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INTEGRATED MANAGEMENT OF *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD INFECTING OKRA BY *BACILLUS THURINGIENSIS* BERLINER ISOLATES AND CHEMICAL FERTILIZERS

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Abstract

In the present studies three *Bacillus thuringiensis* isolates viz. BT-64, BT-16 and BT-14 alone and in combination with three different fertilizers namely urea, Di--ammonium phosphate (DAP) and potash were applied in soil @ 0.1% (w/w). Second stage juveniles (J2) of root-knot nematodes (*Meloidogyne javanica* (Treub) Chitwood) were released around roots of okra (*Abelmoschus esculentus* (L.) Moench. cv. Arka anamica) 15 days after seedling emergence. The effect of the treatments on the growth parameters and nematode population / g roots of okra plants were examined. All treatments significantly reduced the root galls, egg masses/root system and eggs/egg mass. Different developmental stages of *M. javanica* in okra roots were also recorded to assess the effect of combined treatments. Populations of J2, J3, J4 and female nematodes /g root declined with combined treatments of Bt isolates and chemical fertilizers.

Introduction

Okra (*Abelmoschus esculentus* L. (Moench) is considered to be one of the world's oldest crops and is cultivated in almost all the Inter-tropical and Mediterranean regions. In Pakistan, it is grown throughout the country. Okra is an important source of vitamins and essential mineral salts including calcium, which lacks in the diet of poor people of most of the developing countries of the world. The total area under okra cultivation in Pakistan is about 13.9 thousand hectares with production of 0.12 million tonnes (Anonymous, 2014). Root-knot nematodes (*Meloidogyne* spp.) are destructive plant parasitic nematodes parasitizing on thousands of plant species. Annual losses due to root-knot nematodes exceeding over US$100 billion globally (Oka *et al*., 2000). Root-knot nematodes (RKN) may cause annual vegetable yield losses of about 10% (Koenning *et al*., 1999). Root-knot nematodes are difficult to control in fields due to wide host range and their methods of adaptability in soil. However, use of chemical based pesticides are common practice to reduced root-knot nematodes parasitism. Biological control consider as safe and eco-friendly alternatives of hazardous chemical pesticides (Hallman *et al.,* 2009).

Bacillus thuringiensis (Bt) is a commonly found rhizobacteria that have ability to synthesize large crystal proteins which are toxic to most of the insects and nematodes (Wei *et al.,* 2003). Several *in vitro* and *in vivo* experiments have been conducted to test the effectiveness of Bt strains against these microbes by increasing mortality and decreasing penetration, development, and reproduction of RKN (Sharma, 1994; Carneiro *et al*., 1998). Many studies emphasized that nematode population was greatly suppressed. Evidence has been provided that integrating biological control using microbial antagonists with other feasible methods is amongst the most pragmatic strategies of managing the nematodes (Mostafa 2001; Kiewnick and Sikora 2005). Using two or more methods together for the management of soil borne diseases is attracting attention of researchers to develop successful combinations to reduce losses of soil borne diseases and increase plant yield and productivity (Akhtar and Siddiqui, 2007; Siddiqui and Akhtar, 2008). *Bacillus thuringiensis* is commonly found in soil and have been used against variety of plant pathogens as a biological agent (Esnard *et al*., 1998; Kloepper *et al*., 2004; Khan *et al*., 2010). The use of *Bacillus* species with fertilizers was previously reported against plant pathogenic soil-borne fungi in crop plants (Sheikh *et al*., 2006). Siddiqui *et al*. (2001) studied *Pseudomonas fluorescens* with fertilizers to control root-knot nematode infection in tomato plant.

The purpose of the present investigation was to find out better combination of Bt isolates and chemical fertilizers for the management of root-knot nematode disease in okra.

Materials and Methods

Nematode inoculum culture

The inoculum of *Meloidogyne javanica* was obtained from heavily galled roots of Egg plant (*Solanum melongena* L.) maintained in a pot culture in the screen house of the Department of Botany University of Karachi. Eggs were obtained from the roots of Egg plant using Hussey and Barker (1973) extraction method.

Culture of Bt isolates and fertilizers

Three Bt isolates (Bt-64, Bt-16 and Bt-14) were isolated from the rhizosphere of different cultivated plant species. Bt isolates were multiplied on Luria Bertani (LB) broth for 48h at shaking incubator (140 rpm) at 37ºC. For each isolate broth was centrifuged for 20 minutes at 4000x*g* cell residue washed twice with sterilized distilled water and re-suspended to form bacterial cell filtrate and used. Three different fertilizers namely Urea, Di-ammonium phosphate (DAP) and Potash (P) were mixed in soil @0.1% in each pot. Urea was used as a nitrogen source, DAP considered as phosphate source and Potash for potassium.

Greenhouse experiment

Soil obtained from experimental plots of the Department of Botany, University of Karachi was sieved through a 2mm sieve and filled in 8cm diameter plastic pots @ 300g soil/pot. The soil was treated with chemical fertilizers. After 2 days the pots were drenched with 48 h old cultures of *Bacillus thuringiensis* isolates (Bt-64, $11x10^9$, Bt-16, $10x10^9$ and Bt-14, $11x10^9$ CFU). Soil without fertilizers and bacteria were served as control. Each treatment was replicated three times. Next day five seeds were sown in each pot, after emergence two seedlings were maintained in each pot. Two thousands of second stage juveniles were inoculated in each pot after two weeks of emergence. Pots were kept in randomized fashion on green house benches. Experiment was terminated after 45 days of nematode inoculation and observations on plant growth parameters and root-knot infection were recorded. Data were subjected to analysis of variance (ANOVA) followed by the least significant difference (LSD) test at P = 0.05 and Duncan's multiple range test according to Sokal and Rohlf (1995).

Results

Plant height in okra was significant in most of the treatments as compared to controls. All the Bt isolates used in combination with chemical fertilizers enhanced plants height. However, maximum plant height was observed when Bt-14 used in combination with potash (27.0 cm) followed by combined use of Bt-14 and urea (26.75 cm) and Bt-16 with urea (25.75 cm) (Fig. 1 A). Plant fresh weight of okra was significantly (P<0.05) enhanced when Bt isolates were used in combination with fertilizers. Maximum plant weight (4.45 g) was observed in combined use of Bt-16 and DAP, followed by Bt-64 and combined use of BT-16 with urea (Fig.1B).

Galls / root system was significantly (*P*<0.001) reduced in combined use of fertilizers and Bt isolates. Maximum reduction in galls/root system by 74% was recorded in combined use of Bt-64 and urea, followed by Bt-16 with urea, Bt-16 with potash and Bt-16 with DAP exhibiting a reduction in galls / root system by 71, 70 and 65%, respectively (Fig. 2 A). Egg masses/root system were significantly (*P*<0.001) decreased following combined use of bacterial isolates and chemical fertilizers. Maximum reduction in Egg masses/ root system by 80% was recorded in combined use of Bt-64 and urea, followed by Bt-16 with potash, Bt-16 with DAP and Bt-16 with urea exhibiting a reduction in Egg masses/ root system by 75, 73 and 70%, respectively (Fig. 2 B). Eggs / egg mass significantly *P*<0.001 reduced among the treatments. Maximum reduction in Eggs / egg mass by 42% was observed in combined use of Bt-64 and potash followed by Bt-14 with potash and Bt-16 with DAP, reducing eggs/egg mass by 42 and 41%, respectively (Fig.3).

Root-knot development was significantly reduced in plants treated with combined use of bacterial isolates and fertilizers. J2 population / g root was significantly (*P*<0.001) reduced in combined treatments of B.t isolates with fertilizers. Maximum reduction in J2 population / g root by 86% was exhibited by combined use of Bt-64+potash followed by Bt-14+potash, and Bt-16+potash reducing J2 population/ g root by 83 and 81%, respectively (Fig. 4 A). J3 /g root was significantly reduced following combined use of Bt isolates and chemical fertilizers. Maximum reduction in J3 /g root was 83% exhibited in combined use of Bt-64+potash, followed by Bt-64+urea, Bt-64 and Bt-64+DAP reducing J3 /g root by 71, and 63% respectively. (Fig. 4 B). J4 /g root was significantly decreased following combined treatment of bacterial isolates and inorganic fertilizers combined use of Bt-64+potash reduced the J4 /g root by 84% followed by Bt-64+urea, Bt-16+urea and Bt-64 +DAP, showing a reduction in J4 / g root by 82 and 72%, respectively (Fig. 5 A). Numbers of female nematodes / g root was significantly reduced by the combined use of bacterial isolates and inorganic fertilizers *P*<0.001. Bt-64+urea and Bt-64+potash reduced females population/g root by 84%, followed by Bt-64+DAP, BT-16+urea showing a reduction females population/g root by 74%). Bt-64 and Bt-16+DAP exhibited 72% reduction in females population/g root (Fig. 5 B).

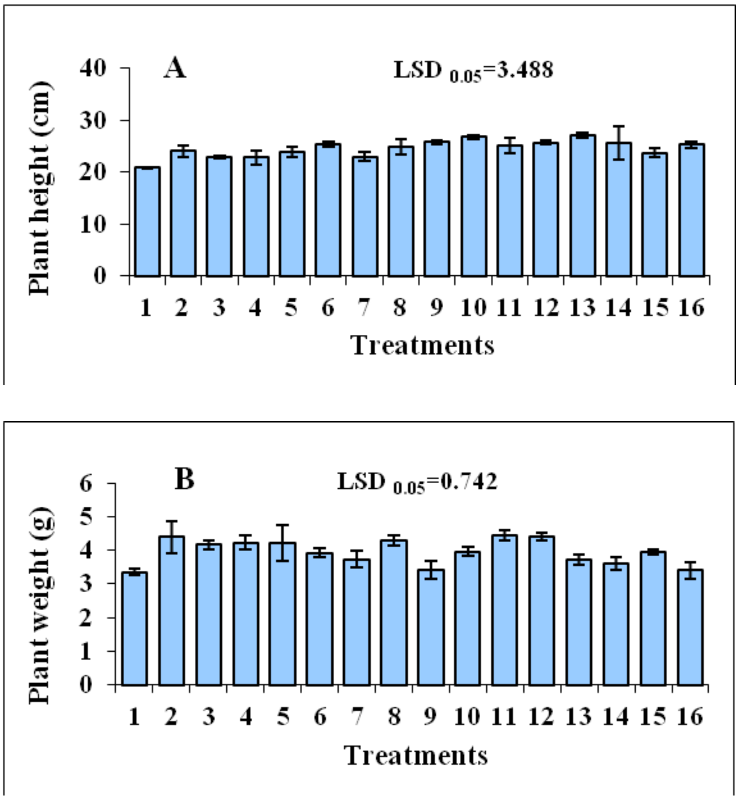


Fig. 1. Effects of *Bacillus thuringiensis* isolates and chemical fertilizers on (A) plant height and (B) plant weight in okra.

Treatments: 1=Control, 2= BT-64, 3=BT-16, 4= BT-14, 5=Urea, 6=Potash, 7=DAP, 8=BT-64+Urea,9=BT-16+Urea,10=BT-14+Urea,11=BT-64+Potash,12=BT-16 +Potash, 13=BT-14+Potash,14=BT-64+DAP, 15=BT-13+DAP, 16=BT-14+DAP,
*(Histogram without bars shows Zero standard error)

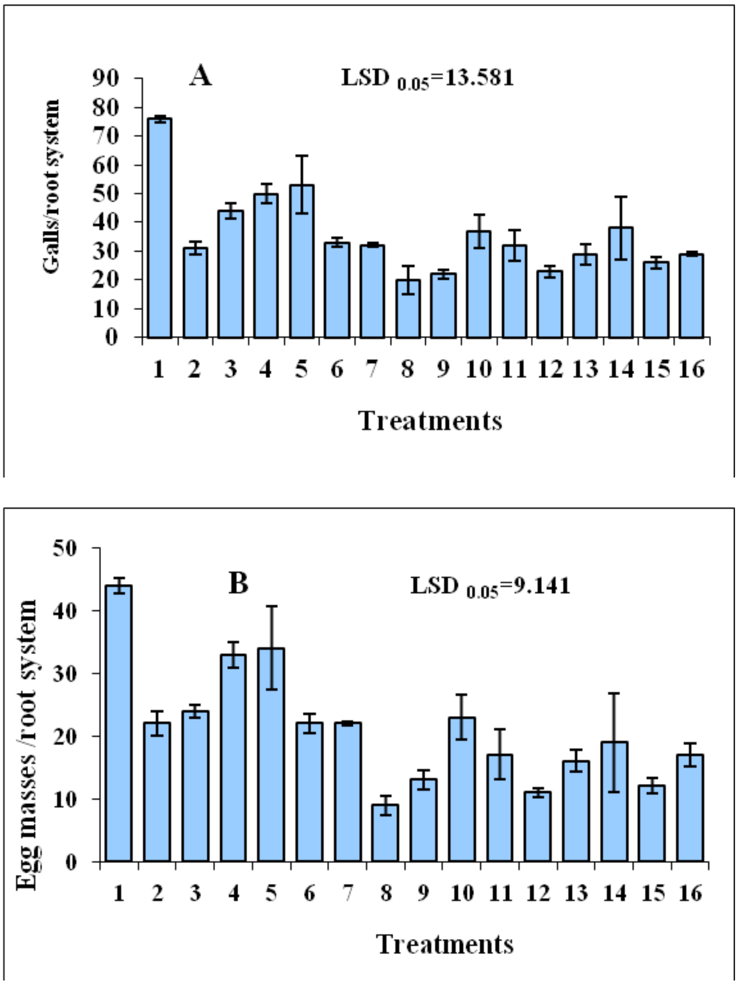


Fig. 2. Effects of *Bacillus thuringiensis* isolates and chemical fertilizers on Galls / root system (A) and Egg masses / root system (B) of okra.

Treatments: 1=Control, 2= BT-64, 3=BT-16, 4= BT-14, 5=Urea, 6=Potash, 7=DAP, 8=BT-64+Urea, 9=BT-16+Urea, 10=BT-14+Urea,11=BT-64+Potash,12=BT-16 +Potash, 13=BT-14+Potash, 14=BT-64+DAP, 15=BT-13+DAP, 16=BT-14+DAP,
*(Histogram without bars shows zero standard error)

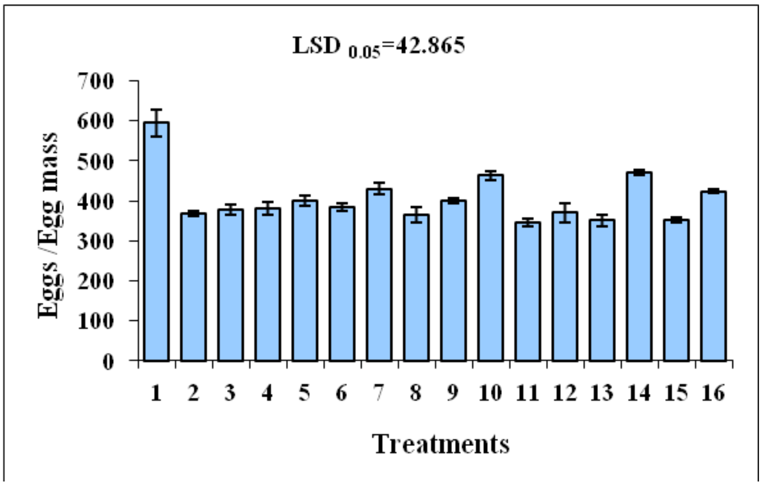
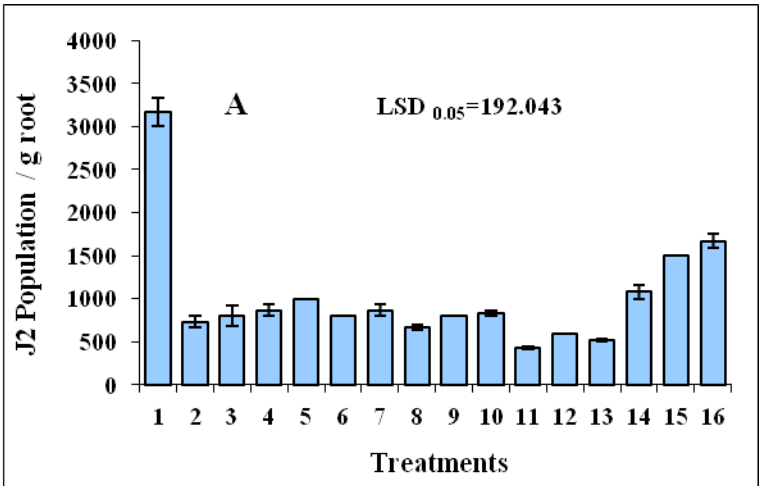


Fig. 3. Effects of *Bacillus thuringiensis* isolates and chemical fertilizers on Eggs / Egg mass in okra.

Treatments: 1=Control, 2= BT-64, 3=BT-16, 4= BT-14, 5=Urea, 6=Potash, 7=DAP, 8=BT-64+Urea, 9=BT-16+Urea, 10=BT-14+Urea,11=BT-64+Potash,12=BT-16 +Potash, 13=BT-14+Potash, 14=BT-64+DAP, 15=BT-13+DAP, 16=BT-14+DAP,
*(Histogram without bars shows zero standard error)



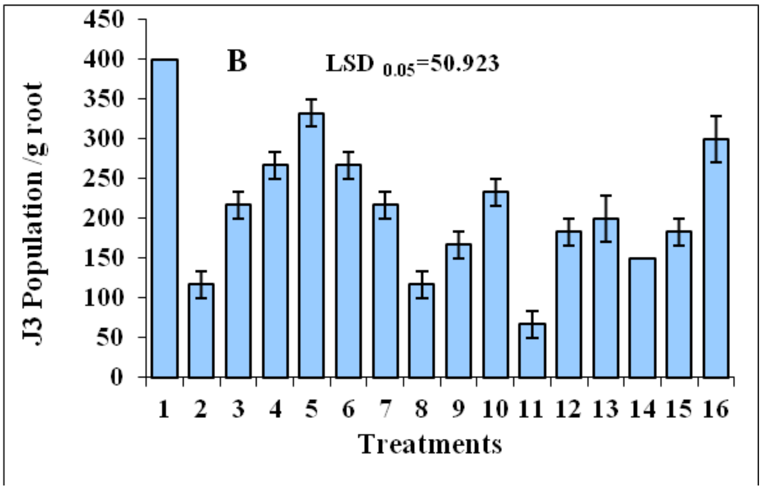
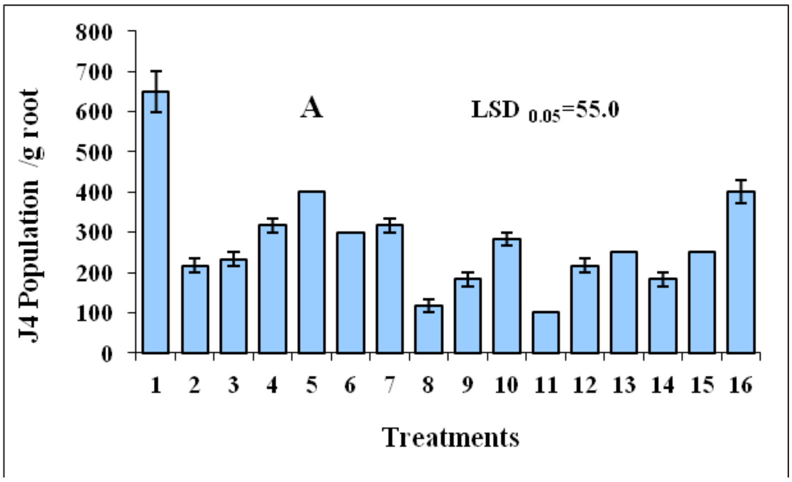


Fig. 4. Effects of *Bacillus thuringiensis* isolates and chemical fertilizers on J2 (A) and J3 (B) population/ g root of okra.

Treatments: 1=Control, 2= BT-64, 3=BT-16, 4= BT-14, 5=Urea, 6=Potash, 7=DAP, 8=BT-64+Urea, 9=BT-16+Urea, 10=BT-14+Urea,11=BT-64+Potash,12=BT-16 +Potash, 13=BT-14+Potash, 14=BT-64+DAP, 15=BT-13+DAP, 16=BT-14+DAP,
*(Histogram without bars shows zero standard error)



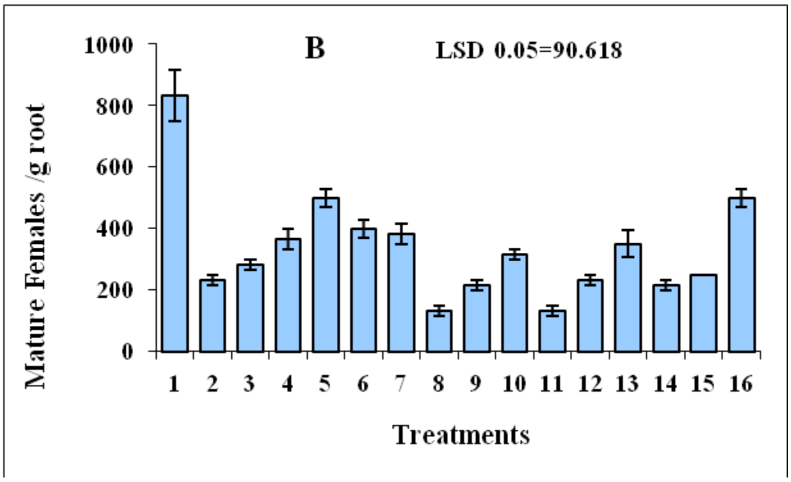


Fig. 5. Effects of *Bacillus thuringiensis* isolates and chemical fertilizers on J4 (A) and
Female (B) population /g root of okra.

Treatments: 1=Control, 2= BT-64, 3=BT-16, 4= BT-14, 5=Urea, 6=Potash, 7=DAP, 8=BT-64+Urea, 9=BT-16+Urea, 10=BT-14+Urea,11=BT-64+Potash,12=BT-16 +Potash, 13=BT-14+Potash, 14=BT-64+DAP, 15=BT-13+DAP, 16=BT-14+DAP,
*(Histogram without bars shows zero standard error)

Discussion

The Integrated management of RKN with combined use of antagonistics with organic and inorganic fertilizers have been reported by (Abbasi and Zaki, 2012; Naheed and Dawar, 2015; Kheir *et al*., 2009; Hoda *et al*., 2013; Radwan *et.al,* 2004; Radwan, 2007). Plant growth promoting rhizobacteria (PGPR) especially belonging to the genera *Pseudomonas* and *Bacillus* have demonstrated potential for disease suppression without negative effects on the plant or the environment, and hence they have considerable agricultural value (Hamida *et al*., 2015; Osman *et al*., 2012). *Bacillus* species increased plant growth by enhancing availability and efficiency of nutrient utilization (Idriss et al., 2002). Furthermore, the application of inorganic fertilizers also responsible to increase bacterial population in soil as well as colonization in roots of crop plants (Siddiqui *et al*., 2001), Severity of root-knot nematode infection was higher in controls, which showed maximum number of knots (76) , egg masses (44) per root system, eggs/egg mass (594) J2,J3, J4 and Mature females/ g root (3167, 400, 650 and 833), on the other hand, least number of knots per root system (20) was recorded in combined treatment of Bt-64 and Urea, s followed by Bt-16 in combination with urea (22) ($P<0.001$). Similarly, least egg masses count (9) and eggs/egg mass (345) were observed in plants treated with Bt-64+urea followed, by combined treatment of Bt-16 + potash ($P<0.001$). The use of rhizobacteria in combination with fertilizers has been to be reported a successful strategy to reduced RKN infection on crop plants under glasshouse conditions (Siddiqui *et al*., 2001). Increase in nematicidal efficacy of microorganisms appears possible when such bio-control agents are integrated with either organic and inorganic amendments or nematicides into an integrated control package (Ashraf and Khan, 2010; Radwan *et al*., 2004; Radwan 2007; Abbasi and Zaki, 2012; Naheed and Dawar, 2015). Under the conditions of this experiment, application of *B. thuringiensis*, alone or combination with the tested fertilizers, provided an effective means for reducing nematode population and could be an alternative control option for the management of RKN. Further work will be needed to determine their effectiveness under field conditions. These findings confirm results previously obtained by Radwan *et al*., (2004); Abbasi and Zaki, (2012).

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