* as soil drenching and seed dressing in the suppression of root knot nematodes on mung bean and cow pea. There is need to characterize nematicidal compound (s) produced by *Bacillus* species in control of root knot nematodes instead of use of pesticides, which are costly and hazardous.

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