

EVALUATION OF *BACILLUS THURINGIENSIS* ISOLATES AGAINST ROOT-KNOT NEMATODES FOLLOWING SEED APPLICATION IN OKRA AND MUNGBEAN

M. QASIM KHAN, M. WASEEM ABBASI, M. JAVED ZAKI
AND SHAKEEL AHMED KHAN*

Department of Botany, University of Karachi, Karachi-75270, Pakistan

**Department of Microbiology, University of Karachi, Karachi-75270, Pakistan*

Abstract

Bacillus thuringiensis, an insecticidal bacterium is widely used as a biological control agent for a number of insects. During a survey, 10 isolates of *Bacillus thuringiensis* were isolated from rhizosphere of okra, brinjal, tomato, cotton, cabbage, onion and water melon crops cultivated in Karachi, Thatta, Hyderabad and Nawabshah. These isolates were evaluated for their nematocidal potential under laboratory and green house experiments. In laboratory studies, eggs and Juveniles were exposed to different BT isolates at 50% concentration of cell free filtrate for different time intervals. The cell free filtrate not only inhibited the hatching of eggs but also killed the second stage juveniles at different time intervals. In green house studies seed treatment of okra (*Abelmoschus esculentus* (L.) Moench var Arka anamika) and Mung bean (*Vigna radiata* [L.] Wilczek cv.MN-95) with cell suspension of BT isolates improved plant growth and reduced number of galls, Egg masses, Eggs/egg mass, Nematode population /g roots and Nematode population /200g soil as compared to non-treated control.

Introduction

The root-knot nematodes (*Meloidogyne* spp.) are world wide in distribution attacking many economically important crops. The use of chemicals is still an effective measure to control plant diseases, however due to toxicity and high cost of the chemicals, alternative control measures have been investigated. A number of bacterial species have been used as biological control agents (Ali *et al.*, 2002; Siddiqui & Shaukat, 2002; Tian *et al.*, 2007). These bacteria may enhance plant growth and suppress nematode population (Siddiqui, 2002; Sikora & Hoffmann-Hergarten, 1993, Zuckermann *et al.*, 1993).

Bacillus thuringiensis was first discovered in Japan (Ishiwata, 1901) from diseased silk-worm larvae identified as sotto disease of silk worm. A German biologist Berliner (1911) isolated it from pupae of Mediterranean flour moth *Ephastia kuchmiella* living in stored grains in the city of Thuringen and hence named it as *Bacillus thuringiensis*. *B. thuringiensis* is a rod shaped Gram positive, aerobic, spore forming bacterium that has been used as a biological insecticide for many years and is a uniquely safe and effective tool for the control of a wide variety of insect pests (Trailer, *et al.*, 1992, Borgonie *et al.*, 1996; Nester, *et al.*, 2002; Wei *et al.*, 2003). Available information depict that *B.thuringiensis* is a versatile pathogen capable of infecting protozoa, nematodes, flatworms, mites and insects. (Feitelson, 993). *B. thuringiensis* is characterized by the production of parasporal crystals composed of protein molecules known as delta-endotoxins that are toxic to insect pests (Höfte & Whiteley, 1989). This unique property of producing delta-endotoxins distinguished *B.thuringiensis* from other spore forming bacteria. There are only a few studies on the use of *B. thuringiensis* as a biocontrol agent of plant parasitic nematodes (Esnard *et al.*, 1998; El-Sherif *et al.*, 1995 Sharma, 1994). Keeping in view the importance of *B.thuringiensis* as a biological control agent, *In vitro* and green house studies were conducted to evaluate the nematocidal potential of indigenous BT isolates against root-knot nematode.

Materials and Methods

Survey, isolation and identification of *Bacillus thuringiensis*: During a survey in 2007, soil samples were collected from okra, brinjal, tomato, cotton, cabbage, onion and watermelon crops cultivated at different localities of Pakistan including Karachi, Thatta, Hyderabad and Nawabshah. Soil samples were collected by removing upper layer of the soil. A 50g sample was taken in sterile containers, the container were sealed and brought to laboratory for further use. Serial dilution technique was used for the isolation of *Bacillus thuringiensis* from rhizosphere soil. One g soil sample was dispensed in 9ml sterile water. From the final dilution, 100 μ l aliquot was transferred to Petri plates and 10ml Lauria Bertani (LB) agar (trypton 10g, yeast extract 5g, sodium chloride 5g, and agar 20g dissolved in one liter sterile distilled water and autoclaved at 121°C for 15 min) was poured in Petri plates. The plates were incubated at room temperature 30 \pm 5°C for 24 hours. In order to identify the selected isolate, different biochemical characteristics (gram staining, spore staining, aerobic growth, catalase test, starch Hydrolysis test, methyle red test, nitrate reduction test and growth on T₃ medium) were studied according to Bergey's Manual of Systematic Bacteriology. The isolated bacterial isolates were grown in LB broth at 37°C for 72hrs in shaking incubator and centrifuged twice at 3000 g for 20 min. The cell pellets were discarded and cell free culture filtrate was collected in a sterile beaker before use.

Laboratory studies: To determine the effect of cell free culture filtrate on egg hatching and juveniles mortality, eggs of *Meloidogyne javanica* (Treub) Chitwood were obtained from the egg plant roots as suggested by Hussey & Barker (1973). Egg suspension was prepared in sterile water and 50% concentration was prepared by taking one ml of culture filtrate and one ml of egg suspension (50-75 eggs/ml) in 3cm cavity blocks. For mortality test, eggs of *M. javanica* were placed in sterile water. Three days after incubation the hatched juveniles were collected in a beaker and 50% concentration was prepared by taking one ml of the juvenile's suspension (45-65 juveniles/ml) and one ml culture filtrate. The cavity blocks containing sterile water served as control. After 24, 48, 72 and 96hrs, numbers of hatched juveniles and 24, 48 and 72hrs dead juveniles were counted and mean percentage hatched eggs and dead juveniles was estimated (Cayrol *et al.*, 1989).

Greenhouse studies: As most of the BT isolates inhibited hatching and caused juvenile mortality *In vitro*, BT-8, BT-9, BT-10, BT-14 and BT-64 were selected for greenhouse studies as seed treatment on Okra (*Abelmoschus esculentus* (L.) Moench cv. Arka anamica) and Mung bean (*Vigna radiata* [L.] Wilczek cv.MN-95). The bacterial isolates were grown on LB broth for 2 days in a shaking incubator and centrifuged twice at 3000 g for 20 min and pellets of cells were used for seed treatments. In two sets of experiments Okra and Mungbean seeds after surface sterilization in 1% Na(OCl)₂ for 2 min were rinsed several times with sterile distilled water and treated with 2 days old bacterial cell suspension of different BT isolates using 1% gum arabic as a sticker. After treatment 5 seeds were sown in each pot containing 300g of soil. Seed treated with sterile water served as control. Each treatment was replicated thrice. Pots were kept in completely randomized design on green house bench. After germination only two seedlings were maintained / pot. Ten days after seedling emergence 1500 J₂ of root knot nematodes were introduced in each pot. Plants were uprooted and plant growth parameters and root knot infection was recorded after 45 days of nematode inoculation.

Data analysis: Data were analyzed and subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) depending upon the experimental design. The follow up of FANOVA included Least Significant Difference (LSD). Duncan's multiple range test was also used to compare the treatment means (Sokal & Rohlf, 1995).

Results

Survey and isolation of *Bacillus thuringiensis*: During survey in 2007, 40 soil samples were collected from okra, brinjal, tomato, cotton, cabbage, onion and watermelon crops cultivated in different localities of Sindh province including Karachi, Thatta, Hyderabad and Nawabshah. Ten *Bacillus thuringiensis* isolates BT-6, BT-7, BT-8, BT-9, BT-10, BT-13, BT-14, BT-15, BT-16 and BT-64 were isolated from the collected samples.

Laboratory studies: *In vitro* studies revealed that cell free culture filtrate of BT isolates at 50% concentration, significantly ($p < 0.001$) reduced the egg hatching and increased the mortality of J_2 root knot nematode. Bacterial isolates BT-10, BT-14, BT-16 and BT-64 did not show egg hatching even after 96 hrs exposure and exhibited significant ($p < 0.001$) reduction in the egg hatching as compared to untreated control. Other bacterial isolates were also found to be effective in reducing egg hatching at different time intervals as compared to control (Fig. 1). At 50% concentration, cell free filtrate of all tested bacterial isolates significantly ($p < 0.001$) caused mortality of *Meloidogyne javanica* juveniles to a varying extent. However, BT-16 and BT-64 caused 100% juveniles mortality after 24 hrs time interval, as compared to untreated control (Fig. 2).

Greenhouse studies

Effect of BT isolates on plant growth and root knot infection in okra: Seed dressing of okra seeds with BT isolates significantly ($p < 0.001$) increased shoot length in okra plants. BT-64 showed 43% increase in shoot length, followed by BT-14 showing an increase in shoot length by greater than 25%, as compared to control. Shoot weight in okra plants was significantly ($p < 0.001$) increased by 35% when treated with BT-64 isolate, followed by Bt-14 isolate. Maximum increase in root length greater than 36% was observed in BT-64 isolate as compared to untreated control. Root weight was increased by 72% in Bt-64 strain as compared to untreated control.

All the BT isolates used in the experiment significantly ($p < 0.001$) reduced number of galls/ root system. Maximum reduction in gall formation by 76% was observed in BT-64 isolate, followed by BT-14 showing a reduction in number of galls/ root system by greater than 59% as compared to control. BT isolates significantly ($p < 0.001$) reduced number of egg masses/root system. Maximum reduction in egg masses/root system by 64% was recorded in BT-64 isolate, followed by BT-14 reducing the egg masses/root system by 52% as compared to untreated control. All the BT isolates significantly ($p < 0.001$) reduced the number of eggs/egg mass, as compared to untreated control. BT-64 significantly reduced number of eggs/egg mass by greater than 54%. Nematode population/g root was significantly ($p < 0.001$) reduced by all the bacterial isolates. However, maximum reduction in nematode population by 52% was observed in BT-64 isolate. Use of bacterial isolates as seed dressing significantly ($p < 0.001$) reduced the nematode population in soil. Maximum reduction by greater than 51% was revealed in BT-64 as compared to untreated control (Table 1).

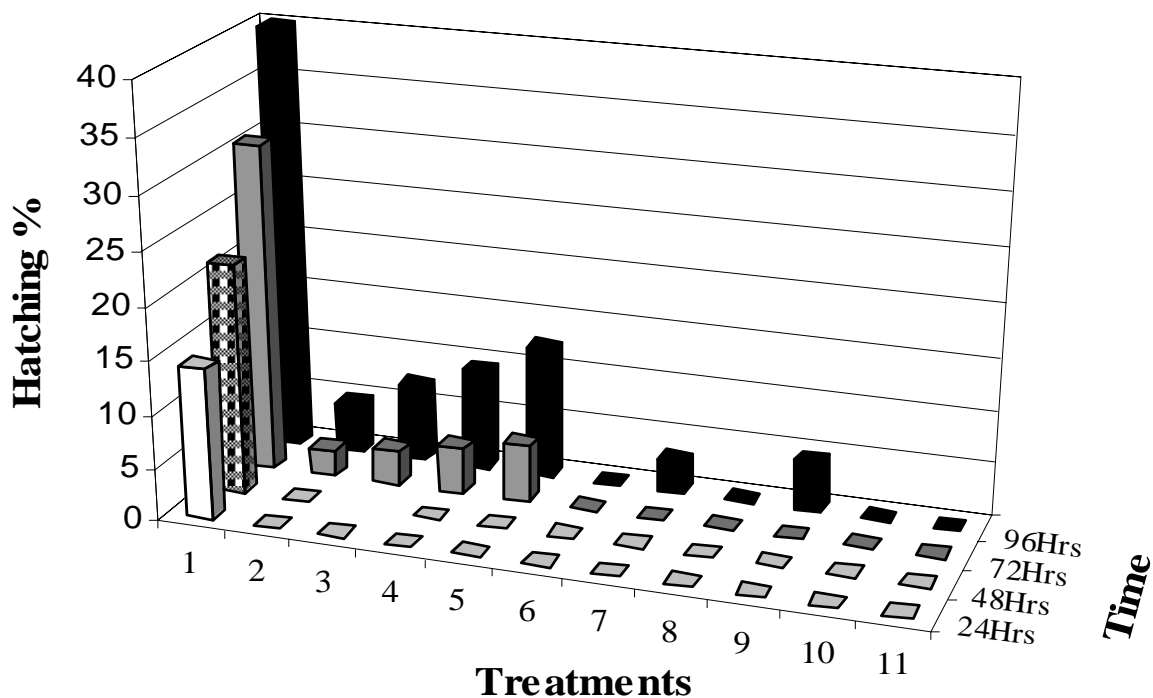


Fig. 1. Effect of BT isolates at 50% concentration on egg hatching of root knot nematodes.

1= Control, 2= BT6, 3= BT7, 4= BT8, 5= BT9, 6= BT10, 7= BT13, 8= BT14, 9= BT15, 10= BT16, 11= BT64.
 Treatments: LSD (0.05) = 1.302, Significant (p<0.001)
 Time: LSD (0.05) = 0.781, Significant (p<0.001)

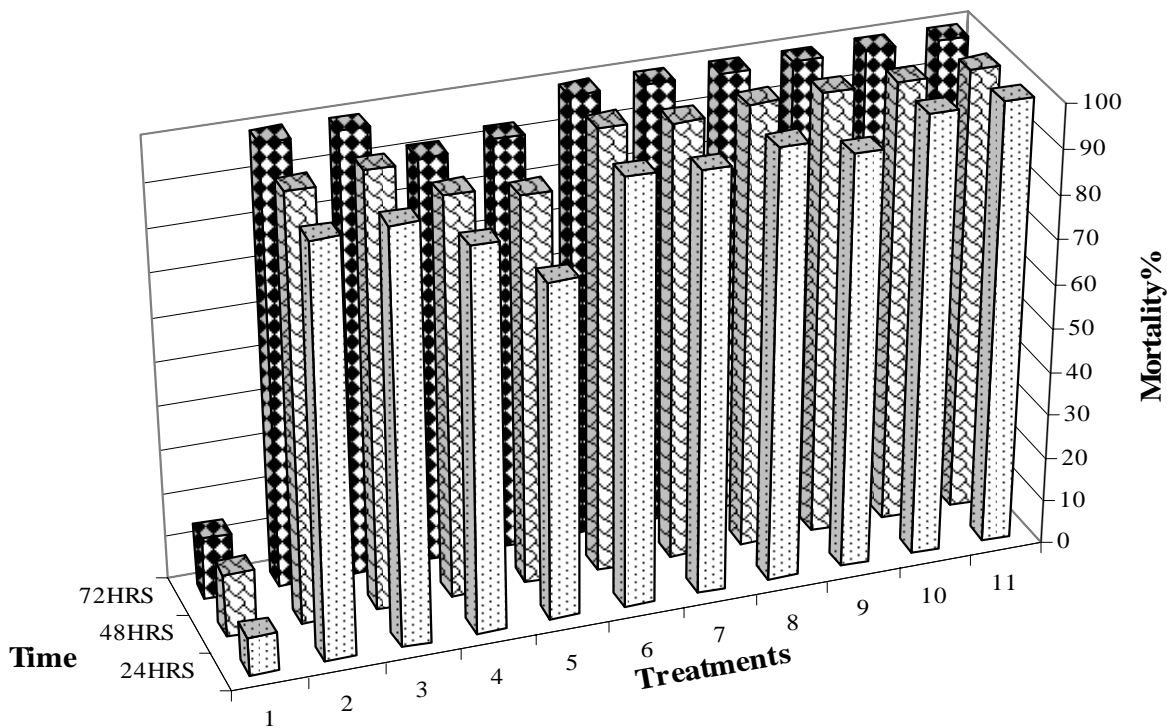


Fig. 2. Effect of BT isolates at 50% concentration on mortality of root knot nematode juveniles.

1= Control, 2= BT6, 3= BT7, 4= BT8, 5= BT9, 6= BT10, 7= BT13, 8= BT14, 9= BT15, 10= BT16, 11= BT64
 Treatments: LSD (0.05) = 3.679, Significant (p<0.001)
 Time: LSD (0.05) = 1.92, Significant (p<0.001)

Effect of BT isolates on plant growth and root knot infection in mung bean: Seed dressing of mung bean seeds with BT isolates showed an increase in plant growth parameters. Shoot length was significantly ($p < 0.001$) increased by 42% in seed treated with BT-64, followed by BT-14 showing 24% increase in shoot length as compared to control. Shoot weight with respect to bacterial isolates was significantly ($p < 0.001$) increased as compared to control. BT-64 isolate revealed maximum shoot weight 97%, followed by BT-14 showing 94% increase in shoot weight. Bacterial isolates significantly ($p < 0.001$) increased the root length in BT-64 maximum increase in root length by 46% was recorded. Root weight data revealed non significant difference among bacterial isolate. However, in BT-64 root weight was significantly increased by 71% as compared to control.

Effect of BT isolates revealed significant ($p < 0.001$) reduction in number of galls/root system as compared to un-treated control. A significant reduction in number of galls/root system by 79% was estimated in BT-64 isolate. Egg masses/root system were significantly ($p < 0.001$) reduced by use of bacterial antagonists as compared to untreated control. Number of eggs/egg mass significantly ($p < 0.001$) reduced by the use of BT isolates. Maximum reduction by 59% was estimated in BT-64 isolate as compared to untreated control. Nematode population/g root revealed that all the bacterial isolates used in the studies significantly ($p < 0.001$) decreased nematode population/g root. In BT-64 isolate nematode population/g root was reduced by 54%. Other BT isolates were also found effective in reducing nematode population/g soil as compared to untreated control. Similarly all bacterial antagonists revealed significant reduction in nematode population in soil as compared to control. Maximum reduction by 51% was recorded in BT-64 isolate as compared to untreated control (Table 2).

Discussion

Substantial work has been done regarding the use of bacteria as biological control agents of soil-borne pathogenic fungi. However, less work has been reported on the potential of *Bacillus thuringiensis* to control plant parasitic nematodes in crop plants. In the present studies, 10 isolates of *B. thuringiensis* (BT-6, BT-7, BT-8, BT-9, BT-10, BT-13, BT-14, BT15, BT-16 and BT-64) were isolated from the cultivated fields of different localities of Sindh province (Karachi, Thatta, Hyderabad, Nawabshah). *Bacillus thuringiensis* is a Gram positive rod shaped spore forming bacterium. It showed no growth in anaerobic conditions. Previous studies also showed that, *B. thuringiensis* seems to be indigenous to many environments (Bernhard *et al.*, 1997). In the present studies BT isolates were found effective in inhibiting egg hatching and causing mortality of *Meloidogyne* spp., *In vitro*. Cell free culture filtrate of BT isolates at 50% concentration was found lethal to nematode larvae, also inhibited egg hatching at different time intervals. Production of metabolites by rhizosphere bacteria (Oostendorp & Sikora, 1990), affect the vitality of second stage juveniles (Becker *et al.*, 1988). Most rhizobacteria act against plant-parasitic nematodes by producing toxins. The effects of these toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as direct killing of nematodes (Siddiqui & Mahmood, 1999). BT produces crystal proteins that target nematodes (Wei *et al.*, 2003). There are six proteins (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21) known to be toxic to larvae of a number of free living or parasitic nematodes (Kotze *et al.*, 2005).

Table 1. Effect of *Bacillus thuringiensis* isolates on plant growth and root-knot infection in okra (*Abelmoschus esculantus* (L) Moench.).

Treatments	Shoot length cm	Shoot weight gm	Root length cm	Root weight gm	No of galls/ Root system	No. of egg masses/Root system	No of eggs/ Egg mass	Nematodes /g root	Nematodes /200 g soil
Control	11.66 c	1.25 d	7.66 c	0.16 e	20 a	18 a	198 a	234 a	900 a
<i>Bacillus thuringiensis</i> BT-8	12.17 c	1.43 c	8.16 bc	0.23 de	16 b	13 b	155 b	192 b	595 b
<i>Bacillus thuringiensis</i> BT-9	13.00 c	1.51 bc	8.50 bc	0.35 bc	10 d	10 d	121 d	150 d	512 d
<i>Bacillus thuringiensis</i> BT-10	12.83 c	1.50 bc	8.50 bc	0.30 cd	12 c	11 c	139 c	169 c	541 c
<i>Bacillus thuringiensis</i> BT-14	15.50 b	1.61 b	9.00 b	0.42 b	8 e	9 d	104 e	131 e	494 d
<i>Bacillus thuringiensis</i> BT-64	20.50 a	2.00 a	12.0 a	0.60 a	5 f	7 e	90 f	112 f	441 e
LSD p<0.05	1.664	0.163	1.005	0.073	0.839	1.185	4.193	6.748	20.610

Table 2. Effect of *Bacillus thuringiensis* isolates on plant growth and root-knot infection in mung bean (*Vigna radiata* (L), Wilczek).

Treatments	Shoot length cm	Shoot weight gm	Root length cm	Root weight gm	No of galls/ Root system	No. of egg masses/Root system	No of eggs/ Egg mass	Nematodes /g root	Nematodes /200 g soil
Control	10.83 d	0.95 d	7.5 e	0.20 d	16 a	16 a	193 a	224 a	844 a
<i>Bacillus thuringiensis</i> BT-8	11.83 cd	1.18 c	8.5 d	0.28 cd	10.b	10 b	140 b	168 b	560 b
<i>Bacillus thuringiensis</i> BT-9	12.83 bc	1.41 b	8.8 d	0.36 bc	7 d	8 cd	102 d	132 d	484 d
<i>Bacillus thuringiensis</i> BT-10	11.83 cd	1.23 c	10.5 c	0.38 b	9 c	8 cd	116 c	145 c	520 c
<i>Bacillus thuringiensis</i> BT-14	14.33 b	1.55 b	11.8 b	0.45 b	5 e	7 d	95 e	120 e	464 e
<i>Bacillus thuringiensis</i> BT-64	18.86 a	1.78 a	14.00 a	0.68 a	2 f	6 e	78 f	104 f	417 f
LSD p<0.05	1.664	0.167	0.938	0.083	0.938	1.109	4.979	5.610	9.837

Under greenhouse conditions seed treatment of okra and mungbean with BT isolates was found very effective to control root knot nematode infection in okra and mungbean. In mungbean, BT-64 and BT-14 showed substantial decrease in root knot infection. Colonization of roots by rhizosphere bacteria has been reported to reduce nematode invasion (Oostendorp & Sikora, 1989). *Meloidogyne incognita* galling on tomato, cucumber and clover was suppressed following application of bacterial soil drenches (Zavaleta-Mejia & Van Gundy, 1982). Similarly, Sikora (1988) reported that treatment of sugarbeet with *Bacillus subtilis* controlled *M. incognita*, *M. arenaria* and *Rotylenchulus reniformis*. Suggested mechanisms include the production of metabolites which reduce hatch and attraction and/or degradation of specific root exudates which control nematode behavior (Sikora & Hoffmann-Hergarten, 1993). Use of bacteria as seed treatment reduced nematode penetration to root plant system of potato and sugar beet (Racke & Sikora, 1992; Oostendorp & Sikora, 1989). Mode of action of some bacteria towards phyto-nematodes has also been documented (Oostendorp & Sikora, 1990; Devidas & Regberger, 1992; Hasky-Gunther *et al.*, 1998).

Acknowledgement

Research work was conducted under a grant received from Higher Education Commission, Pakistan (20-683/r & D / 06), which is sincerely acknowledged.

References

- Ali, N.I., I.A. Siddiqui, S.S. Shaikat and M.J. Zaki. 2002. Nematicidal activity of some strains of *Pseudomonas* spp. *Soil. Biol. Biochem.*, 34: 1051-1058.
- Becker, J.O., E. Zavaleta-Mejia, S.F. Colbert, M.N. Schroth, A.R. Weinhold, J.G. Hancock and S.D. Van Gundy. 1988. Effects of rhizobacteria on root knot nematodes and gall formation. *Phytopath.*, 78: 1466-1469.
- Berliner, E. 1911. Ueber die Schlaftsucht der Nelmottenraupe (*Ephesiakuhniella* Zell) und ihren Erreger *Bacillus thuringiensis* n.sp. *Zeitschrift für angewandte Entomol.*, 21: 29-56.
- Bernhard, K., P. Jarrett, M. Meadows, J. Butt, D.J. Ellis, G.M. Roberts, S. Pauli, P. Rodgers and D. Burges. 1997. Natural isolates of *Bacillus thuringiensis*: worldwide distribution, characterization and activity against insect pests. *J. Invertebr. Pathol.*, 70: 59-68.
- Borgonie, G., R. Van Driessche, F. Leyns, G. Arnaut, D. De Waele and A. Coomans. 1995. Germination of *Bacillus thuringiensis* spores in bacteriophagous nematodes (Nematoda: Rhabditida). *J. Invertebr. Pathol.*, 65(1): 61-67.
- Cayrol, J.C., C. Djian and I. Pijarowski. 1989. Studies on the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Rev. Nematol.*, 12: 331-336.
- Devidas, P., and L.A. Rehberger. 1992. The effect of exotoxin (*thuringiensis*) from *Bacillus thuringiensis* on *Meloidogyne incognita* and *Caenorhabditis elegans*. *Plant and Soil*, 145: 115-120.
- El-Sherif, M.A., A. H. Ali and M.I. Barakat. 1995. Suppressive bacteria associated with plant parasitic nematodes in Egyptian agriculture. *Jap. J. Nematol.*, 24(1): 55-59.
- Esnard, J., N. Marban Mendoza and B.M. Zuckermann. 1998. Effect of three microbial broth cultures and organic amendment on growth and population of free living and plant parasitic nematodes on banana. *Europ. J. P. Pathol.*, 104(3): 457-463.
- Feitelson, J.S. 1993. The *Bacillus thuringiensis* family tree. In: *Advance engineered pesticides* (Ed.) L Kim. Marcel Dekker, Inc. NY. USA. pp. 63-71.
- Hasky-Gunther, K., S. Hoffmann-Hergarten and R.A. Sikora. 1998. Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). *Fund. App. Nematol.*, 21: 511-517.

- Höfte, H. and H.R. Whitefley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.*, 53: 242-255.
- Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. *Pl. Dis. Repr.*, 61: 328-331.
- Ishiwata, S. 1901. On a kind of severe flacherue (sotto disease). *Dainihan Sanshi, Kaiho*, 114: 1-5.
- Kotze, A.C., O. Grady, J. Gough, J.M. Pearson, R. Bagnall, D.H. Kemp and R.J. Akhurst. 2005. Toxicity of *Bacillus thuringiensis* to parasitic and free-living life stages of nematodes parasites of livestock. *Int. J. Parasitol.*, 35: 1013-1022.
- Nester, E.W., L.S. Thomashow, M. Metz and M. Girton. 2002. 100 years of *Bacillus thuringiensis*, a Critical Scientific Assessment, ASM/Washington. D.C.
- Oostendorp, M. and R.A. Sikora. 1989. Seed treatment with antagonistic Rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. *Rev. Nematol.*, 12: 77-83.
- Oostendorp, M. and R.A. Sikora. 1990. *In vitro* interrelationships between rhizosphere bacteria and *Heterodera schachtii*. *Rev. Nematol.*, 13: 269-274.
- Racke, J. and R.A. Sikora. 1992. Wirking der planzengesundheitsfordenden Rhizobakterien *Agrobacterium radiobacter* und *Bacillus sphaericus* auf den *Globodera pallida* Befall kartoffel und das Pflanzenwachstum. *J. Phytopath.*, 134: 198-208.
- Sharma, R. D. 1994. *Bacillus thuringiensis* a biocontrol agent of *Meloidogyne incognita* on barley. *Nematologia Brasileria*, 18: 79-84.
- Siddiqui, I. A. and S. Ehteshamul-Haque. 2000. Use of *Pseudomonas aeruginosa* for the control of root-rot root-knot disease complex in tomato. *Nematol. Medit.*, 28: 189-192.
- Siddiqui, I.A. 2002. *Use of growth promoting bacteria in the control of root infecting fungi and root-knot nematodes of crop plants*. Ph.D. Thesis. Dept of Botany, Univ. of Karachi, Karachi, Pakistan.
- Siddiqui, I.A. and S.S. Shaukat. 2002. Mixtures of plant diseases suppressive bacteria enhance biological control of multiple tomato pathogens. *Biol. Fert. Soils*, 36: 260-268.
- Siddiqui, Z.A. and I. Mahmood. 1999. Role of bacteria in the management of plant parasitic nematodes: a review. *Bioresource Technol.*, 69: 167-179.
- Sikora, R.A. 1988. Interrelationship between plant health promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. *Med. Fac. Landbouww. Rijksuniv. Gent*, 53/2b, 867-878.
- Sikora, R.A. and S. Hoffmann-Hergarten. 1993. Biological control of plant parasitic nematodes with plant health promoting rhizobacteria. pp. 166-172. In: *Pest management Biotechnology based technologies*. (Eds): R.D. Lumsden and J.L. Vaughn, American Chemical Society, Washington. USA.
- Sokal, R.R. and F.J. Rohlf. 1995. *Biometry: The Principals and Practices of Statistics in Biological Research*. Freeman, New York. pp. 887.
- Tailer, R.J., G. Tippett, S. Gibbs, D. Pells, L. Jurdan and S. Ely. 1992. Identification and characterization of a novel *Bacillus thuringiensis* toxin entomocidal to Coleopteran and Lepidopteran larvae. *Mol. Microbiol.*, 21: 1217.
- Tian, B., J. Yang and Ke-Qin Zhang. 2007. Bacteria used in the biological control of plant parasitic nematodes: population, mechanisms of action and future prospects. *Microbiol. Ecol.*, 61: 197-213.
- Wei, J.Z., K. Hale, L. Carta, E. Platzer, C. Wong, S.C. Fang and R.V. Aroian. 2003. *Bacillus thuringiensis* Crystal proteins that target nematodes. *PNAS.*, 100: 2760-2765.
- Zavaleta-Mejia, E. and S. D. Van Gundy. 1982. Effects of rhizobacteria on *Meloidogyne* infection. *J. Nematol.*, 14: 475A-475B.
- Zuckermann, B.E., M. B. Dicklow and N. Ascosta. 1993. A strain of *Bacillus thuringiensis* for the control of plant parasitic nematodes. *Biocont. Sci. Technol.*, 3: 41-46.