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EFFECTS OF BIO-PRIMING OF LEGUMINOUS AND NON-LEGUMINOUS CROP SEEDS IN THE MANAGEMENT OF ROOT ROT FUNGI AND GROWTH OF CROP PLANTS

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ABSTRACT

Biopriming of leguminous and non-leguminous seeds was used as an alternative technique to fungicidal treatments for controlling root infecting fungi and increasing growth of leguminous and non-leguminous plants. To investigate the effect of bio-priming on okra (*Abelmoschus esculentus* L.), Sunflower (*Helianthus annuus* L.), Peanut (*Arachis hypogaea* L.) and Chickpea (*Cicer arietinum*L.), seeds were treated with different microbial antagonists like *Trichoderma harzianum*, *Rhizobium meliloti*, *Bacillus* sp. and *Paecilomyces variotii* for different time periods (5, 10, 20 and 40 minutes) were sown in plastic pots under screen house conditions. Results showed that *Macrophomina phaseolina* (Tassi) Goid and *Fusarium* spp were significantly suppressed when seeds were bio-primed with *R. meliloti*, *T. harzianum* and *Bacillus* sp. conidial/cell suspensions at 5, 10 and 20 minutes. Growth parameters were maximum in magnitude when seeds were bio-primed with *R. meliloti* and *T. harzianum* for 10 minutes.

Keywords: Bio-priming, Root rot fungi, Control, Leguminous and non-leguminous crops.

INTRODUCTION

Control of soil borne pathogens depends mainly on fungicidal applications, but fungicides are known to cause hazards to the human health via environmental pollution. Bio-priming of seeds is gaining importance in management of many plant pathogens as another alternative to chemical fungicides in recent times (Callan *et al*., 1990;Conway *et al*., 2001).Bio-priming is a new technique of seed treatment that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) of disease control (Reddy, 2013). The soil borne fungal pathogens like *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium solani, F. oxysporum*, *Pythium* spp., cause various root rot disease complexes on many crops resulting in death of plants. Apart from many soil borne root infecting fungi, there are many beneficial microorganisms possessing antagonistic properties encountered in the soil environment which are reported to inhibit the root rot pathogens. Bio-priming of seeds with microbial antagonists are capable of colonizing the rhizosphere and potentially providing benefits to the plant beyond the seedling emergence stage (Nancy *et al*., 1997).

Micro-organisms providing protection to roots against fungal pathogens is based on using naturally occurring rhizosphere-associated bacteria and fungi with antagonistic properties as biological control agents (Whipps, 1997; Emmert and Handlesman, 1999). Such microbial antagonists are able to build a protective layer around the roots of plants which helps to prevent infection of the hyphae by soil-borne pathogens (Weller, 1988). Antagonistic microorganisms form an important functional group of plant associated beneficial microorganisms which are involved in plant growth promotion and pathogen defense (Berg *et al.,* 2003). According to Harman (2000) *Trichoderma* strain (T-22) improved root growth and plant development when applied in pathogen free soil which may due to the production of growth regulators (Windham *et al.,* 1986).

Apart from fungal microbial antagonists, antagonistic bacteria are numerically the most abundant group of microorganisms in the soil associated with plant root systems. Application of bacteria either as seed dressing or as soil drenching has shown a significant suppression of root infecting pathogens on leguminous and non-leguminous plants (Zaki and Ghaffar, 1987; Ehtesham-ul-Haque *et al*., 1990). Many plant growth promoting rhizobacteria e.g., *Rhizobium* spp., have a beneficial effect on plants including biological control of soil borne pathogens, induce systematic resistance to plant pathogen, improvement of nutrient and water uptake of plant (Seuk Bae *et al*., 2000). Similarly *Bacillus thuringiensis* is also a plant growth promoting bacterium which produces bacteriocin thuricin compounds. *B. thuringiensis* (commonly known as 'Bt') is an insecticidal bacterium, marketed worldwide for control of many important plant pests responsible for some severe diseases of crop plants all over the world (Gray *et al*., 2006). The aim of the present investigation was to determine the periodic effect of bio-priming against root rot fungi and on growth of okra, sunflower, chickpea and peanut.

MATERIALS AND METHODS

Collection of Microbial antagonists: Cultures of *Rhizobium meliloti*, *Paecilomyces variotii, Trichoderma harzianum* and *Bacillus* sp. were obtained from the Karachi University Culture Collection (KUCC). The fungal cultures (*P. variotii* and *T. harzianum*) were grown on PDA medium supplemented with antibiotics (Penicillin @ 1000,000 units/L and Streptomycin @ 0.2 g/L and the plates were incubated at room temperature. The bacterial cultures (*R. meliloti* and *Bacillus* sp) were grown on PDA medium without supplemented with antibiotics and the plates were incubated at room temperature for bio-priming of seeds.

Preparation of pots: Soil used for the experiment was obtained from the experimental plots of the Department of Botany, University of Karachi and passed through 2mm sieve to discard particles. The soil was transferred in 8mm diam., plastic pots @ 300gm/pot soil. The soil used was sandy loam (sand, silt, clay; 70, 19, 11% respectively), pH ranged from 7.5-8.1 with moisture holding capacity (MHC) of 24.04% (Keen and Raczkowski, 1922), total nitrogen 1.5% (Mackenzie and Wallace, 1954), total organic matter 24%. Soil had natural infestation of 4-6 sclerotia/g of *M. phaseolina* as found by wet sieving dilution technique (Sheikh and Ghaffar, 1975), 6-10% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm ,1955) and 3700 cfu g^{-1} *Fusarium* spp., as assessed by soil dilution technique (Nash and Snyder, 1962).

Bio-priming of seeds: Peanut, Chickpea, Sunflower and Okra seeds were bio-primed with 48h-old cultures of *R. meliloti* (158 x 10^7cells/ml), *Bacillus* sp. at (65 x 10^7 cells/ml) and 5 day old cultures of *P. variotii* (19 x 10^3 conidia/ml) and *T. harzianum* (186 x 10^3 conidia/ml)for 5, 10, 20 and 40 minutes and non-primed seeds were used as control. 5 seeds were sown in 8 cm diam., plastic pots and each containing 300g soil and watered regularly to maintained sufficient moisture required for the growth of plants. The pots were kept under screen house in randomized complete block design with three replicates per treatment at different time intervals. Pots containing un-treated seeds were also kept under screen house which served as control. Germination and growth parameters like shoot length, root length, shoot weight and root weight was observed. No. of nodules of leguminous plants and colonization percentage was also recorded after 30 days of seed germination.

Determination of root infecting fungi: To determine the incidence of root rot fungi, one cm long root pieces of leguminous and non-leguminous crop plants after washing in running tap water were surface sterilized with 1% Ca $(OCl)_2$ and transferred on PDA (Potato dextrose agar) containing plates supplemented with Penicillin @ 200 mg and streptomycin @ 200 mg/Litre (5 root pieces per plate). Petri dishes were incubated at room temperature for 5 to 7 days and colonization of roots by root infecting fungi was recorded after incubation period.

Statistical analysis: 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 to compare treatment means, using Statistica software according to Sokal and Rohlf (1995).

RESULTS

In peanut, significant increase ($p<0.001$) in growth parameters like root length, shoot length and shoot weight was observed when seeds were bio-primed with *Bacillus* sp, *T. harzianum* and *P. variotii* conidial/cell suspensions and 10 minutes time interval was found to be effective when peanut seeds were bio-primed with microbial antagonists (Fig. 1). Root length, shoot length significantly increased ($p<0.001$) and significant enhancement in shoot weight ($p<0.01$) was also observed when chickpea seeds were bio-primed with *R. meliloti, P. variotii* and *T. harzianum* conidial/cell suspensions at 5 and 10 minutes time interval (Fig. 2). Significant reduction ($p<0.01$) in root rot fungi like *Fusarium* spp was also seen when chickpea seeds were treated with *R. meliloti* and *T. harzianum* conidial/cell suspensions at 5 and 10 minutes time interval (Fig. 2). In okra, growth parameters like root length and shoot length significantly increased ($p<0.001$) when okra seeds were bio-primed with *R. meliloti* and *T. harzianum* cell/conidial suspensions (Fig. 3). Significant enhancement in root weight ($p<0.5$) and shoot weight ($p<0.001$) of okra were seen when okra seeds were treated with *Bacillus* sp, *R. meliloti, T. harzianum* and *P. variotii* conidial/cell suspensions at 5 and 10 minutes time interval (Fig. 3). Root rot fungus like *M. phaseolina* showed significant suppression when okra seeds were treated with *Bacillus* sp and *T. harzianum* cell/conidial suspensions at 5 and 20 minutes time interval (Fig. 3). In sunflower root length, shoot length, root weight and shoot weight significantly enhanced ($p<0.001$) when sunflower seeds were treated with *R. meliloti, Bacillus* sp, *T. harzianum* and *P. variotii* conidial/cell suspensions at 5, 10, 20, and 40 minutes (Fig. 4). Of the different time periods and microbial antagonists was used for bio-priming of leguminous and non-leguminous seeds 10 minutes treatment with *R. meliloti* and *T. harzianum* was found to be most effective for the control of root rot fungi and growth of crop plants.

Peanut

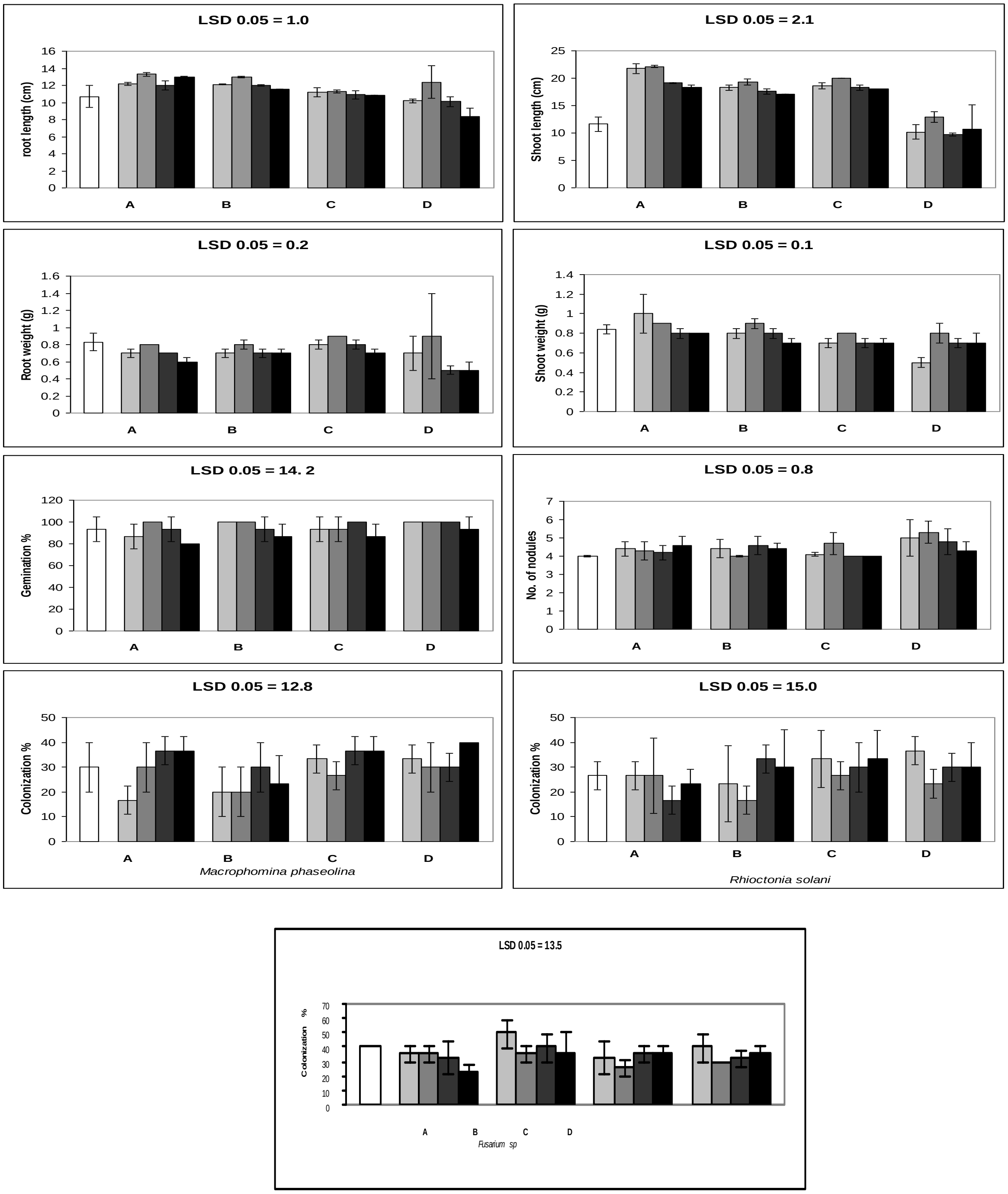

Fig. 1. Bio-priming of Peanut seeds with bacterial and fungal antagonists in the control root rot fungi and growth of plants.

A.	*Bacillus* sp.	C.	*Paecilomyces variotii*
B.	*Rhizobium meliloti*	*D.*	*Trichoderma harzianum*

Time Periods: Control ☐ 5min ☐ 10 min ☐ 20 min ☐ 40 min ☐

Chickpea

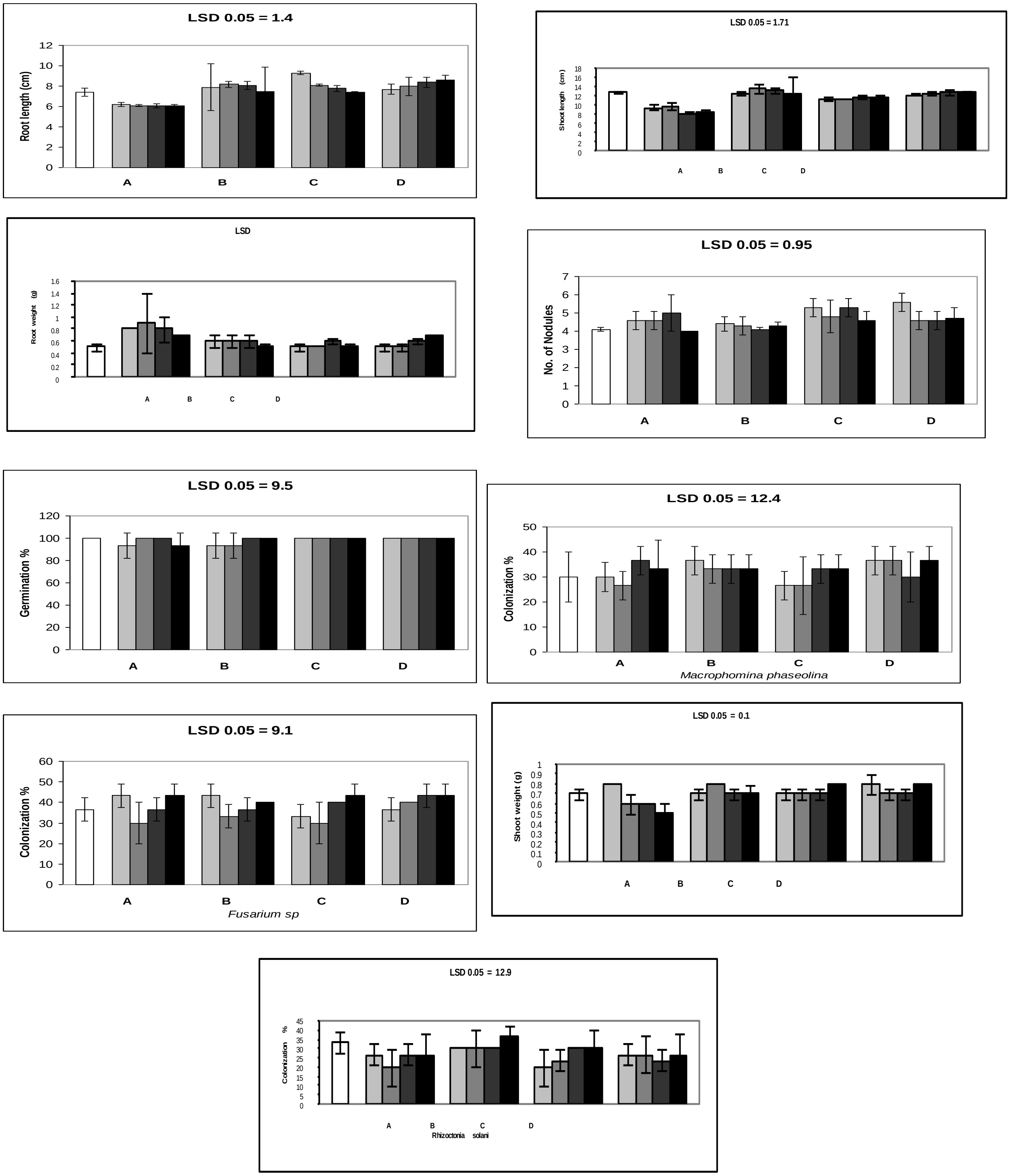

Fig. 2.Bio-priming of Chickpea seeds with bacterial and fungal antagonists in the control root rot fungi and growth of plants.

A. *Bacillus* sp. C. *Paecilomyces variotii*

B. *Rhizobium meliloti* *D.* *Trichoderma harzianum*

Time Periods: Control 5min 10 min 20 min 40 min

Okra

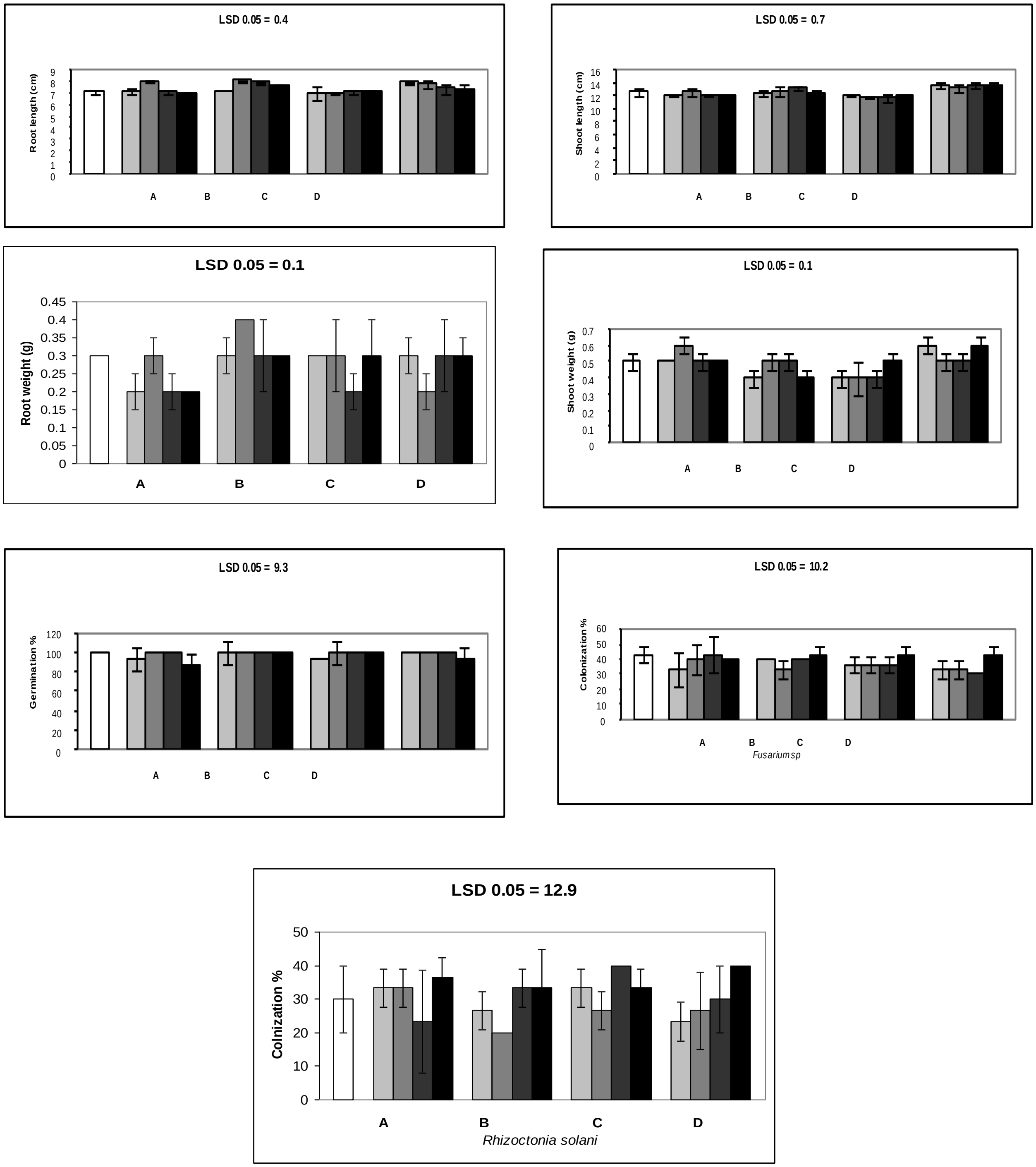

Fig. 3. Bio-priming of okra seeds with bacterial and fungal antagonists in the control root rot fungi and growth of plants.

A. *Bacillus* sp. C. *Paecilomyces variotii*
B. *Rhizobium meliloti* *D.* *Trichoderma harzianum*

Time Periods: Control ☐ 5min ☐ 10 min ☐ 20 min ☐ 40 min ☐

Sunflower

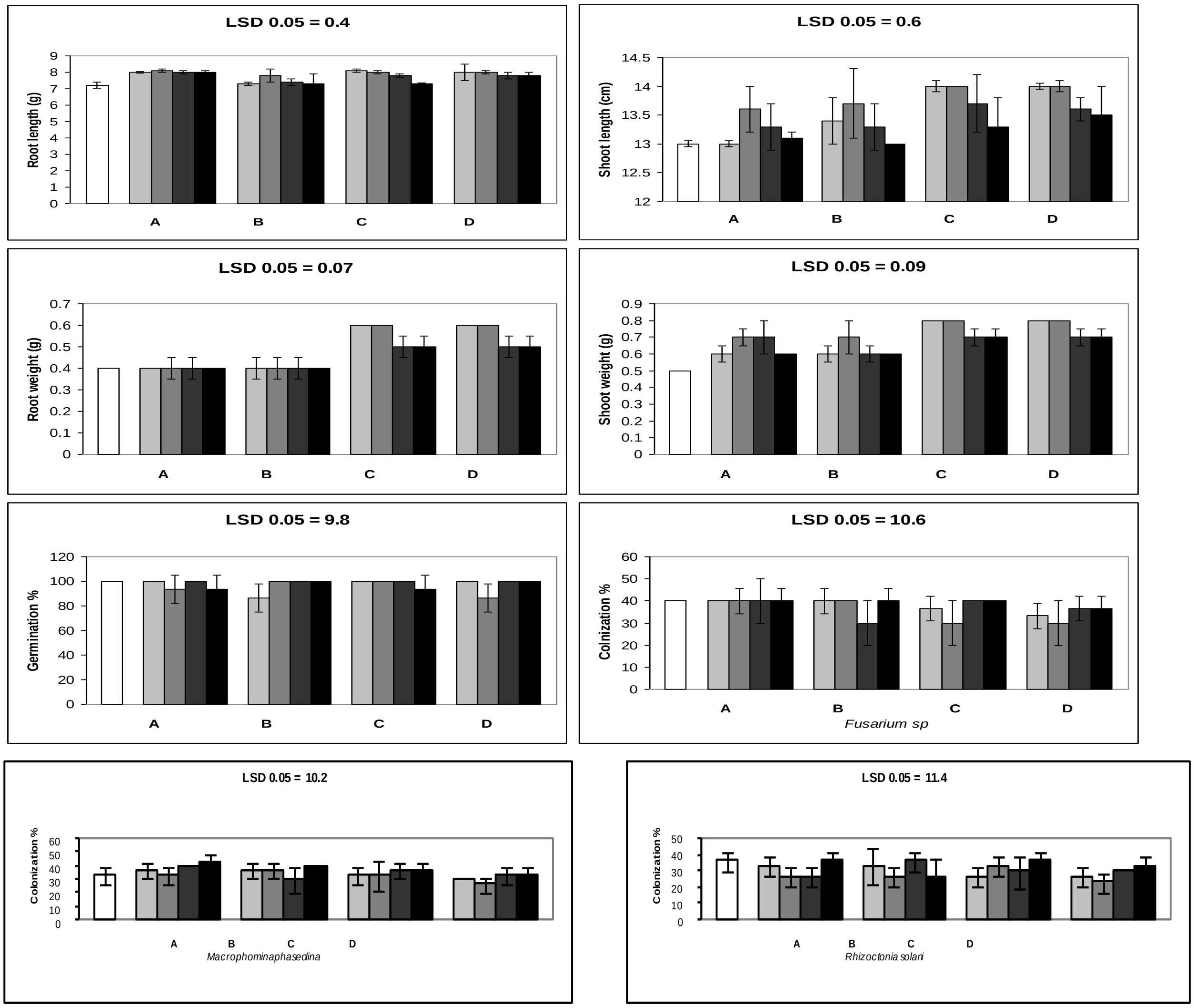

Fig. 4. Bio-priming of Sunflower seeds with bacterial and fungal antagonists in the control root rot fungi and growth of plants.

A. *Bacillus* sp. C. *Paecilomyces variotii*

B. *Rhizobium meliloti* *D.* *Trichoderma harzianum*

Time Periods: Control 5min 10 min 20 min 40 min

DISCUSSION

In the present study, bio-priming of leguminous (chickpea, peanut) and non-leguminous seeds (okra, sunflower)at different period of time with fungal and bacterial antagonists such as *Paecilomyces varioti*, *Trichoderma harzianum*, *Rhizobium meliloti* and *Bacillus* sp. enhanced the growth parameters and significantly reduced the incidence of root rot fungi like *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. In peanut, significant increase in growth parameters like root length, shoot length and shoot weight was observed when seeds were bio-primed with *Bacillus* sp, *T. harzianum* and *P. variotii* conidial/cell suspensions and 10 minutes time interval was found to be effective when peanut seeds were bio-primed with microbial antagonists. Harman (2000) reported that *Trichoderma* (strain T-22) improves root growth and plant development in diseased free plants. This may due to the ability of antagonistic fungi to solubilize nutrients (Altomare *et al.*, 1999). Formulations of *T.*

harzianum, G. virens and *T. viride* both as soil application and seed treatment against *R. bataticola,* causative agent of dry root rot disease on chickpea under field and green house conditions were found to be most effective in suppressing the root rot disease development as well as improving the seed germination, plant growth and grain yield (Dubey *et al.,* 2011). Present results showed that root and shoot length significantly increased and significant enhancement in shoot weight was also observed when chickpea seeds were bio-primed with *R. meliloti, P. variotii* and *T. harzianum* conidial/cell suspensions at 5 and 10 minutes time interval and significant reduction in root rot fungi like *Fusarium* spp was also seen when chickpea seeds were treated with *R. meliloti* and *T. harzianum* conidial/cell suspensions at 5 and 10 minutes time interval. Several studies have shown that fungal and bacterial microbial antagonists can control diseases caused by many root infecting pathogens, including *Fusarium*, *Rhizoctonia* and *Macrophomina.* Treatment of cotton seeds with *Gliocladium virens* and *Bacillus subtilis* reduced the colonization of roots by *Fusarium oxysporum* and reduced *Fusarium* wilt incidence and severity also (Zhang *et al.,* 1996). In the present research, growth parameters of okra like root and shoot length significantly increased when okra seeds were bio-primed with *R. meliloti* and *T. harzianum* cell/conidial suspensions. There was also significant enhancement in root weight and shoot weight of okra seen when okra seeds were treated with *Bacillus* sp, *R. meliloti, T. harzianum* and *P. variotii* conidial/cell suspensions at 5 and 10 minutes time interval and root rot fungus like *M. phaseolina* showed significant suppression when okra seeds were treated with *Bacillus* sp. and *T. harzianum* cell/conidial suspensions at 5 and 20 minutes time interval. Similarly, growth parameters of sunflower significantly increased when sunflower seeds were treated with *R. meliloti, Bacillus* sp, *T. harzianum* and *P. variotii* conidial/cell suspensions at 5, 10, 20, and 40 minutes. *Bacillus subtilis* applied as microcapsules for treatment of tomato seeds reduced the disease incidence and severity caused by *R. solani* and *F. oxysporum* and plant growth and dry weight of roots as well as yield of fruits were also increased significantly due to seed treatment compared with untreated controls (Harnandez*et al.,* 2007). A strain of *T. harzianum* was reported to trigger host defense mechanisms in cucumber plants through induction of defense enzymes (Yedidia *et al.,* 1999). Many studies have confirmed the potential of *T. harzianum* in reducing disease development of *Botrytis cinerea* on cucumber and tomato (Dik and Elad, 1999; O'Neil *et al.,* 1996; Uthkede *et al.,* 2000). Present results suggest growth parameters were maximum when seeds were bio-primed with *R. meliloti* and *T. harzianum* for 10 minutes and root rot fungi like *Macrophomina phaseolina* and *Fusarium* spp. were significantly suppressed when seeds were bio-primed with *R. meliloti*, *T. harzianum* and *Bacillus* sp. conidial/cell suspensions at 5, 10 and 20 minutes.

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