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LONG-TERM RESISTANCE OF BIO-PRIMED SEEDS IN THE SUPPRESSION OF ROOT INFECTING PATHOGENS AND ESTABLISHMENT OF CROP PLANTS

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ABSTRACT

Long-term resistance of bio-primed leguminous and non-leguminous seeds with microbial antagonists (*Trichoderma harzianum* and *Rhizobium meliloti*) was studied for controlling the root rot pathogenic fungi and establishment of crop plants. To investigate the long-term activity of bio-primed okra (*Abelmoschus esculentus* L.), sunflower (*Helianthus annuus* L.), peanut (*Arachis hypogaea* L.) and chickpea (*Cicer arietinum* L.) seeds were stored under different storage temperatures (4°C and room temperature) at different storage periods (0, 30, 60, 90, 180 and 360 days). It was found that seeds bio-primed with *T. harzianum* were effective for 180 days, whereas, *R. meliloti* bio-primed seeds were resistant for 90 days and storage temperature of 4°C was found to be most effective for controlling the root rot fungal pathogens like *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium* spp as well as for increasing the growth of crop plants.

KEYWORDS: Storage, Bio-priming, *Trichoderma harzianum, Rhizobium meliloti*, Root rot fungi.

INTRODUCTION

Use of good quality of seeds is the most important thing in management of diseases (Ahmad, 2001), because good quality seeds play a marked role in better yield production and germination. 30 percent of crop production can be increased by the use of good quality of seeds (Bari, 1993) whereas, ten to fifteen % production was reduced due to the use of poor quality of seeds (Huda, 2001). At the time of storage major part of quality of seeds deteriorates under storage period. Retention of seeds and storage of seed viability is an important tool in agricultural practice. Most of the farmers lack knowledge of seed preservation. They store their seeds just like they store their food grains. Destruction rate depends on conditions of storage such as temperature, moisture, relative humidity, seed moisture contents, and types of storage container, etc. Bio-priming of seeds significantly increased the long-term antifungal resistance of crop seeds during storage. High temperature, moisture and relative humidity in the storage environment occurs as the principle factors involved in deterioration of quality of seeds which influenced the health of seed (Fakir, 1989).

Beneficial microorganisms produced an important functional group of plants which are associated with growth promotion and pathogen defence (Berg *et al.,* 2003). Both quality and crop production can be enhanced by the seed and seedling treatment with antagonistic micro-organisms (Beckers and Conrath 2007, Buensanteai *et al*., 2009). Microbial antagonists can also be useful for controlling the rots of post-harvest and reduced the fungicide use, (Droby *et al*., 1991; Chand-Goyal and Spotts, 1996, 1997). Application of bacteria either as seed dressing or as soil drenching produced 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 eg., *Rhizobium* spp., have a beneficial impact on plants such as biological control of soil borne pathogens, induce systematic resistance to plant pathogen, improvement of plant water and nutrient uptake (Seuk Bae *et al*., 2000). Some strains of beneficial fungus *Trichoderma* are good colonizers of roots which are called as rhizosphere competent. It has also been stated that *T. harzianum* (strain T-22) has the potential of increasing the growth of roots and development of plants in the pathogen absence (Harman, 2000). Present work was therefore carried out for determining the long term resistance of storage of seeds bio-primed with *R. meliloti* and *T. Harzianum* in the control of root infecting pathogenic fungi and growth of crop plants such as okra, sunflower, chickpea and peanut.

MATERIALS AND METHODS

Collection of microbial antagonists: Cultures of *Rhizobium meliloti* and *Trichoderma harzianum* were obtained from the Karachi University Culture Collection (KUCC) for seed bio-priming.

Bio-priming of seeds: Leguminous seeds (peanut and chickpea) and non-leguminous seeds (sunflower and okra) were bio-primed with conidial/cell suspension of 48h-old culture of *Rhizobium meliloti* (158 x 10^7cells/mL) and 5 day old culture of *Trichoderma harzianum* (186 x 10^3 conidia/mL) and air dried on blotter paper.Seeds which are non-primed were acted as control.

Storage of seeds: Leguminous and non-leguminous seeds after bio-priming with conidial/cell suspension of *T. harzianum* and *R. meliloti* were stored in air tight glass containers at room temperature and 4 °C for 0, 30, 60, 90, 180 and 360 days for the determination of long-term activity of bio-primed seeds. Non-primed seeds which acted as control also stored in glass containers at 4°C and room temperature for 0, 30, 60, 90, 180 and 360 days.

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). Five seeds were sown in plastic pots (8 cm diameter) containing soil after 0, 30, 60, 90 and 180 days and placed inside complete randomized block design and watered regularly for maintaining the moisture content.

Determination of growth parameters and root infecting fungi: After 30 days of germination, plants were uprooted carefully and excess soil from roots were washed with tap water and growth parameters were taken. 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, storage of seeds (bio-primed with *T. harzianum* conidial suspension) for 0, 30, 60, 90 and 180 days at 4°C significantly increased ($p<0.001$) the root length and shoot length as compared to the control and seeds stored at room temperature (Fig. 1). Root weight of peanut seeds significantly enhanced ($p<0.001$) when seeds bio-primed with *T. harzianum* and *R. meliloti* conidial/cell suspension stored for 0 and 30 days at room temperature whereas, storage of bio-primed seeds (with *T. harzianum* conidial suspension) for 60, 90 and 180 days at 4°C found to be most significant ($p<0.001$) for root weight of peanut. Shoot weight of peanut elevated significantly ($p<0.001$) when *T. harzianum* bio-primed seeds stored for 60, 90 and 180 days. Storage of peanut seeds bio-primed with *T. harzianum* for 180 days at 4°C significantly reduced the incidence of *M. phaseolina* and *R. solani* whereas storage of peanut seeds for 90 days at 4°C significantly ($p<0.001$) suppressed *Fusarium* sp (Fig. 1). Storage of chickpea seeds bio-primed with *T. harzianum* conidial suspension for 90 days at 4°C significantly increased ($p<0.05$) the germination. Shoot length and root length of chickpea enhanced significantly ($p<0.001$) when *T. harzianum* bio-primed seeds stored for 180 days at 4°C. Root weight of chickpea seeds bio-primed with *T. harzianum* conidial suspension and stored for 90 days at room temperature increased significantly ($p<0.001$) whereas, storage of chickpea seeds for 60, 90 and 180 days at 4°C greatly enhanced ($p<0.001$) the root weight. *T. harzianum* bio-primed chickpea seeds stored for 180 days at room temperature significantly promoted ($p<0.001$) the shoot weight whereas, 4°C temperature was found to be significantly effective ($p<0.001$) for shoot weight when seeds bio-primed with *T. harzianum* and stored for 90 and 180 days respectively. Storage of chickpea seeds (bio-primed with *T. harzianum*) for 90 and 180 days significantly reduced ($p<0.001$) the colonization of *M. phaseolina* followed by *R. solani* and *Fusarium*sp (Fig. 2). In okra, storage of bio-primed seeds (with *T. harzianum* conidial suspension) for 90 and 180 days at 4°C significantly increased ($p<0.001$) the growth parameters of okra and germination was also enhanced significantly ($p<0.001$) when *T. harzianum* primed seeds stored for 60 days at room temperature and 30, 60 and 90 days at 4°C. Root rot fungi like *M. phaseolina* greatly reduced ($p<0.001$) when *T. harzianum* bio-primed seeds stored for 180 days at 4°C (Fig. 3). In sunflower, storage of bio-primed seeds with *T. harzianum* for 60 days at 4°C and for 180 days at 4°C significantly increased ($p<0.001$) the root length and shoot length. Root weight of sunflower enhanced significantly ($p<0.001$) when seeds bio-primed with *R. meliloti* and stored for 0, 30, 60 and 90 days and when seeds bio-primed with *T. harzianum* and stored for 0, 30, 60, 90, and 180 days. Shoot weight of sunflower increased significantly ($p<0.001$) when seeds bio-primed with *T. harzianum* and stored for 90 and 180 days. Storage of sunflower seeds bio-primed with *T. harzianum* for 60, 90 and 180 days at 4°C significantly decreased ($p<0.001$) the colonization of *M. phaseolina* and *R. solani* (Fig. 4). It was found that seeds bio-primed with *T. harzianum* were effective for 180 days, whereas, *R. meliloti* bio-primed seeds were resistant for 90 days and storage temperature of 4°C was found to be most effective for controlling the root rot fungal pathogens like *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium* spp as well as for increasing the growth of crop plants.

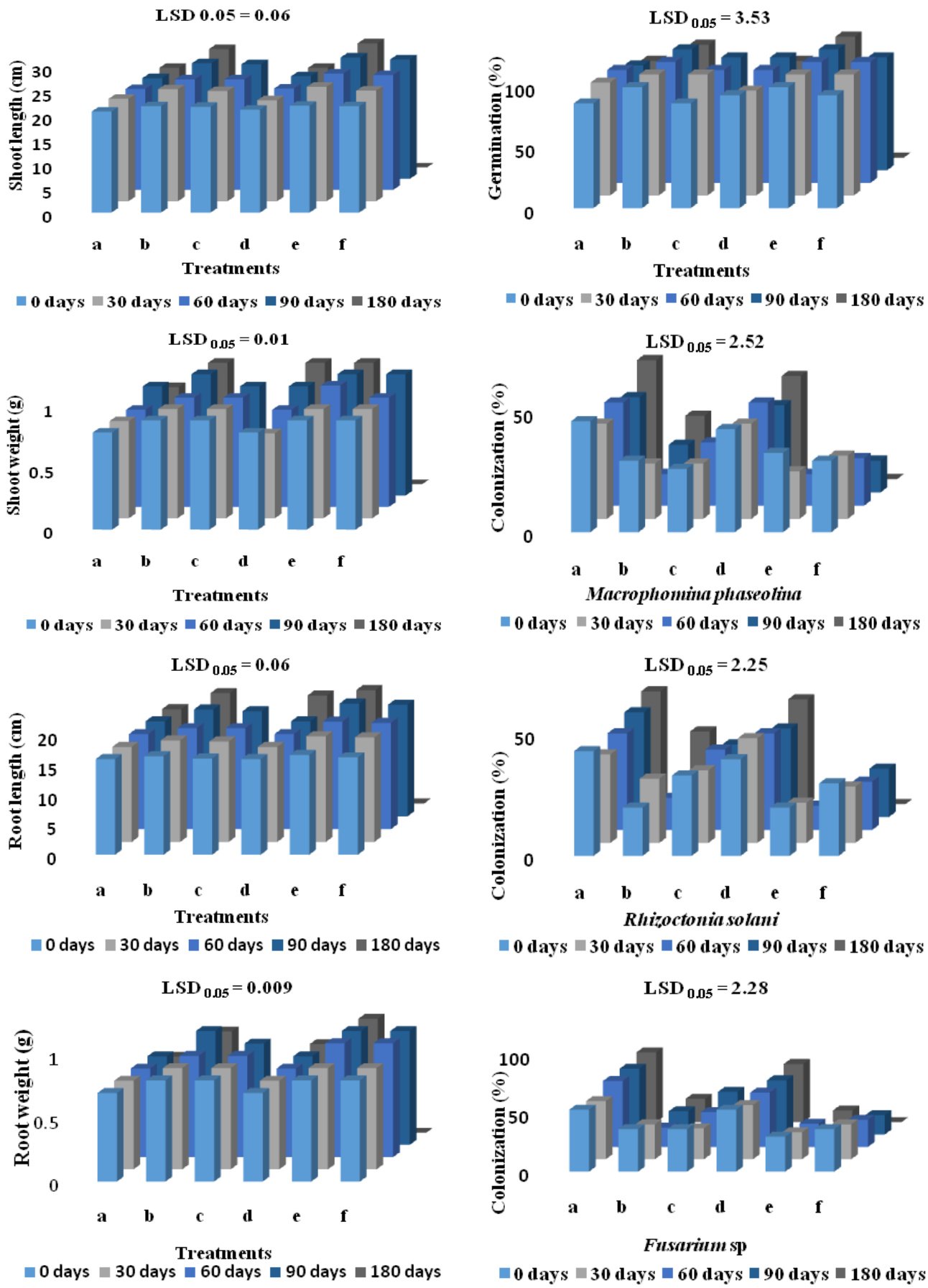

Fig. 1. Long-term resistance of bio-primed 'peanut' seeds in the suppression of root infecting pathogens and establishment of crop plants.
a. Control (Room temp), b. *T. harzianum* (Room temp), c. *R. meliloti* (Room temp)
d. Control (4°C), e. *T. harzianum* (4°C), f. *R. meliloti* (4°C)

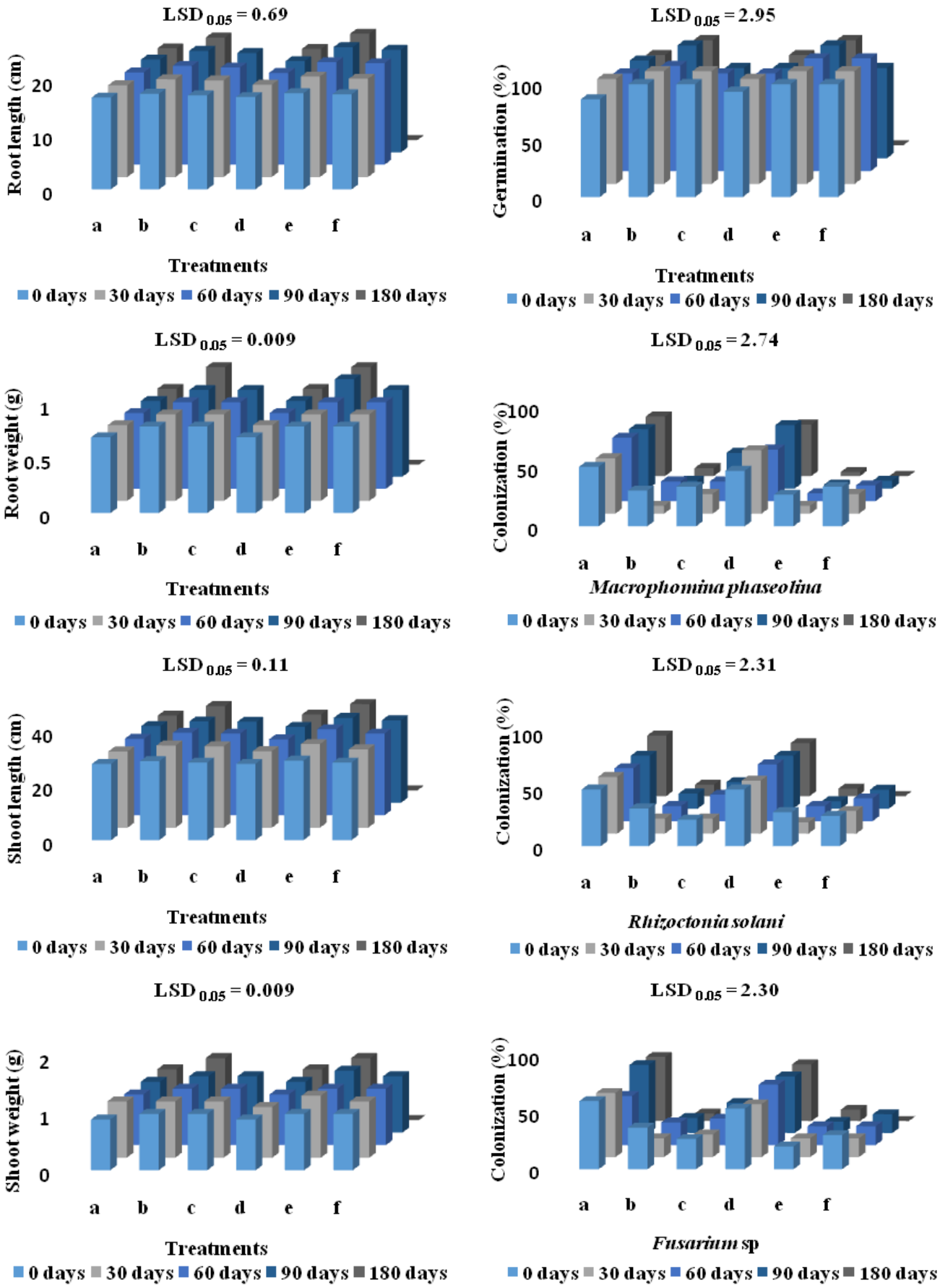

Fig. 2. Long-term resistance of bio-primed 'chickpea' seeds in the suppression of root infecting pathogens and establishment of crop plants.
a. Control (Room temp) b. *T. harzianum* (Room temp) c. *R. meliloti* (Room temp)
d. Control (4°C) e. *T. harzianum* (4°C) f. *R. meliloti* (4°C)

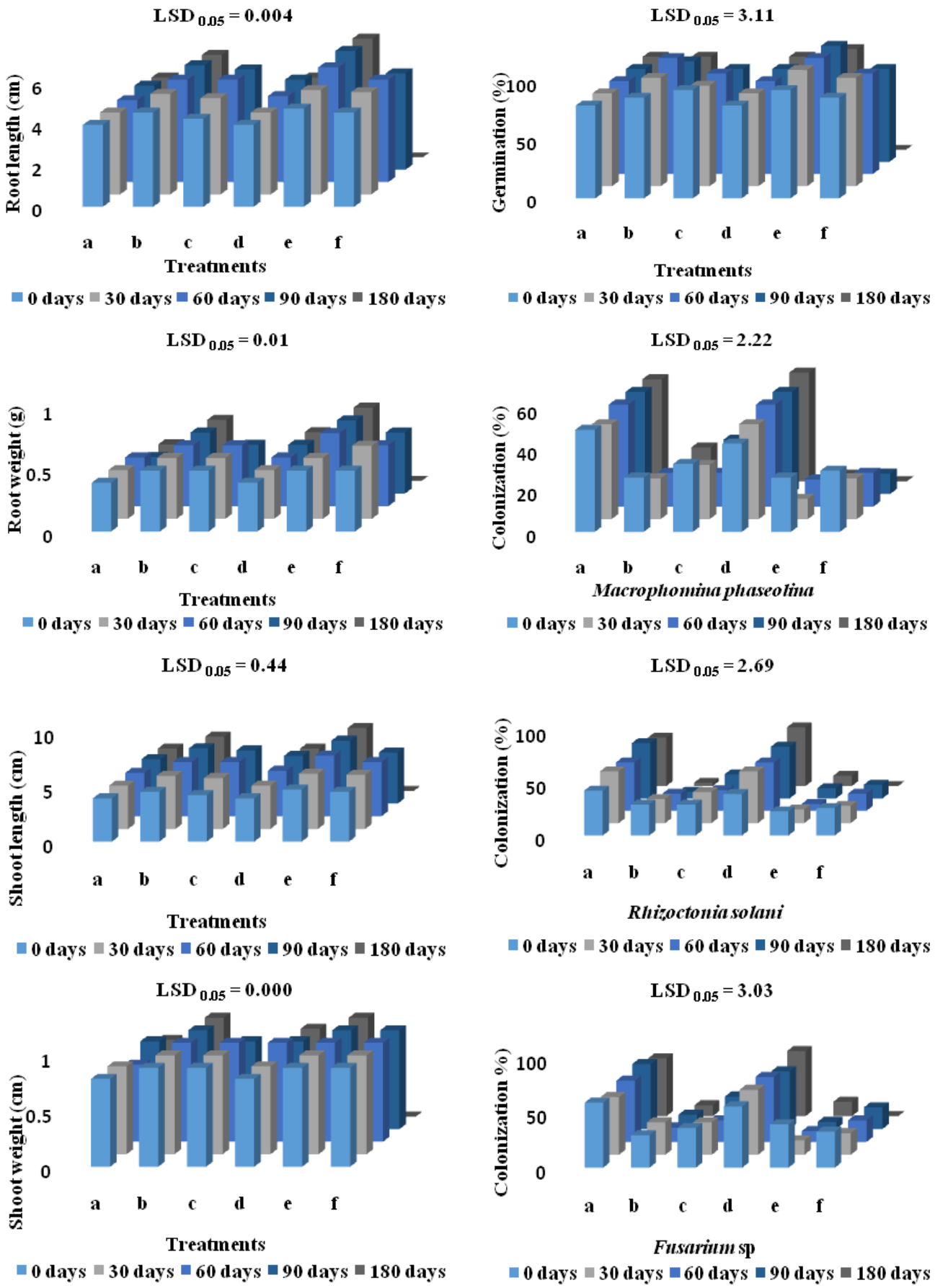

Fig. 3. Long-term resistance of bio-primed 'okra' seeds in the suppression of root infecting pathogens and establishment of crop plants.
a. Control (Room temp) b. *T. harzianum* (Room temp) c. *R. meliloti* (Room temp)
d. Control (4°C) e. *T. harzianum* (4°C) f. *R. meliloti* (4°C)

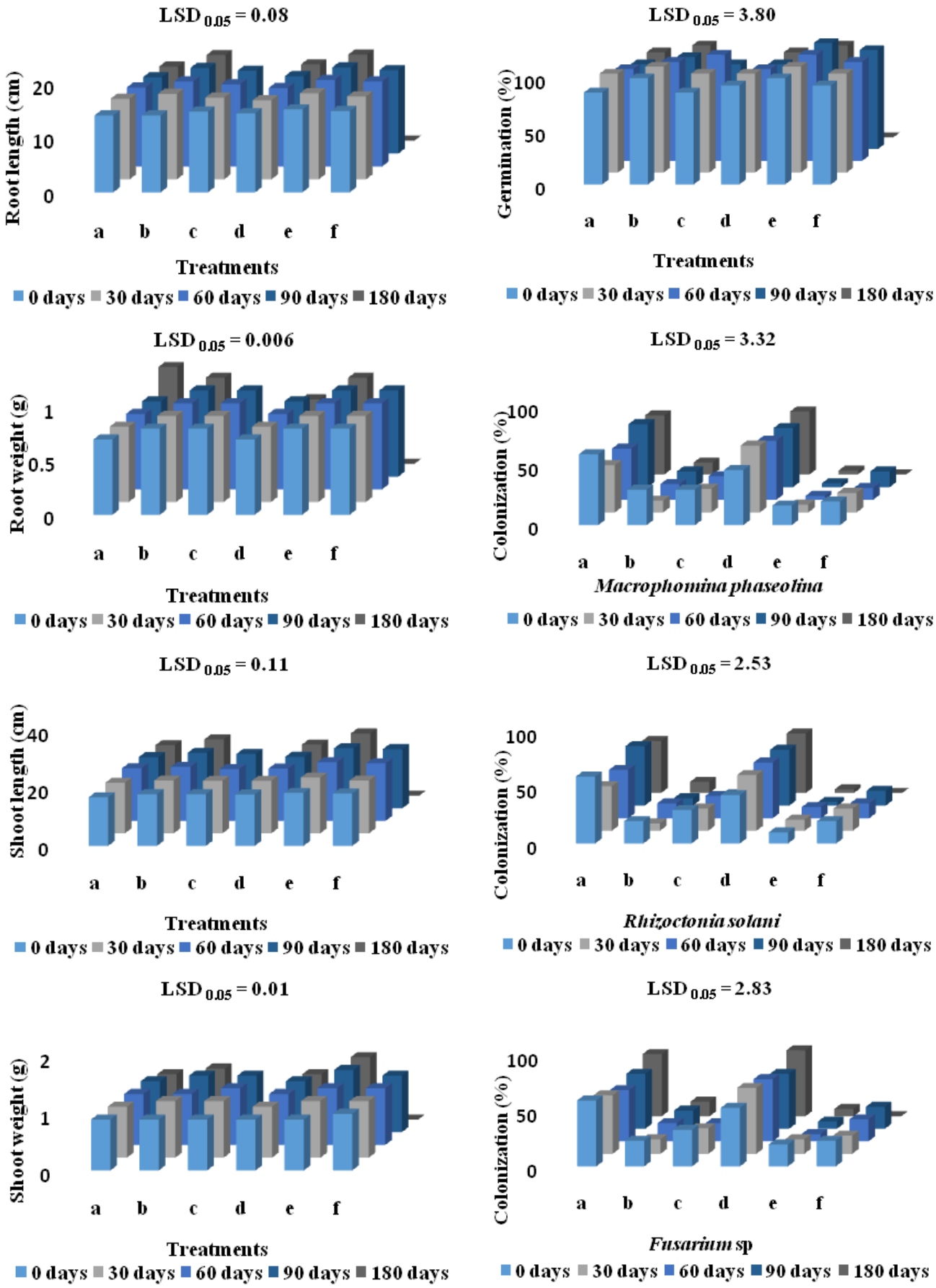

Fig. 4. Long-term resistance of bio-primed 'sunflower' seeds in the suppression of root infecting pathogens and establishment of crop plants.
a. Control (Room temp) b. *T. harzianum* (Room temp) c. *R. meliloti* (Room temp)
d. Control (4°C) e. *T. harzianum* (4°C) f. *R. meliloti* (4°C)

DISCUSSION

Storage of leguminous and non-leguminous seeds after bio-priming with *T. harzianum* and *R. meliloti* for 0, 30, 60, 90 and 180 days at room temperature and 4°C was found to be effective against root rot fungal pathogens and growth of crop plants. Several researchers studied storage of seeds under different storage conditions. Bankole *et al.* (1999) stored seeds of melon in jute bags of polyethylene for 12 months. Storage of peanut seeds bio-primed with *T. harzianum* under different storage conditions increased the growth parameters of peanut as well as reducing the pathogenic fungi. It was found that seeds after bio-priming with *T. harzianum* for 180 days at 4°C reduced the incidence of *M. phaseolina* and *R. solani* whereas storage of peanut seeds for 90 days at 4°C significantly suppressed *Fusarium* sp. El-Mougy and Abdel-Kader (2008) evaluated the long-term activity of bio-priming of seeds with beneficial bacterial and fungal micro-organisms against the faba bean root rot disease and stated that under greenhouse conditions, all the fresh tested and storage of bio-primed faba bean seeds for 60 days were significantly effective for the growth of plants and causing complete suppression of incidence of root rot both at pre and post-emergence plant stages as compared to the control. Storage of chickpea seeds bio-primed with *T. harzianum* conidial suspension for 90 days at 4°C significantly increased the germination. Lin (1999) reported that percentage of germination remained high throughout the period of storage when seeds of cucumber with initial moisture content of 12 % were stored at 20°Cat 70% RH for about 10 months. In okra, storage of bio-primed seeds (with *T. harzianum* conidial suspension) for 90 and 180 days at 4°C significantly increased the growth parameters of okra and germination was also enhanced significantly when *T. harzianum* primed seeds stored for 60 days at room temperature and 30, 60 and 90 days at 4°C. Pradhan and Badola (2012) reported 4°C as the most effective temperature for the longer periods of storage. Storage of sunflower seeds bio-primed with *T. harzianum* for 60, 90 and 180 days at 4°C significantly decreased the colonization of *M. phaseolina* and *R. solani.* The beneficial micro-organisms rapidly multiplied on seed surface in seed treatment methods which reduced the incidence of pathogens (Raguchander *et al*., 1998). Malik and Dawar (2003) stated that *T. harzianum* protects the plant root system from the infection of *F. solani, R. solani* and *M. phasoelina* on a vast number of crops. Present results clearly suggested that, bio-priming of seeds with beneficial micro-organisms has the potential to increase the viability, vigour index, seed health as well as minimize the entry of soil and seed borne pathogens which are safe, non-hazardous, cheaper and should be applied on a large scale.

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