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VARIETAL RESISTANCE OF SUNFLOWER AND OKRA BIO-PRIMED SEEDS AGAINST ROOT INFECTING FUNGI AND ESTABLISHMENT OF CROP PLANTS

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ABSTRACT: *Okra (Abelmoschus esculentus L.) varieties like OH-152, Arka anamika and unknown variety and sunflower (Helianthus annuus L.) varieties like S-278, Hysun-39 and unknown variety after bio-priming with leaf extracts of Acacia nilotica (L.) Willd. ex Delile Sapindus mukorossi (L.) and microbial antagonists (Trichoderma harzianum and Rhizobium meliloti) at different time intervals (10, 20 minutes) were screened against root infecting fungal pathogens (Macrophomina phaseolina, Rhizoctonia solani and Fusarium spp) and growth of crop plants. Results obtained showed that among all the three varieties of sunflower, variety S-278 after bio-priming with A. nilotica leaf extract for 10 minutes and T. harzianum conidial suspension for 20 minutes was found to be most effective for the establishment of plants and completely control the colonization of M. phaseolina followed by Hysun-39 and unknown variety. Whereas in case of okra, variety OH-152 after bio-priming with A. nilotica leaf extract, T. harzianum and R. meliloti cell/conidal suspension for 10 minutes was recorded to be most effective for the complete inhibition of M. phaseolina and significant elevation of growth of plants followed by A. anamika and unknown varieties.*

KEYWORDS: Bio-priming, varieties, sunflower, okra, root rot fungi.

INTRODUCTION

Sunflower found to be as one of the major edible oil crops all over the world. Hatam & Abbasi, 1994, stated that 25-48 % percent of oil is present in seeds of sunflower. Sunflower oil contains about 60 % of poly-unsaturated fatty acids and this is the reason that sunflower oil is also called as premium oil which helps the blood to consume less cholesterol up to a protected level (Satyabrata *et al*., 1988). In Pakistan, during 1991-92, the cultivation area covered by sunflower was 63328 ha with production amount of 83312 tones. However, during 2010-11, the area elevated to 448000 ha with production of 643000 and 244000 tons of seed and oil (Anon, 2012). Moreover, sunflower meal served as a rich source of crude protein for feeding the livestock as a source of vegetable protein. Similarly, Okra as vegetable crop is grown on a commercial level annually all over the tropical and sub-tropical regions of the world for its immature fruit (Martin & Ruberte, 1978). Okra consists of rich amounts of nutrition and is also called as "a perfect villager's vegetable" (Adamou *et al*., 2010; Holser & Bost, 2004). Okra also served as an important source of carbohydrates, minerals, amino acids which play a major role in human diet (Gopalan *et al*., 2007; Farinde *et a*l., 2007; Dilruba *et al*., 2009; Saifullah & Rabbani, 2009). Average production of okra in the world found to be six million tons per year (Burkil, 1997). In Pakistan, it is cultivated on an area of 15,081 hectares with 114,657 million tons of production (Varmudy, 2011).

Both okra and sunflower are economically very important crops and are prone to attack by different pathogens, including bacteria, fungi, nematode and virus. Among all these pathogenic agents the most destructive pathogen for these crops is fungi. It causes heavy yield losses every year. The root rot pathogenic fungi are major threat for these crop as these fungi attack on the plant roots and destroy the proper functioning of the plant to take water and other nutrients uptake. *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* are considered as major root infecting pathogens of these crops. Bio-priming treatment with beneficial micro-organisms and plant extracts is potentially very prominent to induce profound changes in plant health and vigour and to establish more uniform germination of seeds and plants growth associated with bacteria and fungi coatings. Bio-priming is a method that protects the seeds biologically with the help of beneficial organisms from the diseases (Reddy, 2013). The present study gives an account of the screening of bio-primed varieties of sunflower and okra in the inhibition of root infecting fungi and establishment of crop plants.

MATERIALS AND METHODS

Collection of varieties, plant parts and microbial antagonists: Different varieties of sunflower (S-278, Hysun-39, unknown variety) and okra (OH-152, *Arka anamika*, unknown variety) were obtained from different cities of Pakistan. *A. nilotica* and *S. mukorossi* leaf parts were obtained from Campus of University of Karachi, air dried separately under sun exposure and ground in an electric grinder. Cultures of *Rhizobium meliloti* (*Rm*-5) and *Trichoderma harzianum* (*Th*-6) were also obtained from Karachi University Culture Collection (KUCC)

Suspension and extract preparation: Aqueous leaf extracts of *A. nilotica* and *Sapindus mukorossi* were prepared by soaking the leaves powder separately for 24 hours (10 gm powder and 90 ml distilled water). After 24 hours, the suspension was filtered through Whatman's filter paper in order to get aqueous extracts for priming. Whereas the cell/conidial suspensions of *T. harzianum* and *R. meliloti* were prepared by adding the distilled sterilized water into the pure cultures for bio-priming of seeds.

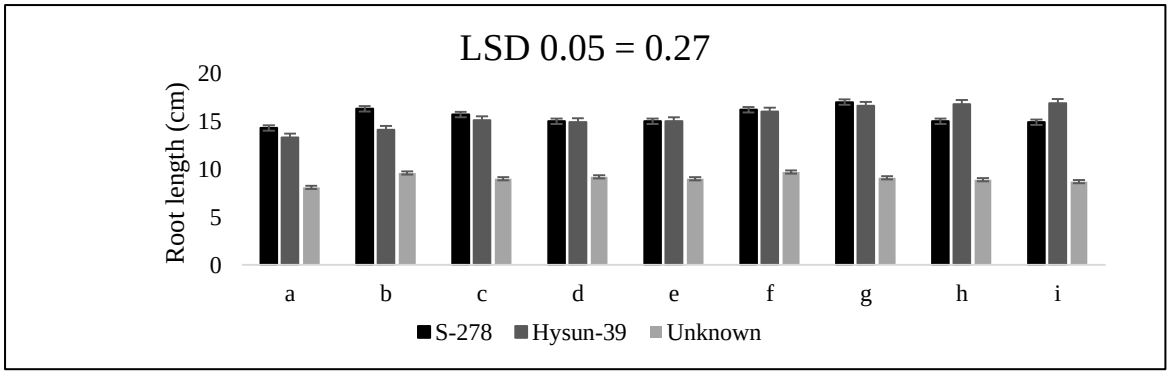
Soil used: Soil used was obtained from experimental plot of Department of Botany, University of Karachi. The sandy loam soil containing (sand, silt, clay, 70, 11 and 10%), pH ranged from 7.1-9.65 with moisture holding capacity (MHC) of 49% (Keen & Raczkowski, 1922), total nitrogen 0.077-0.099% (Mackenzie & Wallace, 1954), 3-7 sclerotia of *M. phaseolina* g-1 as found by wet sieving technique (Sheikh & Ghaffar, 1975), 5-20% of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and *Fusarium* spp., 2000 cfu g-1 as assessed by soil dilution technique (Nash & Synder, 1962).

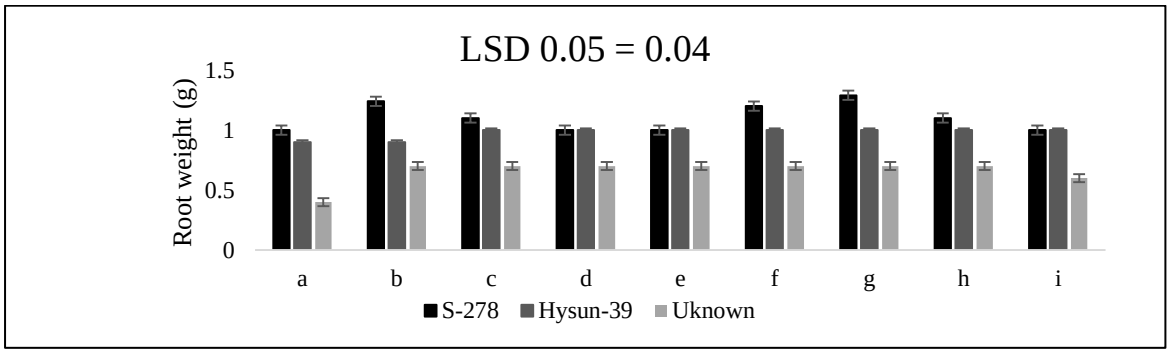
Experimental design: Varieties of sunflower and okra after bio-priming with leaf extracts (*A. nilotica, S. mukorossi*) and cell/conidial suspension of *R. meliloti* (158 x 10^7cells/ml) and *T. harazianum* (186 x 10^3 conidia/ml) for 10 and 20 minutes were sown in 8 cm diam., plastic pots (5 seeds per pot) 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

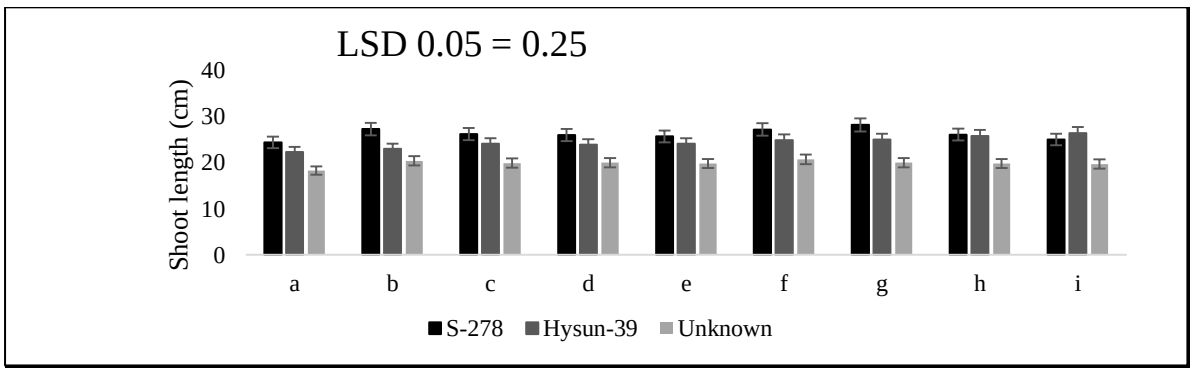
root weight were observed. Colonization percentage was also recorded after 30 days of seed germination.

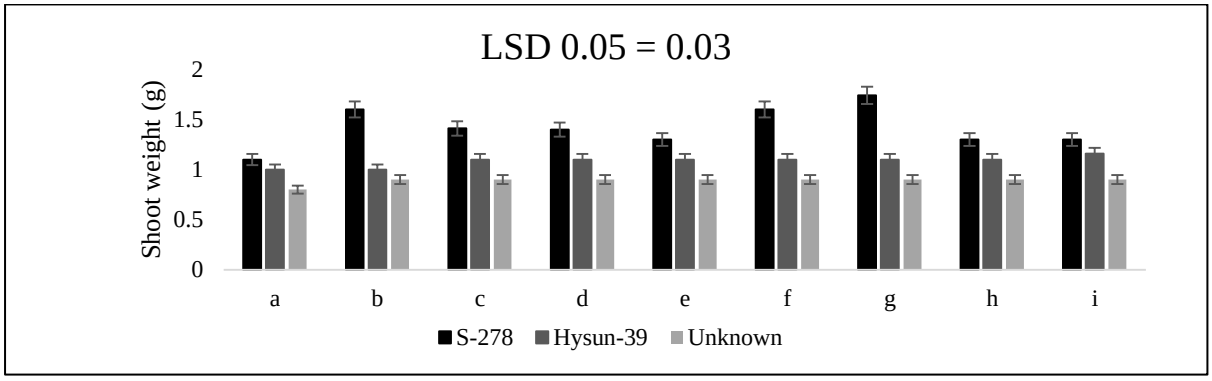
Determination of root infecting fungi: To determine the incidence of root rot fungi, one cm long root pieces of sunflower and okra varieties after washing with running tap water were surface sterilized with 1% Ca $(OCl)_2$ and transferred on PDA (Potato dextrose agar) containing plates supplemented with 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.

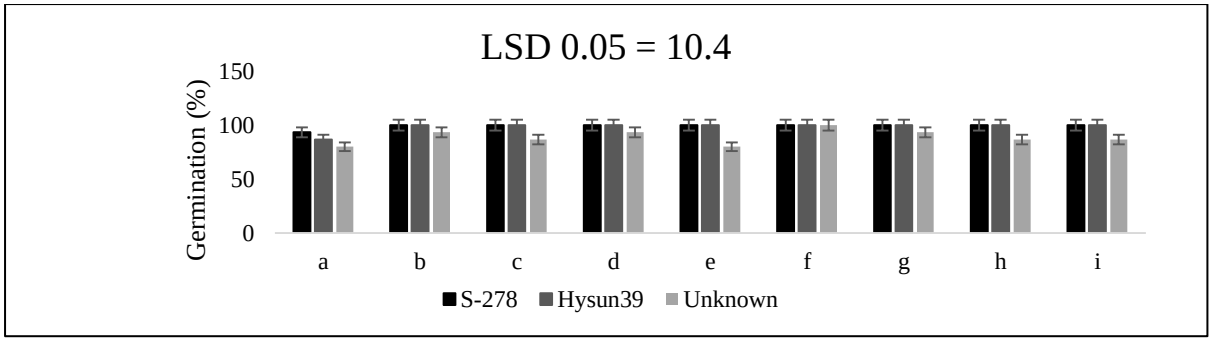
Statistical analysis: Data were subjected to analysis of variance (ANOVA) followed by the least significant difference (LSD) test at P = 0.05 and Duncan's multiple range test to compare treatment means, using statistica software according to Sokal & Rohlf (1995).

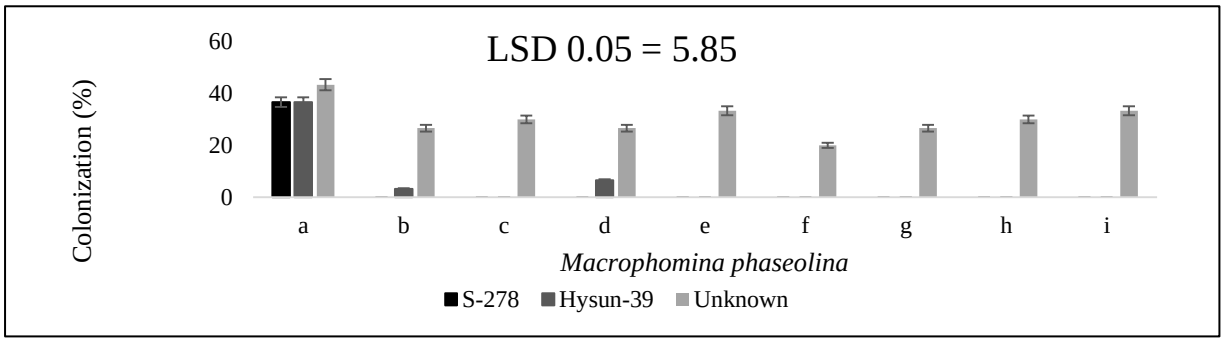


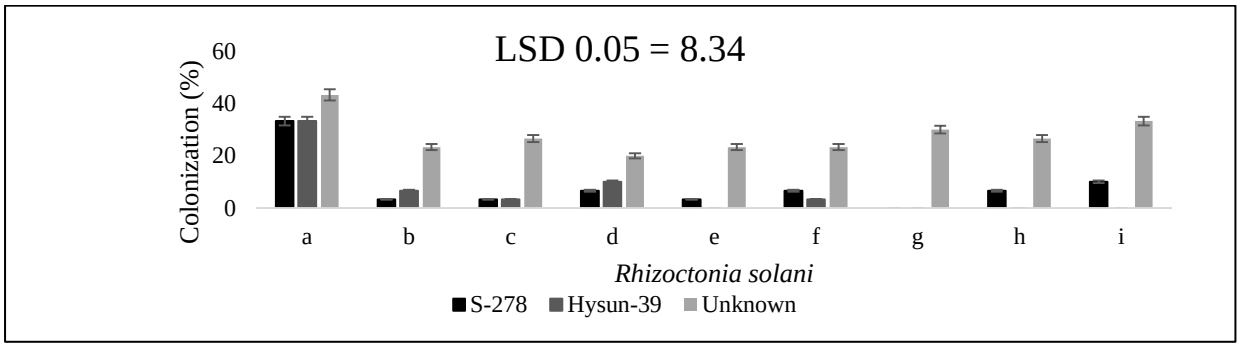





LSD 0.05 = 0.03
Shoot weight (g)
2
1.5
1
0.5
0
a b c d e f g h i
S-278 Hysun-39 Unknown


LSD 0.05 = 10.4
Germination (%)
150
100
50
0
a b c d e f g h i
S-278 Hysun39 Unknown


LSD 0.05 = 5.85
Colonization (%)
60
40
20
0
a b c d e f g h i
Macrophomina phaseolina
S-278 Hysun-39 Unknown


LSD 0.05 = 8.34
Colonization (%)
60
40
20
0
a b c d e f g h i
Rhizoctonia solani
S-278 Hysun-39 Unknown

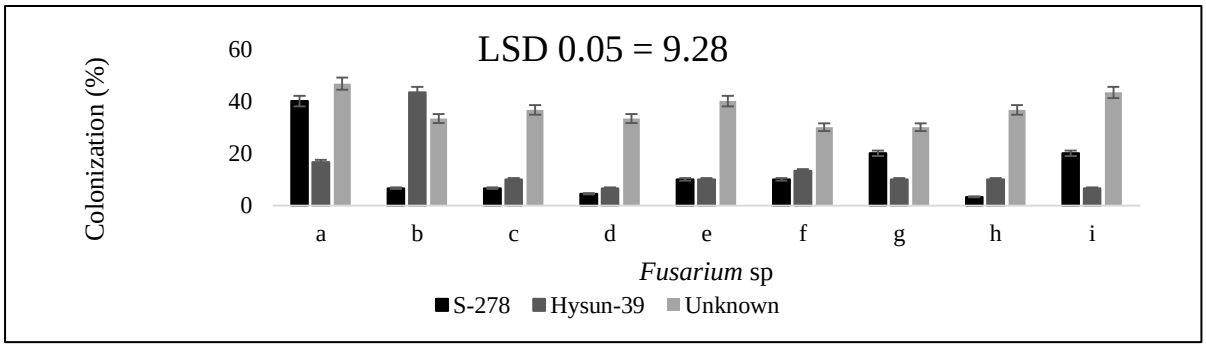
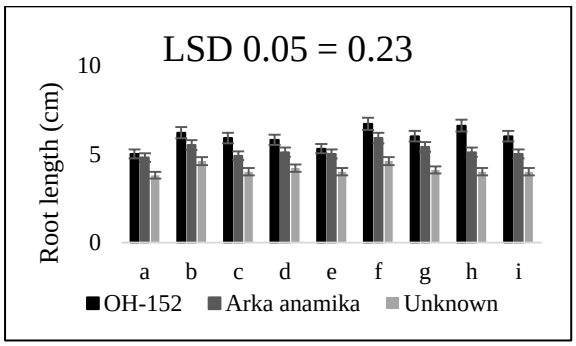
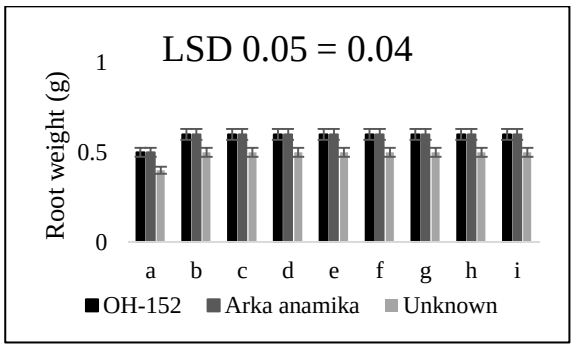
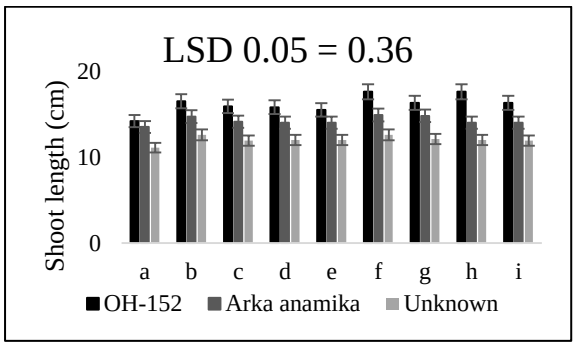


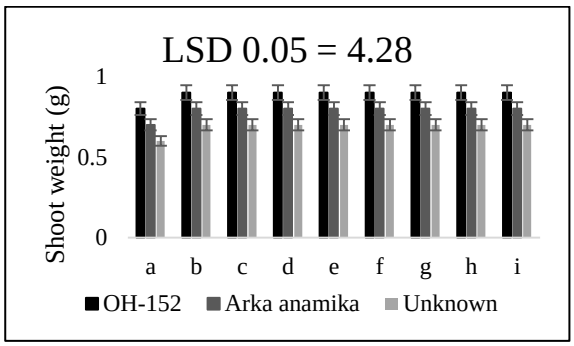
Fig. 1. Efficacy of different bio-primed varieties of sunflower in the control of root rot fungi.

a. Control
b. Bio-priming of seeds with leaves extracts of *Acacia nilotica* (10 mins)
c. Bio-priming of seeds with leaves extracts of *Acacia nilotica* (20 mins)
d. Bio-priming of seeds with leaves extracts of *Sapindus mukorossi* (10 mins)
e. Bio-priming of seeds with leaves extracts of *Sapindus mukorossi* (20 mins)
f. Bio-priming of seeds with conidial suspension of *Trichoderma harzianum* (10 mins)
g. Bio-priming of seeds with conidial suspension of *Trichoderma harzianum* (20 mins)
h. Bio-priming of seeds with cell suspension of *Rhizobium meliloti* (10 mins)
i. Bio-priming of seeds with cell suspension of *Rhizobium meliloti* (20 mins)









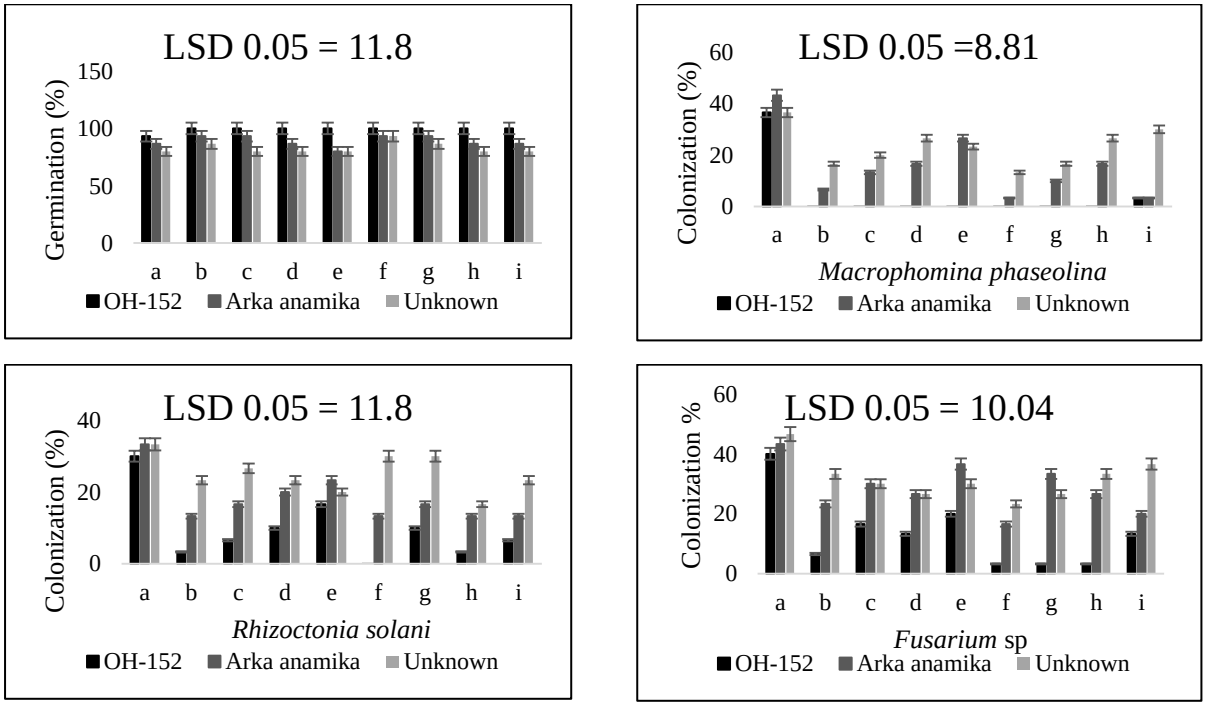


Fig 2. Efficacy of different bio-primed varieties of okra in the control of root rot fungi.

a. Control
b. Bio-priming of seeds with leaves extracts of *Acacia nilotica* (10 mins)
c. Bio-priming of seeds with leaves extracts of *Acacia nilotica* (20 mins)
d. Bio-priming of seeds with leaves extracts of *Sapindus mukorossi* (10 mins)
e. Bio-priming of seeds with leaves extracts of *Sapindus mukorossi* (20 mins)
f. Bio-priming of seeds with conidial suspension of *Trichoderma harzianum* (10 mins)
g. Bio-priming of seeds with conidial suspension of *Trichoderma harzianum* (20 mins)
h. Bio-priming of seeds with cell suspension of *Rhizobium meliloti* (10 mins)
i. Bio-priming of seeds with cell suspension of *Rhizobium meliloti* (20 mins)

RESULTS

Screening of different varieties of sunflower and okra after bio-priming with plant extracts and microbial antagonists for 10 and 20 minutes showed significant results in the control of root rot fungi and promotion of crop plants. Variety S-278 of sunflower when bio-primed with *A. nilotica* leaf extracts for 10 minutes and *T. harzianum* conidial suspension for 20 minutes completely inhibited the incidence of *M. phaseolina* ($p<0.001$) root rot fungus and significantly increase the germination ($p<0.001$) and growth parameters of plants as compared to the control (Fig. 1). In case of Hysun-39 variety of sunflower bio-priming of seeds with *A. nilotica* leaves extracts and *R. meliloti* cell suspension for 20 minutes significantly enhanced ($p<0.001$) the shoot length, root length, shoot weight and root weight and noticeable suppression in the incidence of root rot fungi was also observed (Fig. 1). In unknown variety of sunflower, bio-priming of seeds with *A. nilotica* and *T. harzianum* was effective for the suppression of root infecting fungi and promotion of plants but the unknown variety was less effective as compared to the variety S-278 and Hysun39 respectively. Germination, root length, shoot length, root weight and shoot weight was significantly

increased ($p<0.001$) and root rot fungus like *M. phaseolina* was completely suppressed ($p<0.001$) when OH-152 variety of okra bio-primed with *A. nilotica* leaves extracts, *T. harzianum* and *R. meliloti* cell/conidial suspensions for 10 minutes (Fig. 2). Similarly, *Arka anamika* variety of okra when bio-primed with *A. nilotica* leaves extracts and *T. harzianum* conidial suspension showed significantly ($p<0.001$) healthy growth parameters and significant inhibition ($p<0.001$) in the colonization of root rot fungi like *M. phaseolina, R. solani* and *Fusarium* spp (Fig. 2). On the other hand plants of unknown variety of okra were short and less effective in the control of root rot pathogenic fungi as compared to the other two varieties of okra. Of all the varieties of sunflower and okra variety S-278 and OH-152 was found to be most effective after bio-priming with *A. nilotica* leaves extracts and *T. harzianum* conidial suspension for 10 minutes and also play an important role in the complete inhibition of *M. phaseolina* root rot fungus followed by *Rhizoctonia solani* and *Fusarium* spp

DISCUSSION

In the present research, different varieties of sunflower and okra after seed bio-priming with plant extracts and microbial antagonists for 10 and 20 minutes showed significant results in the control of root rot fungi and promotion of crop plants. Seed bio-priming with biocontrol agents not only provide faster germination of seeds but also protects the plants from seed borne and soil borne pathogens with the tremendous increase in seedling growth (Chang & Kommedahl, 1968). According to our results,variety S-278 of sunflower when bio-primed with *A. nilotica* leaf extracts for 10 minutes and *T. harzianum* conidial suspension for 20 minutes completely inhibit the incidence of *M. phaseolina* and significantly increase the germination and growth parameters of plants as compared to the control. Whereas, when Hysun-39 variety of sunflower bio-primed with *A. nilotica* leaves extracts and *R. meliloti* cell suspension for 20 minutes significantly enhanced the shoot length, root length, shoot weight and root weight and noticeable suppression in the incidence of root rot fungi was also observed. Similar results were found when Anis *et al.,* 2011, screened sunflower varieties like Aussie gold-04, Aussie gold-62, Hysun-33, Hysun-39, NK Armoni and S-278 against *Macrophominaphaseolina.*Present research showed that bio-priming of okra cultivar OH-152 with *A. nilotica* leaves extracts, *T. harzianum* and *R. meliloti* cell/conidial suspensions for 10 minutes not only increase the growth of plants but also completely suppressed the rot fungus like *M. phaseolina.* Our results are in accordance with Sharif *et al*., 2000; Din *et al*., 2003; Kon *et al*., 2007; Hamideldin, 2010; Khalil & Amin, 2010 and Mengistu & Yamoah, 2010 who found that bio priming seed treatments not only increases the vegetative growth, pod yield but also reduces the disease incidence on many crops. Blum, *et al*.,1991 Harman, *et. al*., 1989; Jensen *et al*, 2002 and Jahn & Puls, 1998, also stated that bio-priming has great potential for increasing the efficacy, shelf life and better performance of biological control agents. The varietal screening of okra and sunflower clearly suggested that varieties S-278 of sunflower and OH-152 of okra were more resistant against root infecting pathogenic fungi and significantly increased the growth of plants after bio-priming with *A. nilotica leaf extracts* and *T. harzianum* conidial suspension for 10 minutes.

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