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INTEGRATED CONTROL OF *MELOIDOGYNE JAVANICA* IN OKRA (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH) BY USING COMBINED APPLICATION OF *BACILLUS THURINGIENSIS* ISOLATES AND ORGANIC AMENDMENTS WITH SOME MEDICINALY IMPORTANT PLANTS

Muhammad Qasim Khan, Muhammad Waseem Abbasi, Marium Tariq, Muhammad Javed Zaki and D. Khan

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

ABSTRACT

Pot experiment was conducted containing powder of dried leaves of *Guaicum officinale* L., *Sapindus trifoliatus* L. or *Barleria acanthoides* Vahl. in soil in combination with three *Bacillus thuringiensis* Berliner isolates viz. B.t.-64, B.t.-16 or B.t.-14 to observe their efficacy on growth and root knot nematode *Meloidogyne javanica* associated with roots of okra. Leaf powder was applied in soil at the rate of 1 % w/w and bacterial cell suspension was inoculated around the roots after 10 days of growth of seedlings. The combination of Bt-64 and 1% w/w leaf powder of *S. trifoliatus* was found to be the most effective treatment in reducing root-knot nematodes and improving growth in okra plants.

Key words: *Bacillus thuringiensis*, root-knot nematode, soil amendment with leaf powder, okra.

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 for its young fruits. The vegetable 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. Pakistan is among the leading okra producing countries, and several cultivars are cultivated throughout the year on thousands of hectares. Its yields are very variable, ranging from 1.7 to 19 $t{\cdot}ha^{-1}$ (Tiendrebeogo *et al*., 2010). Per hectare yield of okra in Pakistan (8.8 t) is very low as compared to Cyprus (17.8 t), Jordan (17.7 t), Egypt (14.1 t) and Barbados (11.1 t) (Anonymous 2006). In Pakistan, root knot nematode is found throughout the okra producing regions where it substantially affects growth and yield (Mukhtar *et al*., 2013). Sikora and Fernandez (2005) reported severe attack of root-knot disease caused by *Meloidogyne* spp. The yield losses caused by root-knot nematodes are due to the build-up of inoculum of this pathogen (Kayani *et al*., 2013).

Plant parasitic nematodes are responsible for crop losses every year worldwide of worth of 125 billion dollars (Chitwood, 2003). Most losses are mainly due to the root-knot nematodes, *Meloidogyne* species (Wesemael *et al.*, 2010). About 2000 species of plants are susceptible to infection by root-knot nematodes all over the world. Root-knot nematode damage results in poor growth, a decline in quality, yield of the crop and have the ability to break the resistance of host plant and make it more susceptible to other pathogens (Back *et al.,* 2002; Castello, 2003; Manzanilla-Lopez and Bridge, 2004). Various strategies have been extensively used to manage phytonematodes in infested areas such as organic amendments, biological control and chemical nematicides. The chemical control is widely used for the management of root-knot nematodes. However, synthetic nematicides are now being reappraised with respect to environmental hazard, high costs, limited availability in many developing countries and their diminished effectiveness following repeated applications (Dong and Zhang, 2006). An urgent need to find alternative methods which are safe and environment friendly.

Biological agents and organic soil amendments have been used successfully as effective alternative methods for controlling root-knot nematodes (Stirling, 1991; D'Addabbo, 1995; Akhtar and Malik, 2000; Oka, 2010). However, biological control alone is often inadequate and/or insufficient to maintain nematode populations below their economic threshold under normal agricultural conditions. Therefore, it must be integrated with other management means (Akhtar, 1997; Wu *et al*., 1998; Hildalgo-Diaz and Kerry, 2008). Organic amendments of soil enhanced the activity of biocontrol agents causing suppression of plant pathogens (Sitaramaiah, 1990). An increase in nematicidal efficacy of biocontrol agents appears possible when such bio-control agents are integrated with either organic amendments or nematicides into an integrated control package (Radwan, 1999; 2007; Radwan *et al*., 2004; Ashraf and Khan, 2010).

Organic amendments are generally used to improve crop productivity and increase soil fertility, has also shown suppressive effect on plant pathogenic fungi and nematodes (Alam *et al*., 1980; Tariq *et al*., 2007; Abbasi *et al*., 2008). A number of weedy plants such as *Acalypha indica, Achyranthes aspera, Argemone mexicana, Parthenium hysterophorus, Ricinus communis*, and *Trianthema portulacastrum* are reported to contain Nematostatic and nematicidal potential – *A. mexicana* and *A. aspera* being the highly toxic against root-knot nematode, *Meloidogyne incognata* (Khan *et al.,* 2017), in form of inhibition of egg hatching and mortality of J2 juveniles, presumably due to the occurrence of alkaloids, flavonoids, tannins, saponins, phytosterols, mucilage gum in the aqueous extracts of these weeds. Indeed, numerous plant species, representing 57 families, have been shown to contain nematicidal compounds (Sukul, 1992).

In present studies, green house experiments were carried out to study the effects of soil organic amendment with leaf powder of some local plants on the efficacy of selective *Bacillus thuringiensis* isolates BT-64, BT-16 and BT-14 to control root-knot nematode, *Meloidogyne javanica*.

MATERIALS AND METHODS

Soil was obtained from experimental field of Department of Botany, University of Karachi. Soil was observed for physical properties like texture (sandy loam), pH (8.1), moisture holding capacity (29%) and total nitrogen (0.077- 0.099%) (Mackenzie and Wallace, 1954; Keen and Raczkowski, 1922). Pots (8 cm diameter) were filled with 300 kg soil and amended with dried leaves powder of *Guaicum officinale* L., *Sapindus trifoliatus* L. and *Barleria acanthoides* Vahl. at 1% w/w. Two week after amendment, 25 ml aqueous cell suspension of *B. thuringiensis* isolates were drenched in each pot (BT-64 at 6 x 10^8 CFU mL^{-1}, BT-16 at 10 x 10^8 CFU $mL^{-1}$and BT-14 at 17x 10^7 CFU mL^{-1}). Five okra (*Abelmoschus esculentus* (L.) Moench. Var. Arka anamika) seeds were sown in each pot. On emergence, two seedlings were maintained in each pot. After 10 days of emergence approximately 2000 freshly hatched second stage juveniles were introduced in holes made around the roots in each pot. The un-amended but nematode inoculated pots served as control. Treatments and controls were replicated thrice. The pots were placed in a completely randomized design in a green house and watered daily. After 8 weeks of nematode inoculation, plants were gently removed from pots and the roots were carefully washed in running tap water. Plant growth parameters i.e. shoot length, shoot weight, root length, root weight and nematode infection i.e. number of galls, egg masses, eggs/ egg mass, nematode and bacterial population in roots and soil were determined. All growth and infection parameters were estimated and least significant difference was determined at 5 % significance level (Gomez and Gomez, 1984).

RESULTS

Bacillus thuringiensis isolates used separately or in combination with organic amendments significantly increased shoot length. Maximum increase in shoot length (51 %) and shoot weight (by 140%) was observed when *S. trifoliatus* used in combination with BT-64, followed by *S. trifoliatus*+BT-16 (40, 130%) and *S. trifoliatus*+ BT-14 (38, 121%) (Fig. 1). Maximum increase in root length was recorded when combination of *S. trifoliatus* + BT-16 (59%) was used while *S. trifoliatus* **+** BT-64 gave maximum root weight followed by *S. trifoliatus* **+** BT-16, *Gliocladium* + BT-16, and *Gliocladium* + BT-14 (Fig. 2).

Root-knot infection was significantly reduced by using organic amendments of plants and BT isolates as compared to control. Maximum reduction in galls / root system by <61% was recorded when 1 % w/w *S. trifoliatus* was used in combination with BT-64, followed by *G. officinale* + BT-64 and BT-64 (41 and 40% respectively). Egg masses significantly reduced among the treatments. The highest reduction in egg masses / root system was 50% and eggs / egg mass was 57% exhibited in combined use of *S. trifoliatus* + BT-64 followed by *B. acanthoides*+BT-64 (42%), S. *trifoliatus* + BT-16 (51%) and *G. officinale* + BT-16 (48%) (Fig. 3, 4). Nematode population in soil was reduced up to 82% due to combined use of *S. trifoliatus* + BT-64, followed by treatment with *G. officinale* + BT-64 showing a reduction in nematode population soil by 76% (Fig. 4).

A significant difference in root-knot development was observed in soil amended with combination of organic substrates and BT isolates. J2 / g root was significantly reduced by 83% when combination of *S. trifoliatus* and BT-64 used, followed by *G. officinale* +BT-64 and BT-64 showing a reduction in J2 population /g root by 78 and 76% respectively, as compared to the control. However, 87 % reduction in J3 population/g root was recorded when *S. trifoliatus* + BT-64 was used while J4 population/g root and female population/g root were significantly reduced by 89 and 91% due to use of same combination followed by *G. officinale* + BT-64, BT-64 and *B acanthoides* + BT-64, respectively (Fig. 5, 6).

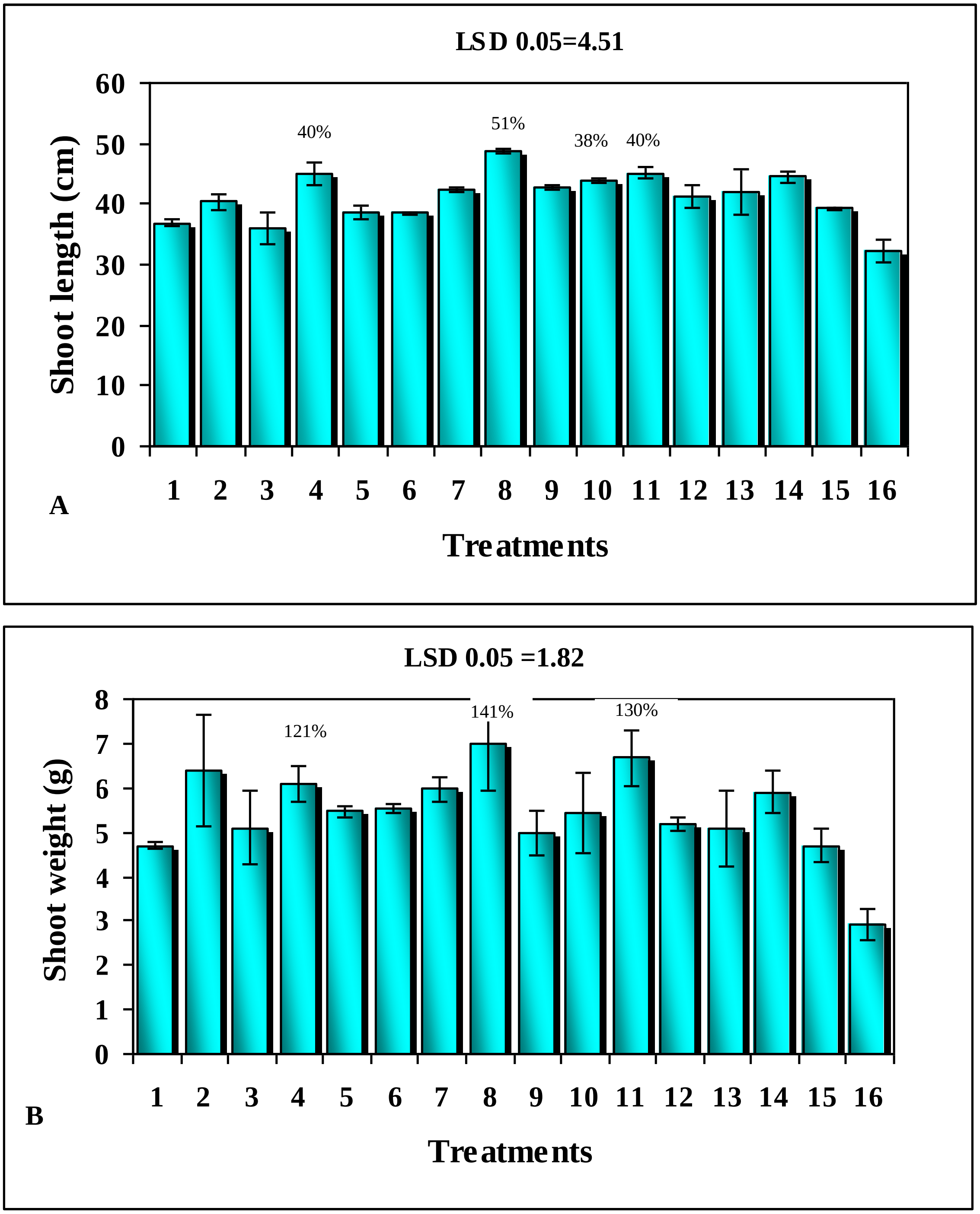
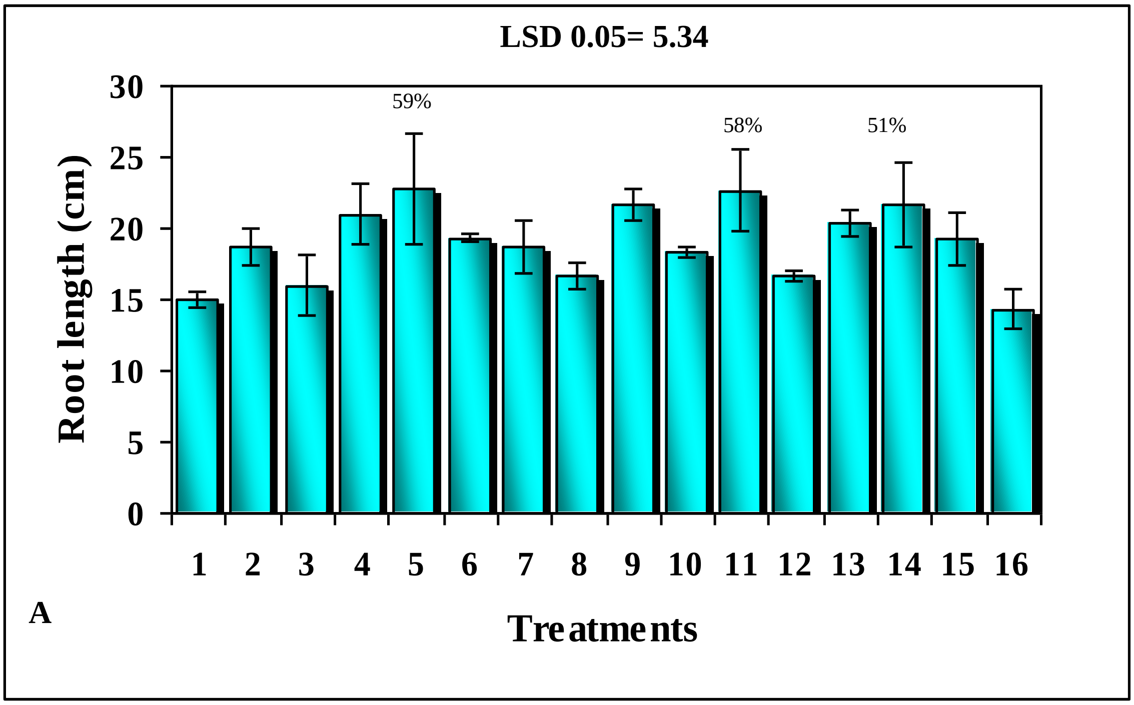


Fig. 1. Effects of *Bacillus thuringiensis* isolates and organic amendments on (A) shoot length and (B) shoot weight in okra. Figures above the bar show percent promotion or reduction over control.

Treatments: 1= *Guaicum officinale*, 2 = *Sapindus trifoliatus*, 3= *Barleria acanthoides*, 4 = BT-64, 5 = BT-16, and 6 = BT-14.

Treatment combinations: 7 = treatments 1+ 4; 8 = treatments 2 + 4; 9 = treatments 3 + 4; 10 = treatments 1+5; 11 = treatments 2 + 5; 12 = treatments 3 + 5; 13 = treatments 1 + 6; 14 = treatments 2 + 6; 15 = treatments 3 + 6 and 16 = Control.



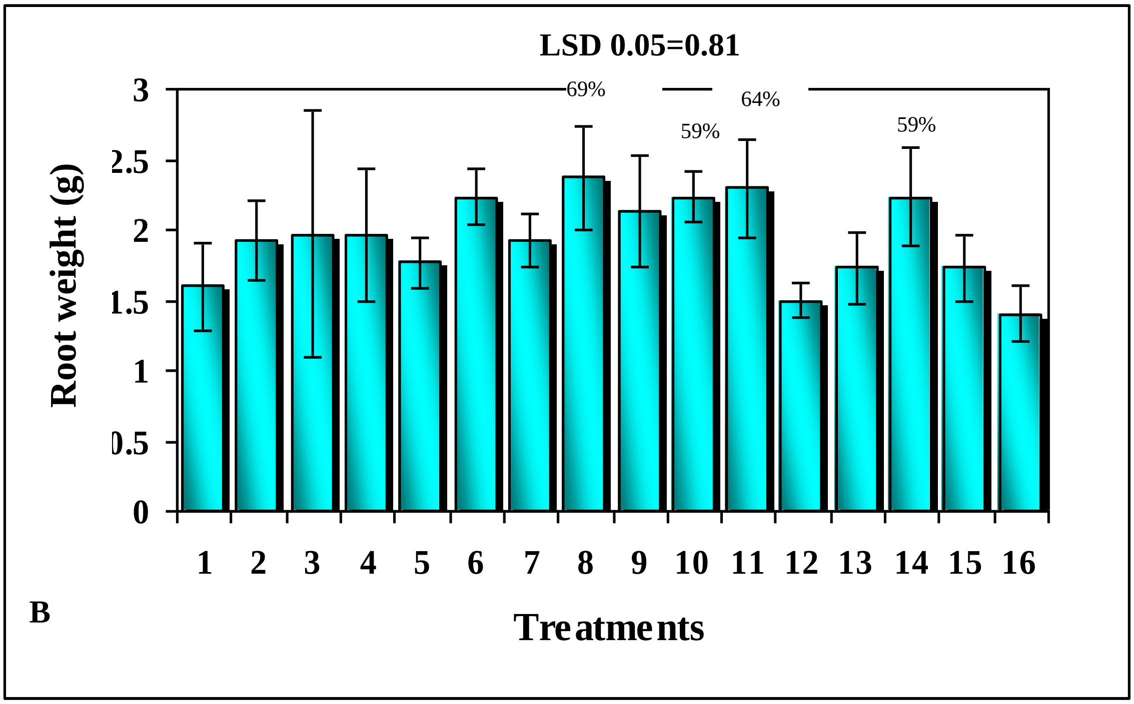
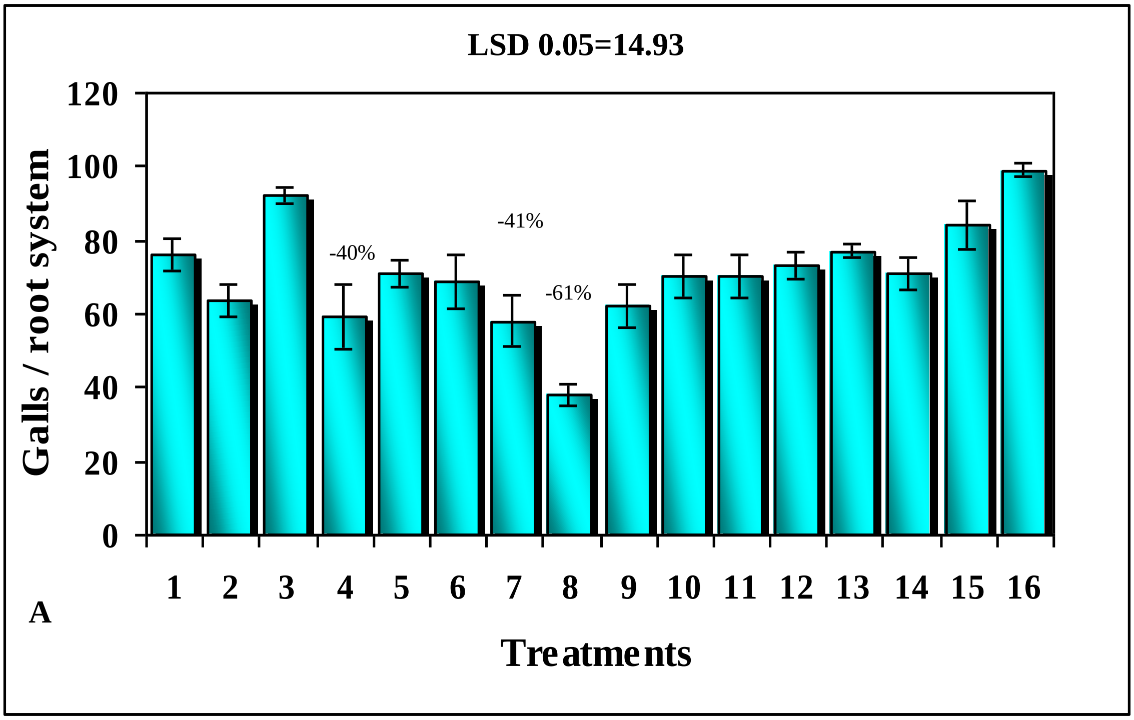


Fig. 2. Effects of *Bacillus thuringiensis* isolates and organic amendments on (A) root length and (B) root weight in okra. Figures above the bar show percent promotion or reduction over control.

Treatments: 1= *Guaicum officinale*, 2 = *Sapindus trifoliatus*, 3= *Barleria acanthoides*, 4 = BT-64, 5 = BT-16, and 6 = BT-14.

Treatment combinations: 7 = treatments 1+ 4; 8 = treatments 2 + 4; 9 = treatments 3 + 4; 10 = treatments 1+5; 11 = treatments 2 + 5; 12 = treatments 3 + 5; 13 = treatments 1 + 6; 14 = treatments 2 + 6; 15 = treatments 3 + 6 and 16 = Control.



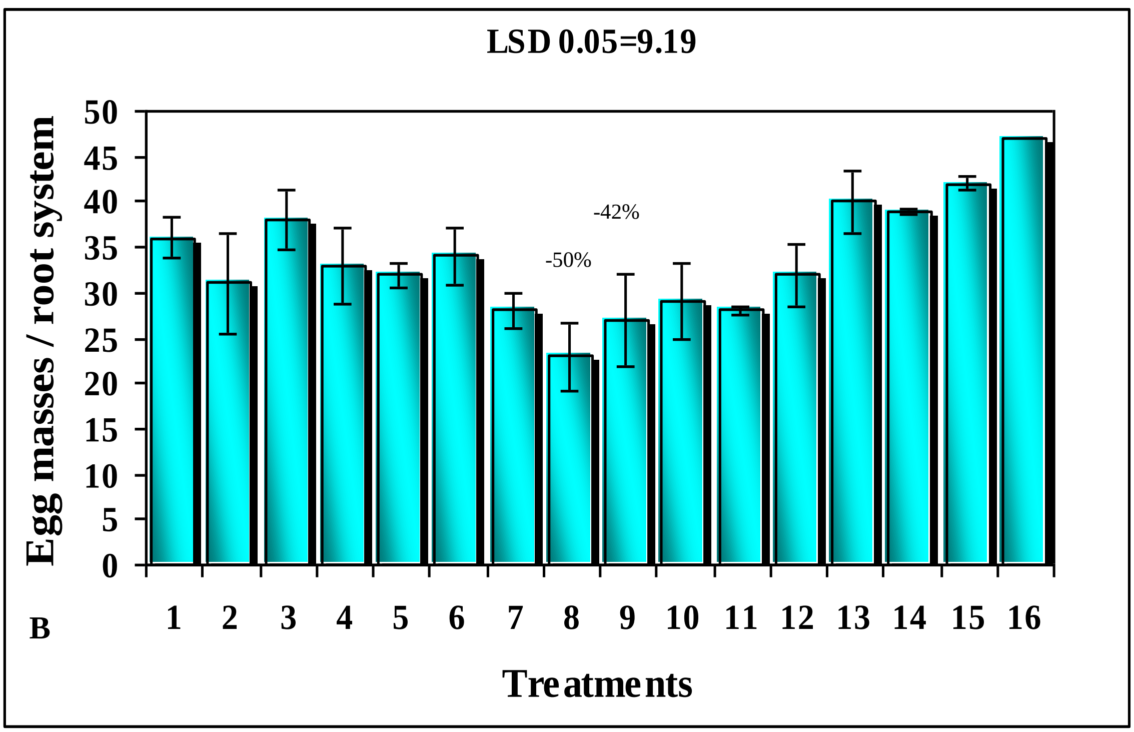


Fig. 3. Effects of *Bacillus thuringiensis* isolates and organic amendments on (A) Galls / root system and (B) Egg masses/root system in okra. Figures above the bar show percent promotion or reduction over control.

Treatments: 1= *Guaicum officinale*, 2 = *Sapindus trifoliatus*, 3= *Barleria acanthoides*, 4 = BT-64, 5 = BT-16, and 6 = BT-14.

Treatment combinations: 7 = treatments 1+ 4; 8 = treatments 2 + 4; 9 = treatments 3 + 4; 10 = treatments 1+5; 11 = treatments 2 + 5; 12 = treatments 3 + 5; 13 = treatments 1 + 6; 14 = treatments 2 + 6; 15 = treatments 3 + 6 and 16 = Control.

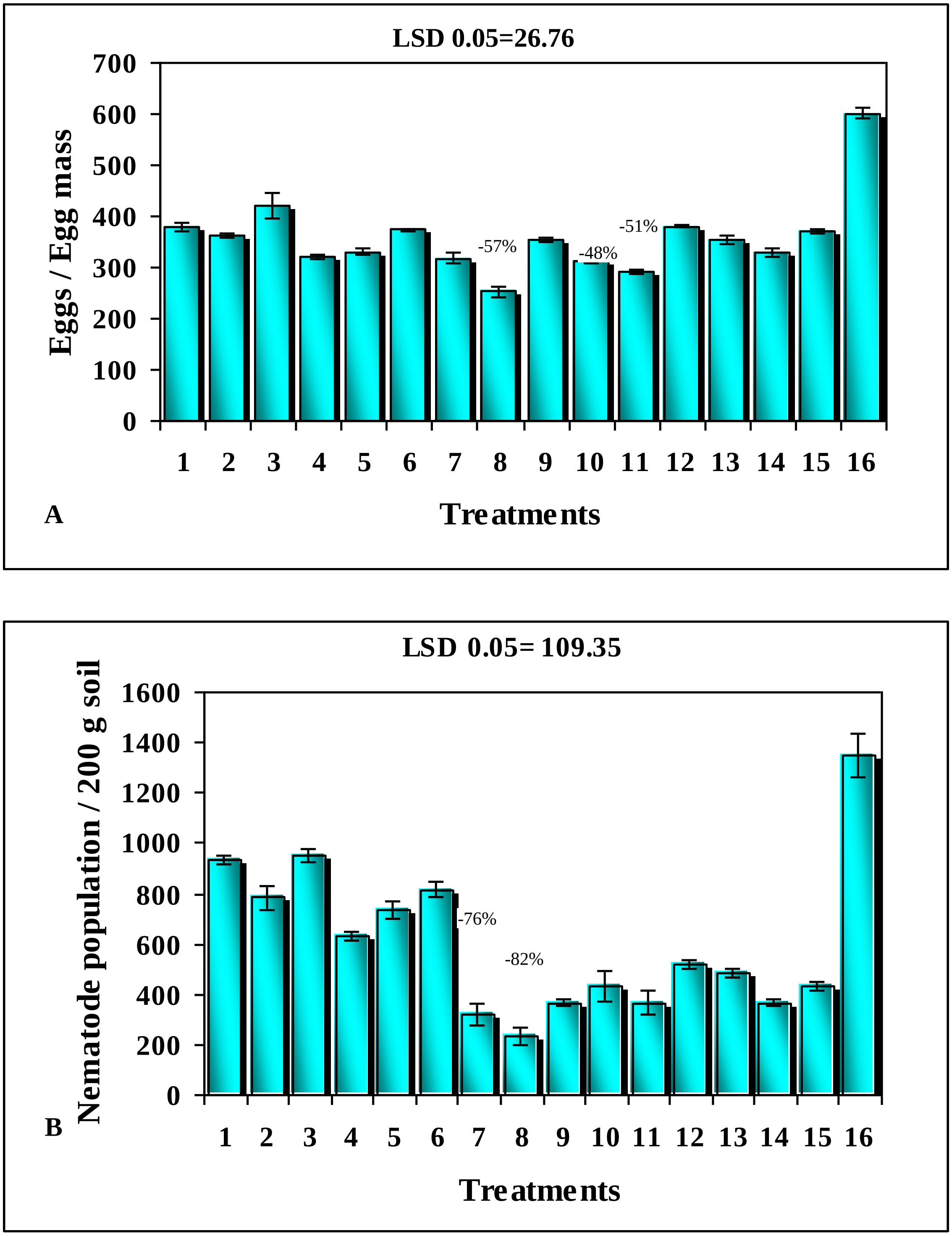
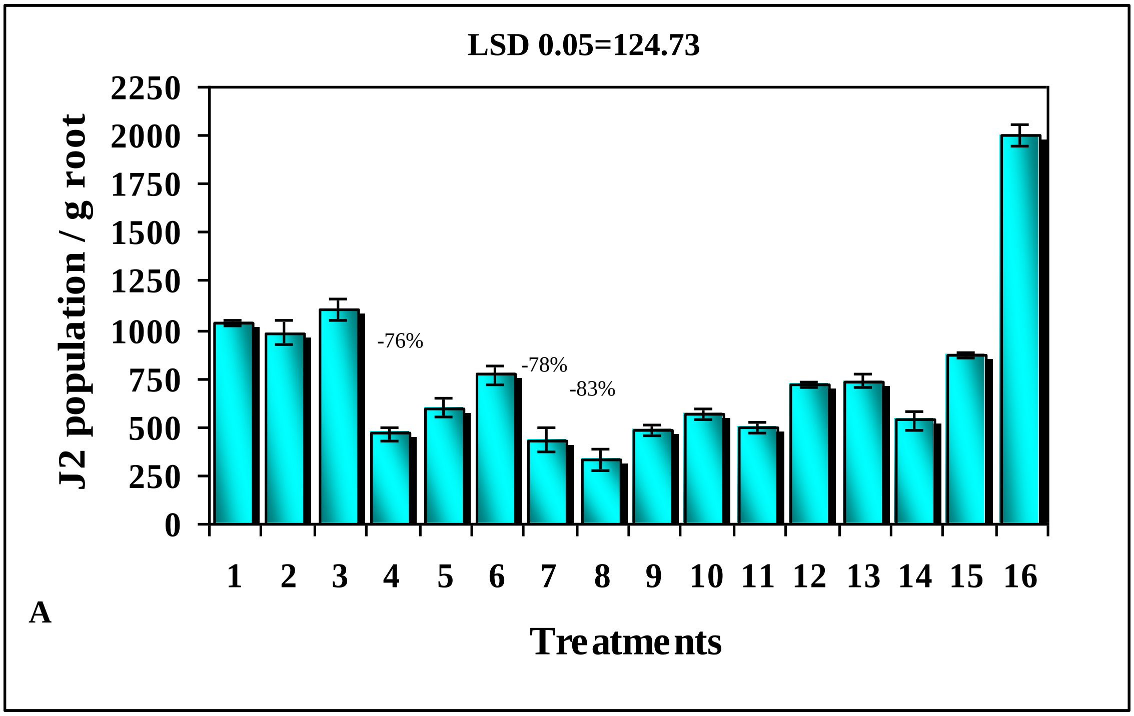


Fig. 4. Effects of *Bacillus thuringiensis* isolates and organic amendments on (A) Eggs / egg mass in okra and (B) Nematode population/200 g soil. Figures above the bar show percent promotion or reduction over control.

Treatments: 1= *Guaicum officinale*, 2 = *Sapindus trifoliatus*, 3= *Barleria acanthoides*, 4 = BT-64, 5 = BT-16, and 6 = BT-14.

Treatment combinations: 7 = treatments 1+ 4; 8 = treatments 2 + 4; 9 = treatments 3 + 4; 10 = treatments 1+5; 11 = treatments 2 + 5; 12 = treatments 3 + 5; 13 = treatments 1 + 6; 14 = treatments 2 + 6; 15 = treatments 3 + 6 and 16 = Control.



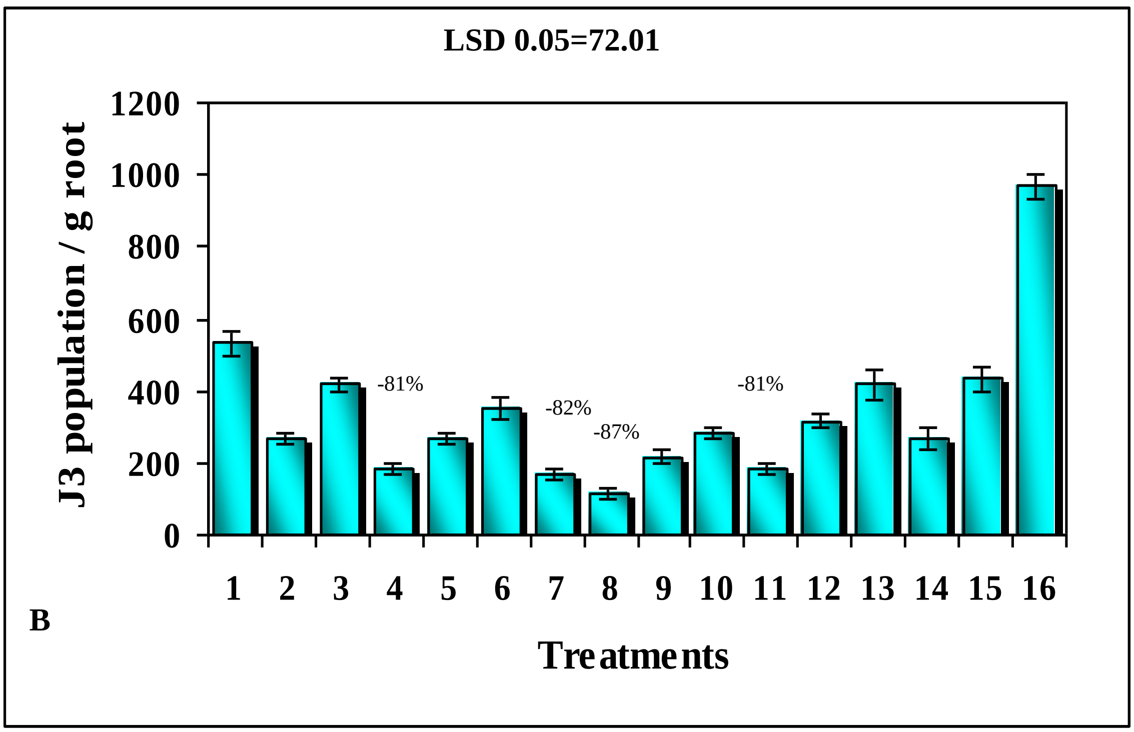


Fig. 5. Effects of *Bacillus thuringiensis* isolates and organic amendments on (A) J2 and (B) J3 population / g root in okra. Figures above the bar show percent promotion or reduction over control.

Treatments: 1= *Guaicum officinale*, 2 = *Sapindus trifoliatus*, 3= *Barleria acanthoides*, 4 = BT-64, 5 = BT-16, and 6 = BT-14.

Treatment combinations: 7 = treatments 1+ 4; 8 = treatments 2 + 4; 9 = treatments 3 + 4; 10 = treatments 1+5; 11 = treatments 2 + 5; 12 = treatments 3 + 5; 13 = treatments 1 + 6; 14 = treatments 2 + 6; 15 = treatments 3 + 6 and 16 = Control.

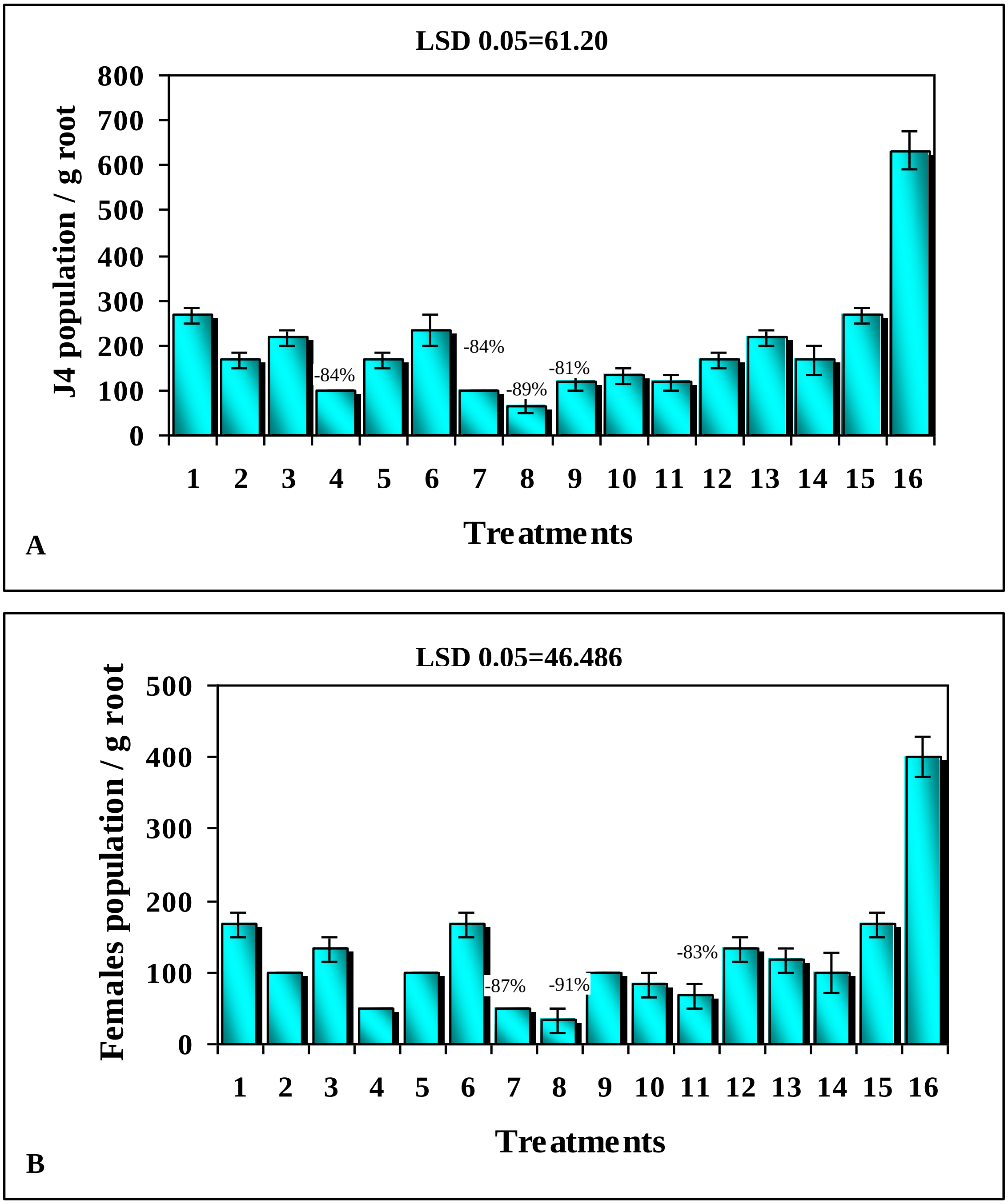


Fig. 6. Effects of *Bacillus thuringiensis* isolates and organic amendments on (A) J4 and (B) females population / g root in okra. Figures above the bar show percent promotion or reduction over control.

Treatments: 1= *Guaicum officinale*, 2 = *Sapindus trifoliatus*, 3= *Barleria acanthoides*, 4 = BT-64, 5 = BT-16, and 6 = BT-14.

Treatment combinations: 7 = treatments 1+ 4; 8 = treatments 2 + 4; 9 = treatments 3 + 4; 10 = treatments 1+5; 11 = treatments 2 + 5; 12 = treatments 3 + 5; 13 = treatments 1 + 6; 14 = treatments 2 + 6; 15 = treatments 3 + 6 and 16 = Control.
(Histogram without error bar shows that standard error was zero)

DISCUSSION

Organic amendments with plant materials have been found to be effective control measure against root-knot nematodes and cause increase in plant growth by improving soil physical and biological conditions (D'Addabbo, 1995). Use of antagonistic organisms in combination with organic amendments is an effective measure to control root-knot nematode (Siddiqui *et al.*, 1999; Ibrahin and Ibrahim, 2000; Tariq and Dawar, 2010). In our studies, organic amendment along with three BT isolates was found to be effective against root-knot nematode infection and for the improvement of growth of crop plants. The combined use of BT-64 and *Sapindus trifoliatus* showed a significant reduction in root-knot nematode infection (galls / root system, egg masses / root system and eggs / egg mass), root-knot nematode development (J2, J3 , J4 and female population/ g root) and increased plant growth. Members of Sapindaceae are known to contain saponins. Saponins are glycosides comprises of two groups i.e., steroidal saponins and triterpenoid saponins occurring worldwide in many plants (Soetan *et al.,* 2006). Biological activities of saponins such as antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer have been reported (Price *et al.*, 1990; Just *et al*., 1998, Arao *et al.*, 1998). Organic amendment provides nutrition for saprophytic bacteria and fungi in soil. Antagonistic organisms also multiply on organic amendment and increase their population in soil which ultimately affect nematode population in soil and thus improve plant growth. Nematode population in soil altered by the use of combined treatment. Release of toxic compounds from plant tissues are also reported to reduce plant parasitic nematode infection. Several plant terpenoids and phenolic compounds are known to have nematicidal properties (Akhtar and Mahmood, 1994; Siddiqui and Shaukat, 2004).

Application of the soil amendment caused significant reduction in the nematode parasitism in roots of okra. Addition of bark powder to soil also enhanced growth of okra plants. Parveen and Bhat (2011) reported nematicidal activities of neem bark powder in pot experiment against *Rotylenchulus reniformis* and *Meliodogynae incognita* on castor. Increase in growth and decreased in nematodes parasitism was recorded in plants amended with *Eucalyptus* spp. *Eucalyptus* might be highly toxic to juveniles of nematode. Dawar *et al*, (2007) reported similar observation. Leaves of *Eucalyptus microtheca* are reported to control plant parasitic nematodes (Elbadri *et al.,* 2008).

Guaicum officinale contains saponins such as guaiacin and guaianin (Ahmad *et al.,* 1990). It has high phenolic content. The twig extract of *G. officinale* under ethyl acetate was reported to be 20.3 ±0.003µg GAE /1 µg extract (Maneechai and Pikulthong (2017). Occurrence of phenolics, NDGA (Nordihydroguiaretic acid) (Polya, 2003) and vanillin (Hoffmann, 1996) is known in *G. officinale*. Khan and Naqvi (2008) have investigated the phytotoxicity of aq. Extracts of various components of *G. officinale* against germination and seedling growth of *Triticum aestivum* var. Kiran. The toxicity appeared to be in following order: Pericarp > Flowers > Yellow leaves > Green leaves > Green fruits > Bark.

The genus *Barleria* is shown to exhibit biological activity. Aqueous, petroleum ether, chloroform and acetone extracts of *Barleria prionitis* leaves have showed antimicrobial activity against several bacteria. Chloroform extract was inhibitorier than other extracts (Khare, 2016). *Barleria acanthoides* has been reported to be inhibitory to root-knot nematodes infecting egg plant (Abbasi *et al.,* 2013).

The genus *Sapindus* is known to contain more than 103 compounds, of these compounds, triterpenoidal saponins of oleanane, dammarane and tirucullane are considered to be the active group that is to be most likely to be responsible for the several biological activities (Goyal *et al.,* 2014). Krishnaveni and Thaakur (2008) have reported aqueous extract of leaf powder of *S. trifoliatus* to contain a number of phytochemicals – alkaloids, carbohydrates, tannins, flavanoids, sterols, saponins. Seeds extract of this species is considered to be great anthelmintic against cestodes possibly due to saponins and flavanoids (Sravanthi *et al.,* 2011). Pai *et al*. (2014) have reported total flavanoids content to be 103.9 mg CE/100g in *Sapindus trifoliatus* seed extract.

From above results it was concluded that although Bt-64 has potential to reduce root knot nematode and promotes growth of crop plants but it was found to be the most effective in combination with the leaf powder *S. trifoliatus* at the rate of 1% w/w.

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