CONTROL OF ROOT-KNOT NEMATODES AND AMELIORATION OF EGG PLANT GROWTH BY THE COMBINED USE OF *BACILLUS THURINGIENSIS* BERLINER AND NEMATICIDES

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Abstract

Root-knot nematodes are considered to be the most destructive plant parasitic nematodes. The use of nematicides for the control of root-knot nematodes is common practice. The use of biological control agents is, however, considered more effective and environmentally safe replacement of chemical nematicides. In the present study, three *Bacillus thuringiensis* Berliner isolates viz. B.t.-64, B.t.-16 and B.t.-14 were used with and without nematicides such as Carbofuran and Fertinemakil to control root knot nematode, *Meloidogyne javanica* associated with egg plant. Nematicides were applied in soil before seedling transplantation and bacterial cell suspension was inoculated around the roots after seedling establishment. The combination of B.t. -64 and Fertinemakil was the most effective treatment in reducing root-knot nematodes and improving the Egg plant growth. It was also effective in enhancing the plant growth and reducing the root-knot nematodes infection in combination with other B.t. isolates (B.t. 16 and B.t. 14).

Introduction

Bacteria are most abundant and common flora of soil. Most of bacteria associated with roots of plant are characterized as plant growth promoting bacteria (PGPB) and found effective for improvement of plant growth by different direct and indirect mechanisms includes production of plant growth hormones, nitrogen fixing ability, enhancing mineral availability in soil (Saharan and Nehra, 2011). Some bacterial species produce metabolites that act directly against variety of pathogens. Different *Bacillus* species are characterized as PGPB. There are reports that have suggested nematicidal properties of *Bacillus* species (Siddiqui, 2002; Li *et al.*, 2005). *Bacillus thuringiensis* Berliner produces parasporal crystals protein known as delta-endotoxins that are toxic to insect pests (Höfte and Whiteley, 1989). This property of producing delta-endotoxins distinguishes *B. thuringiensis* from other spore forming bacteria. *B. thuringiensis* consider as an effective measure for controlling a wide range of insect pests (Nester *et al.*, 2002; Wei *et al*., 2003). There are reports that *B. thuringiensis* (B.t.) is capable of infecting protozoa, nematodes, flatworms, mites and insects (Feitelson, 1993). Use of biological control agents alone or in combination with nematicides is an effective practice to control root knot nematodes in crop plants (Stephan *et al*., 1998; Radwan, 1999; Ameer-Zareen and Zaki, 2002,). Carbofuran is systemic nematicide carbamate and granular form of carbofuran is used. Fertinemakil is an organic nematicide consisting Neem cake and fungicide. In the present study three *Bacillus thuringiensis* (B.t.) isolates with and without nematicides i.e., Carbofuran and Fertinemakil are evaluated to manage root-knot nematodes infection in egg plant (*Solanum melongena* L.) and to improvement of growth of the plants.

Materials and Methods

The experiment was carried out in plastic pots of 8 cm diameter containing 250g soil/pot. The soil was amended with nematicides, Carbofuran and Fertinemakil @ 2g and 4g/250g soil, respectively. After two days of nematicides application three weeks old egg plant seedlings were transplanted in each pot and three days after transplantation rhizosphere soil was drenched with 10 ml suspension of actively growing culture of B.t. isolates. Pots without nematicide amendment and drenched with 10 ml distilled water served as control. Each treatment was replicated thrice. After one week of soil drench the pots were inoculated with around 2000 freshly hatched second stage Juveniles of *Meloidogyne javanica* obtained from infected egg plant. These pots were kept in randomized way on greenhouse bench. Observations on plant growth parameters and root knot nematode infection were undertaken after 45 days of nematode inoculation. The plant growth was measured as plant length and dry weights of root and shoot.

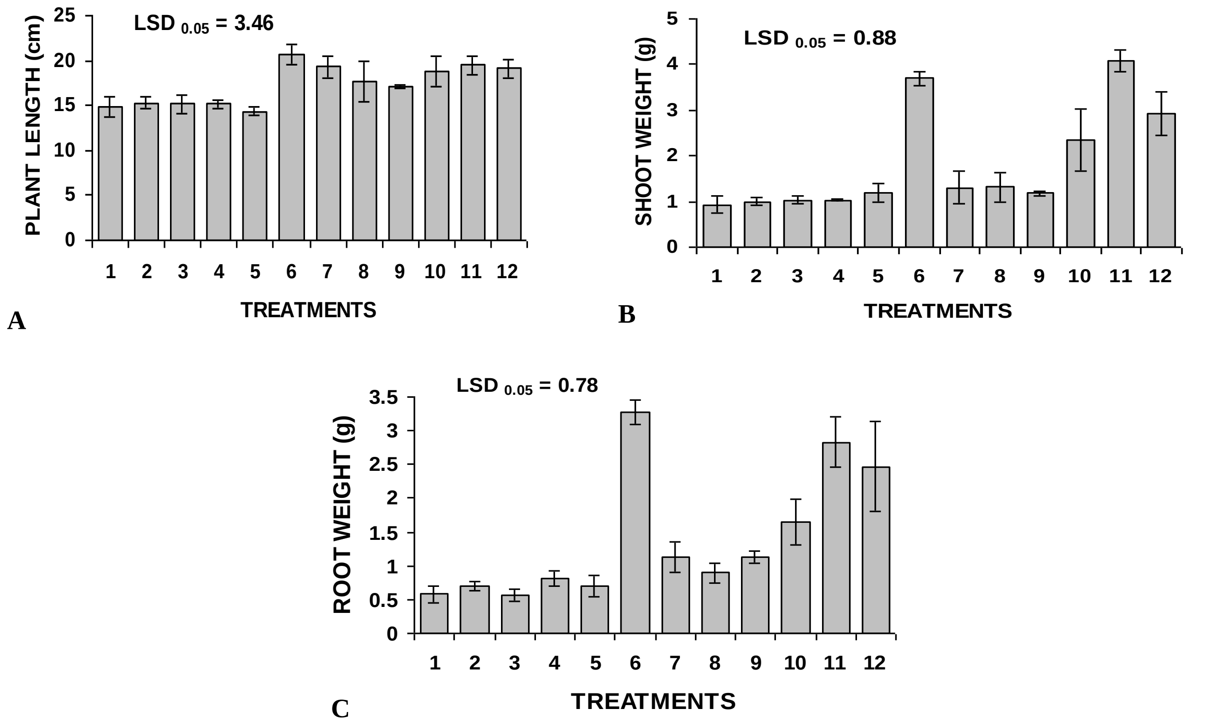


Fig. 1. Effects of *Bacillus thuringiensis* isolates and nematicides on (A) Plant length (B) shoot weight and c, root weight in Egg plant. Treatments: 1=Control, 2 =B.t.-64, 3 =B.t.-16, 4=B.t.-14, 5 = Carbofuran, 6 = Fertinemakil, 7=Carbofuran + B.t.-64, 8 =Carbofuran + B.t.-16, 9 = Carbofuran + B.t.-14, 10 = B.t.-64 + Fertinemakil, 11 = B.t. -16 + Fertinemakil, 12 = B.t.-14 + Fertinemakil.

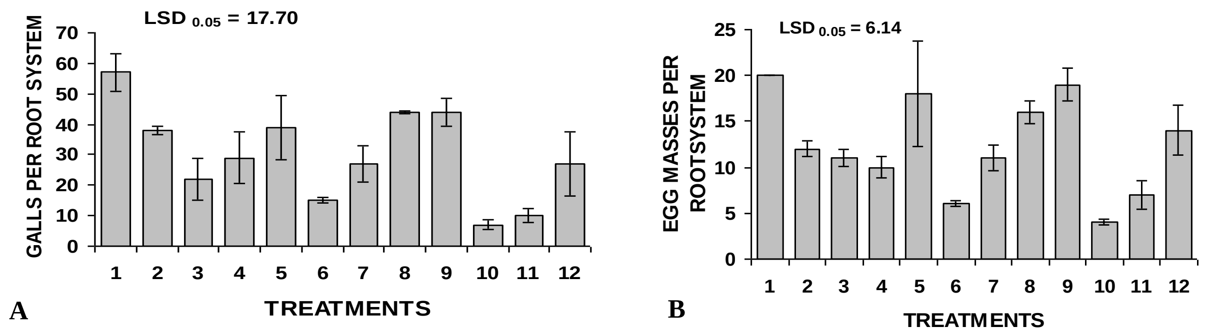
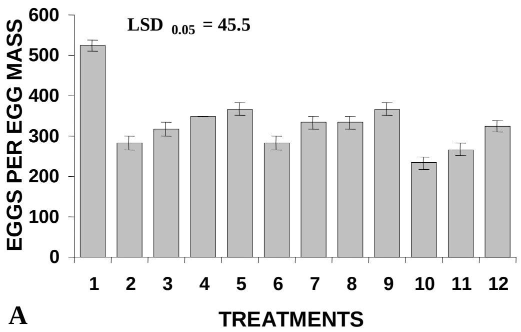


Fig. 2. Effects of *Bacillus thuringiensis* isolates and nematicides on (A) Galls / root system and (B) Egg masses / root system in Egg plant. Treatments: Treatments: 1=Control, 2 =B.t.-64, 3 =B.t.-16, 4=B.t.-14, 5 = Carbofuran, 6 = Fertinemakil, 7=Carbofuran + B.t.-64, 8 =Carbofuran + B.t.-16, 9 = Carbofuran + B.t.-14, 10 = B.t.-64 + Fertinemakil, 11 = B.t. -16 + Fertinemakil, 12 = B.t.-14 + Fertinemakil.

Galls /root system and egg masses / root systems were counted under 4x objective of stereomicroscope. Eggs / egg mass was recorded by detaching 10 egg masses from infected roots using needle and expose to sodium hypochlorite (2%) for 5 minutes and released eggs were counted and to determine number of eggs /egg mass. The number of nematodes / g roots was estimated by staining infected roots with boiling acid fuchsin. After destaining roots pieces were homogenized in a homogenizer at adequate speed for 40 sec. and released nematodes were counted under microscope.

Results and Discussion

None of the B.t. isolates and carbofuran alone made any significant difference in the plant height. Fertinemakil alone increased the plant height by 39%. When nematicides were supplemented with B.t. isolates, there was significant increase in plant height over control. The maximum increase in plant length over control (39%) was recorded in treatment with Fertinemakil, followed by B.t.-64 + Fertinemakil, B.t.-16+ Fertinemakil, B.t.-14+ Fertinemakil and B.t.-64+ Carbofuran showing an increase in plant length by 30%. However most of the treatments when applied alone did not show increase in plant height. Plant growth, in terms of root and shoot weight, was significantly increased in Fertinemakil treated plants ($F = 14.28$ and 13.26, respectively; $p < 0.01$) where an increase of 349 and 375 % over control was observed. Plants treated with B.t.-16 + Fertinemakil showed 366 and 400% increase in root and shoot growth, respectively increase over control (Fig. 1). B.t. isolates when applied in combination with the nematicides showed improvement in plant growth especially treatments of all tested B.t. isolated in combination with Fertinemakil found impressive in the enhancement of plant growth. Application of B.t. isolates with nematicides showed better growth in egg plant. Especially Fertinemakil when applied alone and with B.t. isolates enhanced plant growth. Fertinemakil is an organic nematicide which contains Neem cake as a major constituent. When mixed in soil it might improve organic matter content of soil which in turn should increase nutrients in soil and improve the chemical and physical condition of the soil (Akhtar, 1998).



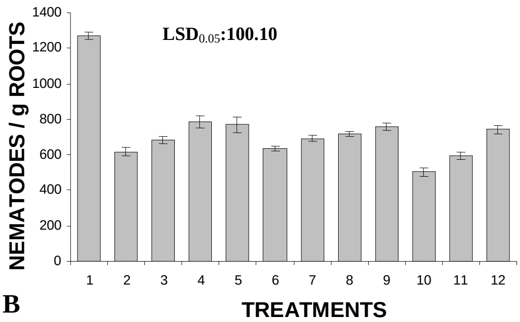


Fig.3. Effects of *Bacillus thuringiensis* isolates and nematicides on (A) Eggs / Egg mass and (B) nematodes population (J2, J3, J4 and adults) / g root in Egg plant. Treatments: Treatments: 1=Control, 2 =B.t.-64, 3 =B.t.-16, 4=B.t.-14, 5 = Carbofuran, 6 = Fertinemakil, 7=Carbofuran + B.t.-64, 8 = Carbofuran + B.t.-16, 9 = Carbofuran + B.t.-14, 10 = B.t.-64 + Fertinemakil, 11 = B.t. -16 + Fertinemakil, 12 = B.t.-14 + Fertinemakil.

Addition of organic matter in soil is also reported to enhance microbial activity in soil which may also increase the population of microbial antagonists. Additionally, plant-based material releases contain several toxic compounds such as terpenoids and phenolic compounds that have nematicidal activity (Siddiqui and Shaukat, 2002; Shaukat *et al*., 2004). Carbofuran is known to reduce saprophytic soil bacteria during early days of application (Suneja *et al*., 2008). This may be the reason of reduced growth of egg plants treated with Carbofuran. Carbofuran in combination with B.t. isolates found effective to induce plant growth but this increase is less then the combined application of B.t. isolates and Fertinemakil.

The number of galls/root system was decreased by 88% in combined use of B.t.-64 + Fertinemakil. B.t. -16 + Fertinemakil and alone Fertinemakil showed a decrease in gall formation by 82 and 73%, respectively. Egg masses / root system was significantly decreased in all the treated plants as compare to control ($F = 6.42$, $p < 0.001$). Maximum reduction in egg masses/root system by 80% was recorded in combined use of B.t.-64 + Fertinemakil, followed by Fertinemakil, B.t. -16 + Fertinemakil, showing 70 and 65% reduction in egg masses / root system, respectively (Fig. 2). The combined use of B.t.-64+ Fertinemakil exhibited maximum reduction in eggs/egg masses (56%), followed by B.t.-16 + Fertinemakil (49%), Fertinemakil and B.t.-64 (45%). Number of penetrated nematodes /g root (including J_2, J3, J4 and adults) was significantly reduced following application of B.t. isolates with and without nematicides application in contrast to untreated control. Nematode population /g root reduced by c 56% when B.t. - 64 was used in combination with Fertinemakil. It was followed by the combination of B.t.-16 with Fertinemakil showing reduction in nematodes population by c 54% (Fig. 3). *Bacillus thuringiensis* isolates B.t.- 64 and B.t.-16 when applied with Fertinemakil proved effective against root knot nematodes infection. In the present study root-knot nematodes infection was measured by different parameters including number of galls / root system, egg masses / root system, eggs / egg mass and nematodes / g roots. Use of B.t.-64 and Fertinemakil was found most effective combination among all treatments and almost all the infection parameters were reduced after this treatment. The reduced number of eggs / egg mass indicated the decreased size of egg masses. It may show the inability in the production of eggs by adult females. It may be due to biological and nematicidal stressful conditions experienced by the nematode. The reduction in nematodes penetrating roots is suggestive of the reduction of nematode population in soil. The reduction of plant-parasitic nematodes in soil and roots is reported after systemic nematicides (Hague & Gowen, 1987). Khan *et al*. (2010) have recorded inhibition in J2 emergence and great J2 mortality *in vitro* by following cell free filtrate application with B.t.-64. This may be due to the production of metabolites against root-knot nematodes. Fertinemakil has been reported to show successful reduction in nematodes infection in rice and mungbean plants (Khan *et al.,* 2000; Khan *et al.*, 2008).

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