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COMBINED EFFECT OF *BACILLUS* SPECIES AND FERTILIZERS ON GROWTH AND ROOT-KNOT NEMATODE INFECTION IN OKRA

M. Waseem Abbasi and M. Javed Zaki

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

ABSTRACT

In the present investigation three *Bacillus* series viz. *Bacillus coagulans* (BA), *Bacillus licheniformis* (BLB2) and *Bacillus cereus* group isolate (BS) alone and in combination with three different fertilizers namely Urea, di-ammonium phosphate (DAP) and potash (P) were applied in soil. J2 of root-knot nematodes released around roots of okra (*Abelmoschus esculentus* (L.) Moench. cv. Arka anam) after 10 days of seedling emergence. *Bacillus* species in combination with inorganic fertilizers significantly increased plant growth. Especially, *B. coagulans* (BA) in combination with all fertilizers significantly (P<0.001) enhanced plant growth. *B. cereus* group isolate (BS) with Potash (P) and Urea most significantly reduced root-knot nematodes infection in okra.

Key-words: *Bacillus* species, fertilizers, root-knot nematodes, okra.

INTRODUCTION

Different synthetic fertilizers have been used to increase fertility of soil all around the world. Use of fertilizers mainly depends upon the nutrients composition of soil. Nitrogen, phosphorous and potassium are very important nutrients for plant growth. Huber (1990) reported that fertilization decreases soil-borne plant diseases by stimulating plant growth and also by altering the soil environment, which influence survival and penetration of pathogens into the roots of plants. Some other reports also suggesting efficacy of fertilizers against different plant pathogenic fungi (Sharma and Kolte, 1994; Dawar and Ghaffar, 2003). However, some studies suggested that by increasing fertilization the rate of secondary metabolism become lower this phenomenon related to the decrease in resistance against pathogens (Herms, 2002). Using two or more methods together for the management of soil borne diseases considering 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* species are commonly found as rhizosphere microorganisms that have been used against variety of plant pathogens as a biological agent (Esnard *et al*., 1998; Kloepper *et al*., 2004; Khan *et al*., 2010). 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* species with fertilizers to control root-knot nematode infection in tomato plant. The purpose of present investigation is to find out better combination against root-knot nematode disease in okra. For this reason *Bacillus* species and different fertilizers applied in soil alone and in combination for the control of root-knot nematodes on okra.

MATERIALS AND METHODS

Culture of *Bacillus* species and fertilizers

Three *Bacillus* species viz. *B. coagulans* (BA), *B. licheniformis* (BLB2) and *B. cereus* group isolate (BS) were used; they were isolated from the rhizosphere of different cultivated plant species. *Bacillus* species were multiplied in Luria Bertani (LB) broth for 48h at shaking incubator at 37ºC and 140rpm. For each isolate broth was centrifuge for 10 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 selected for application in soil. Urea was used as a nitrogen source, DAP considered as phosphate source and Potash for potassium.

Greenhouse experiment

Soil for the experiment was collected from experimental field and pass through a 2mm sieve. In each pot 300g soil was applied with fertilizers viz., Urea, Potash and di-ammonium phosphate (DAP) @ 0.1% w/w. Second day after fertilizers applications 10ml of *Bacillus* species cell filtrates were drenched to the pots containing colony forming units (CFU) *B. coagulans* (BA) $23x10^8$, *B. subtilis* (BS) $4x10^8$ *B. licheniformis* (BLB2) $15x10^8$ /ml CFU. Five seeds were sown in each pot next day of each bacterial application after germination two seedlings were maintained in each pot. About 2000 J2 of root-knot nematodes released around the roots of each plant 10 days after emergence. Experiment was terminated 45 days after nematode inoculation.

Data collection and analysis

Data of plan length, fresh plant weight, number of knots per root system, egg masses per root system and eggs per egg mass recorded. Knots and egg masses were counted by spreading roots under stereomicroscope; large roots were cut into pieces before counting. 10 egg masses were collected with the help of needle from plants of each pot randomly in cavity block containing 2ml distilled water for eggs counting; few drops of sodium hypochlorite was added to dissolve gelatinous mass of egg masses, eggs released into the water were counted under compound microscope after dilution.

Mean and standard error of three replicates of each treatment was calculated and data were subjected to analysis of variance (ANOVA), the follow up of FANOVA included Least Significant Difference (LSD) were performed to compare the treatment means (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

Plant length in okra was significantly increased ($P< 0.001$) in most of the treatments as compared to controls. *B. coagulans* (BA) in combination with all fertilizers used were most significantly ($P<0.001$) enhanced plant length. However, greatest plant length was noticed when BA used in combination with DAP (28.83 cm) which was followed by BA with P (28.5cm) and urea (28.17). It was recorded that fresh weight of okra was significantly ($P<0.05$) enhanced when *B. subtilis* (BS) applied in combination with urea (Fig. 1). Most of the treatments did not show significant increase on plant fresh weight. It was observed that plant length increases with BA and fertilizers, while plant fresh weight most significantly enhanced by the use of urea with all tested species. This showed that BA species have ability to enhance nutrient uptake from soil.

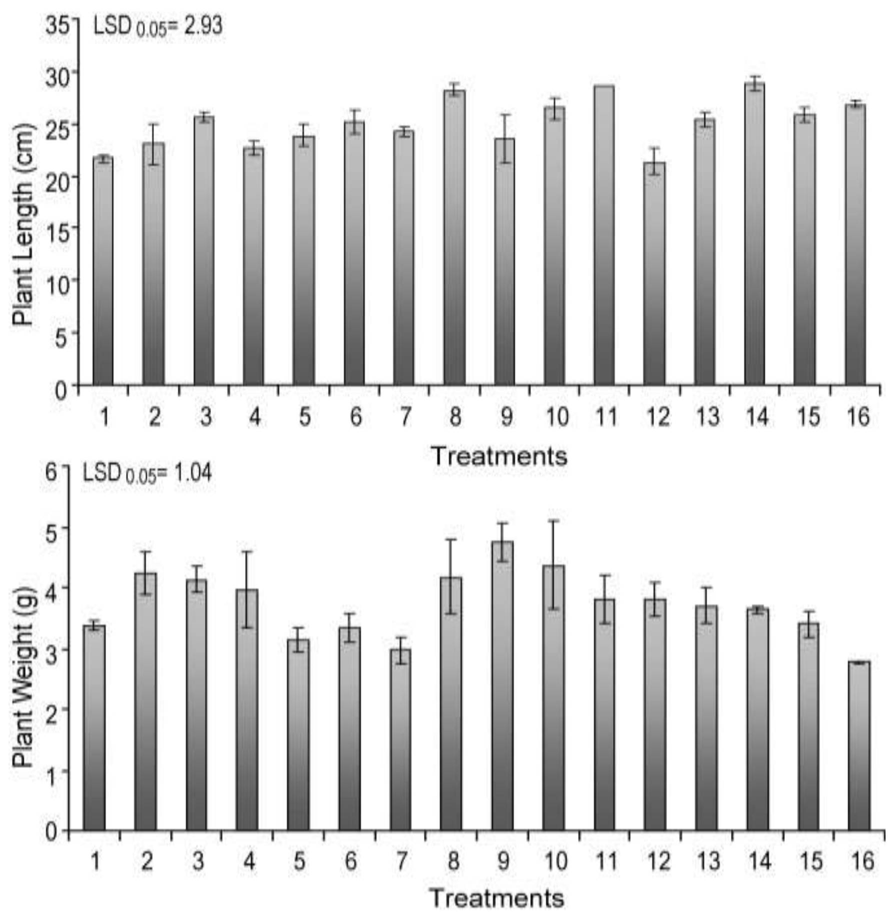

Fig. 1. Combined effect of *Bacillus* species and fertilizers on growth of okra plant.

1=control, 2=Urea, 3=P, 4=di-ammonium phosphate (DAP), 5=*Bacillus coagulans* (BA), 6=*Bacillus subtilis* (BS), 7=*Bacillus licheniformis* (BLB2), 8=BA+Urea, 9=BS+Urea, 10=BLB2+Urea, 11=BA+P, 12=BS+P, 13=BLB2+P, 14=BA+DAP, 15=BS+DAP,16=BLB2+DAP

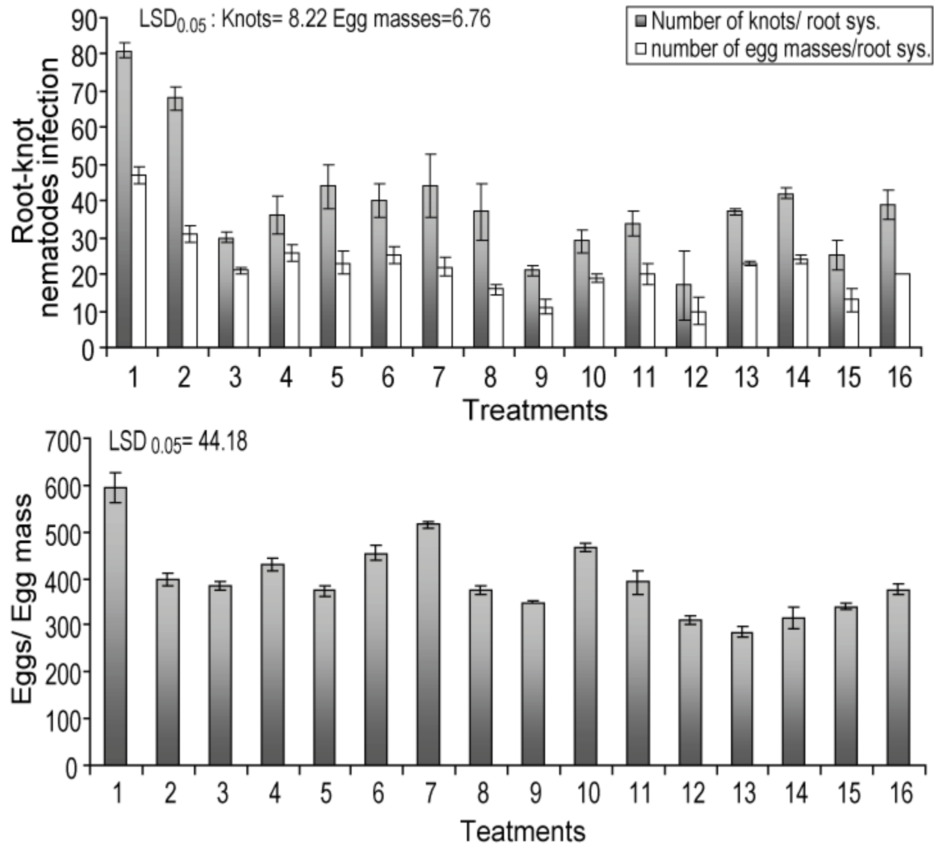

Fig. 2. Combined effect of *Bacillus* species and fertilizers on root-knot nematode infection in okra.

1=control, 2=Urea, 3=P, 4=DAP, 5=*Bacillus coagulans* (BA), 6=*Bacillus subtilis* (BS), 7=*Bacillus licheniformis* (BLB2), 8=BA+Urea, 9=BS+Urea, 10=BLB2+Urea, 11=BA+P, 12=BS+P, 13=BLB2+P, 14=BA+DAP, 15=BS+DAP, 16=BLB2+DAP

Studies have been revealed that some *Bacillus* species increased plant growth when applied in combination with fertilizers (Adesemoye *et al*., 2009) and minerals uptake increased after the application of *Bacillus* species (Datta *et al*., 1982, Rodríguez and Fraga, 1999, Dobbelaere and Okon 2007). *Bacillus* species increased plant growth by enhancing availability and efficiency of nutrient utilization. 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), which eventually directly promoting plant growth and/or inducing resistance against variety of pathogens. On the other hand, use of *Bacillus* species also reducing the rate about 25% of inorganic fertilizers than recommended dose for better plant growth, yield and nutrient uptake in tomato (Adesemoye *et al*., 2009).

Higher severity of root-knot nematode infection was noticed in controls, which showed maximum number of knots (81) and egg masses (47) per root system. on the other hand, least number of knots per root system (17) was recorded in combined treatment of BS and P, which was followed by BS in combination with urea (21) ($P<0.001$). Similarly least egg masses count was observed in plants treated with BS and P together and BS with urea ($P<0.001$) (Fig. 2). Use of rhizobacteria in combination with fertilizers reported a successful strategy to reduced root-knot nematode infection on crop plants under glasshouse conditions (Siddiqui *et al*., 2001). Studies showed that different nitrogenous compounds found nematicidal in pot and field experiments (Oka and Pivonia, 2002). Dawar and Ghaffar (2003) suggested increased antagonistic activity of *Paecilomyces lilacinus* against root infecting fungi in combination with urea and superphosphate in mung bean during greenhouse trials. Another important dependent variable to asses' root-knot nematode infection severity i.e. eggs per egg mass count, which can be correlate with size and potential of egg mass to produce infectious juveniles. Figure 2 revealed lesser number of eggs per egg mass count in all treatments as compared to control ($P<0.001$). Maximum numbers of eggs counted in controls (594 eggs/ egg mass). Eggs of root-knot nematodes reported to destroy after the application of different bacteria (Rodriguez-Kabana *et al*., 1987). Number of eggs/ root system was reduced after the application of endophytes either by inducing pathogenesis related protein production or by producing metabolites that are toxic to nematodes (Roberts *et al*., 1992 ; Hallmann and Sikora 1996; Elmi *et al*., 2000). It was observed that BS isolate with different fertilizers decreases root-knot nematode infection greatly.

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