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Evaluation of Effective Doses for Bio Priming of Leguminous and Non-Leguminous Seeds with Microbial Antagonists and Plant Extracts in the Management of Root Rot Fungi and Promotion of Plants

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Abstract

Bio priming of leguminous and non-leguminous seeds with different doses of microorganisms like *Trichoderma harzianum* and *Rhizobium melilotii* and different concentrations of *Acacia nilotica* and *Sapindus mukorossi* leaves extracts was used to control the root infecting fungi and enhancement of growth parameters of crop plants. In this research, it was found that, when seeds were primed with different concentrations of *A. nilotica* and *S. mukorossi* leaves extracts, 100 % concentration (stock) of leaves extracts was found to be most effective and 100 ml (pure) conidal suspension of *T. harzianum* was found to be most effective for enhancing the growth and suppression of root rot fungi like *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* sp on leguminous and non-leguminous crops.

Keywords: Biopriming; Microorganisms; Plant extracts; Different doses; Root rot fungi; Crop plants

Introduction

Synthetic fungicides are useful in sustaining the production of crops and protecting the plants from fungal pathogens, but the use of these synthetic fungicides are expensive and pose serious threat to human health. Recent efforts have targeted on developing environmentally safe, long-lasting, and effective bio control methods and treatments for the management of plant diseases. Bio-priming of seeds with plant extracts and microbial antagonists is gaining importance in the recent times which not only improves the vigor and seedling establishment but also minimizes the risk of several plant diseases. Natural products of plants are the useful sources of new agrochemicals for the management of plant diseases [1]. Extracts of different plant parts can suppress the populations of foliar pathogens and control the development of diseases, and these plant extracts are potentially environmental safe and can be used as an alternative to chemical fungicides in integrated pest management programs [2]. A number of plant species have been reported to contain natural substances that are poisonous to several plant pathogenic fungi [3]. Dushyent and Bohra, [4], investigated the effect of 11 different extracts of plants on the mycelial growth of *Alternaria solani* and observed that leaf extracts of some plants, i.e. *Tamarix aphylla* and *Salsola baryosma*, completely inhibited the population of pathogens *in vivo*. Wszelaki and Miller, [5] reported that extracts of garlic significantly suppress the early blight disease on tomato. Additionally, several extracts of plants have been shown to possess the antimicrobial activity against fungal pathogens under *in vitro* and *in vivo* methods [1].

Besides plant extracts, microbial antagonists such as bacteria and fungi have also found to be effective for the establishment of crops as well as in the reduction of several pathogenic fungi. Application of antagonistic fungi like *Trichoderma* spp in agriculture has beneficial effects on plants. Species of *Trichoderma* have the ability to colonize the plant roots and its rhizosphere and control pathogens of plants through parasitism and antibiosis production. They not only enhance systemic resistance but also increase the growth parameters of plants [6,7]. Studies have shown that treatment with *Trichoderma* in tobacco plants increased root fresh weight leaf area index [8]. Similarly, Soil bacteria and rhizobacteria comprise an important group of bio-conontrol agents. Many plant growth promoting rhizobacteria e.g., *Rhizobium* spp., have a significant effect on plants including biological control of soil borne diseases, induce systematic resistance to plant pathogen, enhancement of nutrient and water uptake of plants [9]. Resulting secondary metabolites of soil rhizobacteria and plant root system can increase the availability of nutrient and mineral to the plants, improve the ability of plant nitrogen fixation and improve plant health by bio-control of plant pathogens [10]. Present investigation was therefore carried out on the bio-priming of leguminous and non-leguminous seeds with different doses of micro-organisms and plant extracts in the control of root rot fungi and growth of crop plants.

Materials and Methods

Collection of plants and microbial antagonists: Plant parts (leaves) of *S. mukorossi* and *A. nilotica* were collected from University of Karachi Campus, dried separately in full exposure of sunlight and fine powdered in an electric grinder machine. Cultures of *Rhizobium meliloti* (*Rm*-5) and *Trichoderma harzianum* (*Th*-6) were obtained from the Karachi University Culture Collection (KUCC).

Extract preparation: Aqueous extracts of *A. nilotica* and *S. mukorossi* leaves were prepared by soaking the leaves powder separately for 24 hours in distilled water (10 gm powder and 90 ml distilled water). After 24 hr, the suspension was filtered through Whatman's filter paper and different concentrations (100, 50 and 25%) were prepared from these extracts with distilled sterilized water for seed priming.

Conidal/cell suspension preparation: The fungal cultures of *T. harzianum* were grown on PDA (Potato Dextrose Agar) medium supplemented with antibiotics (Penicillin @ 1000,000 units/L and Streptomycin @ 0.2 g/L whereas, the bacterial culture of *R. meliloti* were grown on PDA medium without supplemented with antibiotics

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and the plates were incubated at room temperature for 5-7 days. After incubation period different doses (100, 50 and 75 %) of these micro-organisms were prepared with distilled sterilized water for seed bio-priming.

Experiment set-up: five seeds of chickpea, sunflower, okra and peanut after biopriming with different concentrations of plant extracts and different doses of microbial antagonists were sown in the plastic pots (8 cm diam.,) and each filled with 300 g soil. Each treatment and control (non-primed seeds) were replicated thrice. Soil used for the experiment was taken 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% [11], total nitrogen 0.077-0.099% [12], 3-7 sclerotia of *M. phaseolina* g-1 as found by wet sieving technique [13], 5-20% of *R. solani* on sorghum seeds used as baits [14] and *Fusarium* spp., 2000 cfu g^{-1} as assessed by soil dilution technique [15]. The pots were kept under screen house in randomized complete block design with three replicates per treatment and watered regularly in order to maintain sufficient moisture content required for the growth of plants. Pots containing non-primed seeds were also kept under screen house which served as control. After thirty days of seed germination, growth parameters like shoot length, root length, shoot weight and root weight was observed. Germination 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 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: The data obtained 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, with the help of statistica software according to Sokal and Rohlf [16].

Results

In peanut there was significant ($p<0.001$) increase in growth parameters when seeds were primed with *A. nilotica* and *S. mukorossi* leaves extracts @ 100% concentration (stock) followed by 50 and 25% concentrations as compared to the control and significant reduction in the colonization of *M. phaseolina* ($p<0.001$), *R. solani* ($p<0.001$) and *Fusarium* spp ($p<0.01$) was also found (Figure 1). Similarly, bio-priming of peanut seeds with 100 % pure (stock) of *T. harzianum* (186 $\times 10^3$ conidia/ml) was found to be significantly ($p<0.001$) effective for the enhancement of growth parameters such as root length, shoot length, root weight and shoot weight and suppression of root infecting fungi (Figure 1). Leaves extracts of *A. nilotica* and *S. mukorossi* @ 100 % concentrations was found to be significant ($p<0.001$) for root length, shoot length, root weight and shoot weight of chickpea. Whereas, 100 % pure conidal/cell suspensions of *T. harzianum* and *R. meliloti* (158 $\times 10^7$ cells/ml) was found to be most significant ($p<0.001$) for the growth of chickpea and suppression of root rot fungi such as *M. phaseolina* ($p<0.001$), *R. solani* ($p<0.001$) and *Fusarium* spp ($p<0.001$) (Figure 2). In okra, 100 % concentration of *A. nilotica* significantly ($p<0.001$) promoted the growth parameters like root length, shoot length, root weight and shoot weight as well as germination % was also significantly ($p<0.001$) enhanced. Similarly, bio-priming of okra seeds with *T. harzianum* @ 100 % pure conidal suspension significantly ($p<0.001$) elevated the growth of okra plants as well as significant reduction in the incidence of root infecting pathogenic fungi was also observed (Figure 3). Significant ($p<0.001$) increase in growth parameters like root length, shoot length, root weight and shoot weight was noticed when sunflower seeds were bio-primed with 100 % pure doses of *T. harzianum* conidal suspension whereas, concentrations of *A. nilotica* and *S. mukorossi* leaves extracts @ 100 % was found to be significant ($p<0.001$) for the growth as well as for the reduction of root rot fungi followed by 50 and 25 % concentrations as compared to the control (non-primed seeds) (Figure 4). Of all the tested bio-priming treatments, it was found that 100 % concentrations of *A. nilotica* and *S. mukorossi* leaves extracts and 100 % pure (stock) doses of conidal suspension of *T. harzianum* was found to be most effective for enhancing the growth and suppression of root rot fungi like *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* sp on leguminous and non-leguminous crops.

Discussion

Our results indicated that all the tested concentrations of leaves extracts of *A. nilotica* and *S. mukorossi* and all the tested doses of antagonistic micro-organisms such as *T. harzianum* and *R. melilotii* prominently increased the growth and caused a significant reduction in the colonization of root infecting fungi on leguminous and non-leguminous crops. In peanut and sunflower, significant reduction

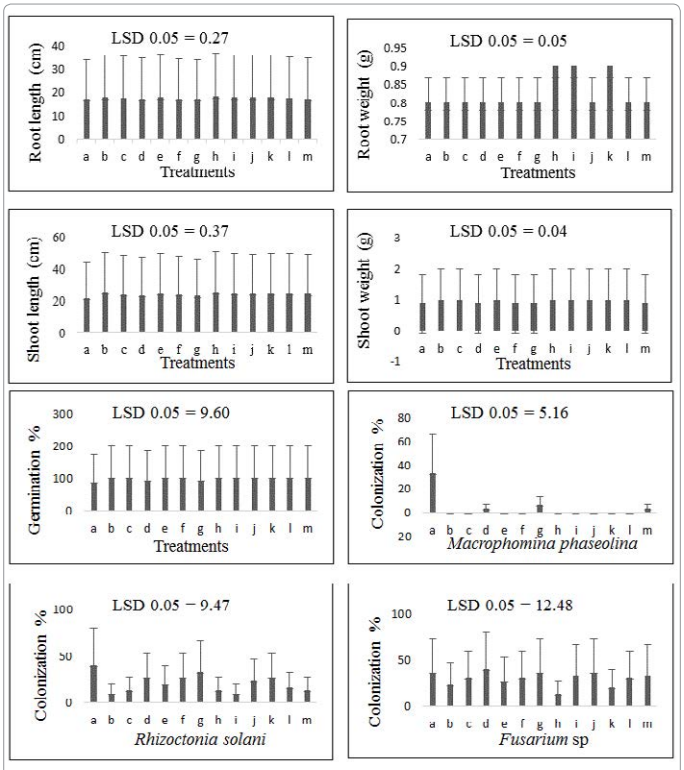

Figure 1: Evaluation of effective doses for bio-priming of peanut seeds with microbial antagonists and plant ectracts in the management of root rot fungi and promotion of plants.

a. Control
b. *A. nilotica* @100 %
c. *A. nilotica* @ 50 %
d. *A. nilotica* @ 25 %
e. *S. mukorossi* @ 100 %
f. *S. mukorossi* @ 50 %
g. *S. mukorossi* @ 25%
h. *T. harzianum* @100 %
i. *T. harzianum* @ 75 %
j. *T. harzianum* @ 25@
k. *R. meliloti* @ 100 %
l. *R. meliloti* @ 75 %
m. *R. meliloti* @ 25 %

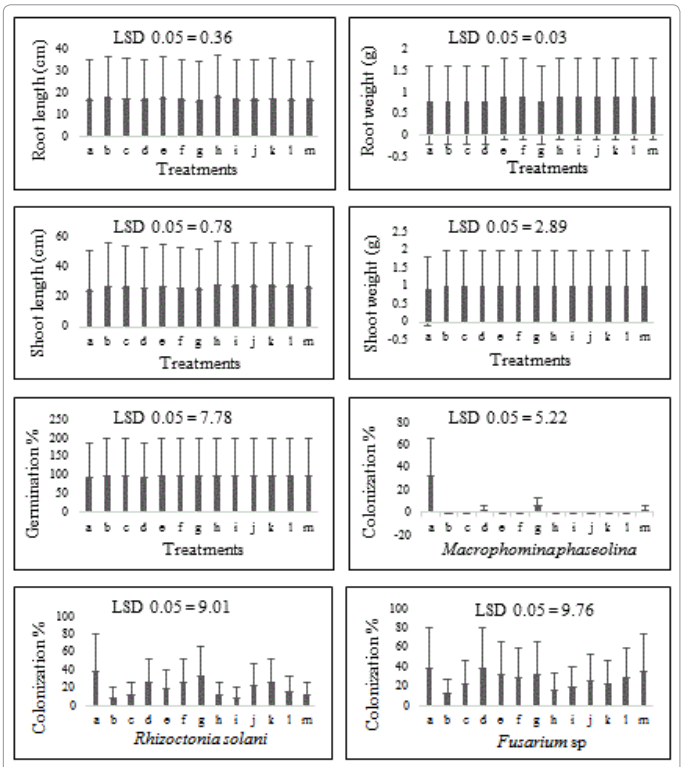

Figure 2: Evaluation of effective doses for bio-priming of chickpea seeds with microbial antagonists and plant ectracts in the management of root rot fungi and promotion of plants.

a. Control
b. *A. nilotica* @100 %
c. *A. nilotica* @ 50 %
d. *A. nilotica* @ 25 %
e. *S. mukorossi* @ 100 %
f. *S. mukorossi* @ 50 %
g. *S. mukorossi* @ 25%
h. *T. harzianum* @100 %
i. *T. harzianum* @ 75 %
j. *T. harzianum* @ 25@
k. *R. meliloti* @ 100 %
l. *R. meliloti* @ 75 %
m. *R. meliloti* @ 25 %

in the incidence of *M. phaseolina*, *R. solani* and *Fusarium* spp was observed when seeds were primed with *A. nilotica* and *S. mukorossi* leaves extracts @ 100% concentration as compared to the control. Several researchers have shown that extracts of plants can control anamorphic fungal plant pathogens [17-19]. Babu et al. [20] investigate the plant extracts and their compounds for the management of fungal pathogen and found that plant extracts significantly inhibited the radial growth of isolated fungus. Vijayan, [21] reported that the bulb extract of *A. sativum,* leaf extract of *Aegle marmelos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*. Our results have shown that bio-priming of peanut seeds with 100 % pure doses of conidal suspension of *T. harzianum* was found to be significantly effective for the enhancement of growth parameters such as root length, shoot length, root weight and shoot weight and for the suppression of root infecting fungi. Khan, [22] reported that seed bio-priming can improve the physiological responses of plants and tolerance of seeds under the stress conditions. According to our results, leaves extracts of *A. nilotica* and *S. mukorossi* @ 100 % concentrations was found to be significant for root length, shoot length, root weight and shoot weight of chickpea followed by 50% and 25 % concentrations and 100 % pure conidal/cell suspensions of *T. harzianum* and *R. meliloti* was found to be most significant for the growth of chickpea and suppression of root rot fungi such as *M. phaseolina*, *R. solani* and *Fusarium* spp. Several authors including Curtis et al. [23]; Krebs et al. [24] & Latha et al. [25] reported that plant extracts from 20 non-host plant species significantly reduced the early blight disease and inhibited the mycelial growth of *A. solani*. 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 [9]. Various strains of *Trichoderma* have been founded to be effective in plant growth and increase biomass production [26-28]. These fungi improved the growth of roots and abiotic stress [28], improve systemic resistance to diseases [5,29] and increase uptake of nutrients [29-31]. Moreover, these fungi increase leaf greenness that is probably due to increased photosynthetic rate [32]. In several cases, seed inoculation with biological agents in combination with priming has been reported to increase and stabilize the efficacy of biological agents [32,33].

Present results showed that germination %, root length, shoot length, root weight and shoot weight were significantly enhanced when okra seeds were primed with *A. nilotica* leaves extracts @ 100 % conc and *T. harzianum* @ 100 % pure conidal suspension separately as well as significant reduction in the incidence of root infecting pathogenic fungi was also observed. Many reports on seed germination, mean germination time, seed vigor, root length, shoot length, primary establishment and seed emergence revealed the beneficial effects of priming [34]. Mahesh et al. [35] have showed antifungal activity

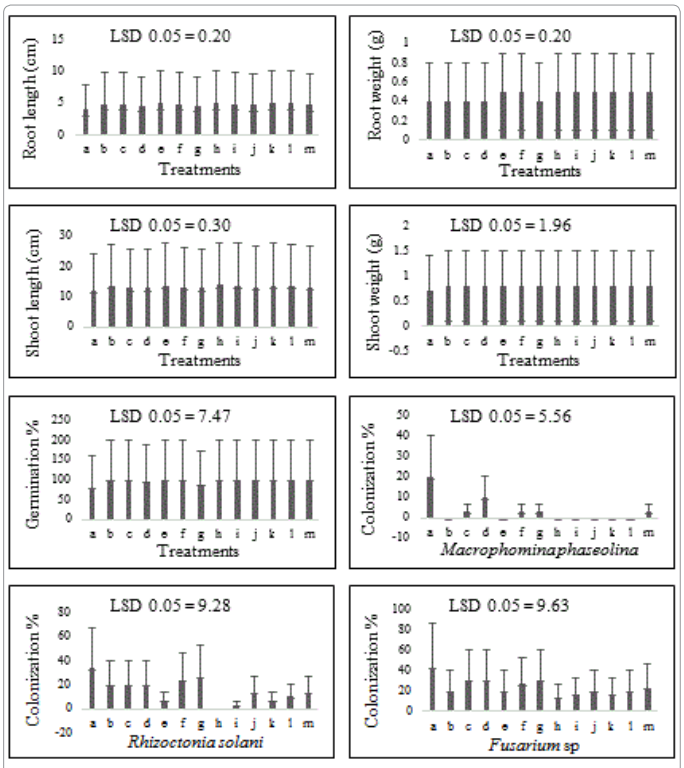

Figure 3: Evaluation of effective doses for bio-priming of okra seeds with microbial antagonists and plant ectracts in the management of root rot fungi and promotion of plants.

a. Control
b. *A. nilotica* @100 %
c. *A. nilotica* @ 50 %
d. *A. nilotica* @ 25 %
e. *S. mukorossi* @ 100 %
f. *S. mukorossi* @ 50 %
g. *S. mukorossi* @ 25%
h. *T. harzianum* @100 %
i. *T. harzianum* @ 75 %
j. *T. harzianum* @ 25@
k. *R. meliloti* @ 100 %
l. *R. meliloti* @ 75 %
m. *R. meliloti* @ 25 %

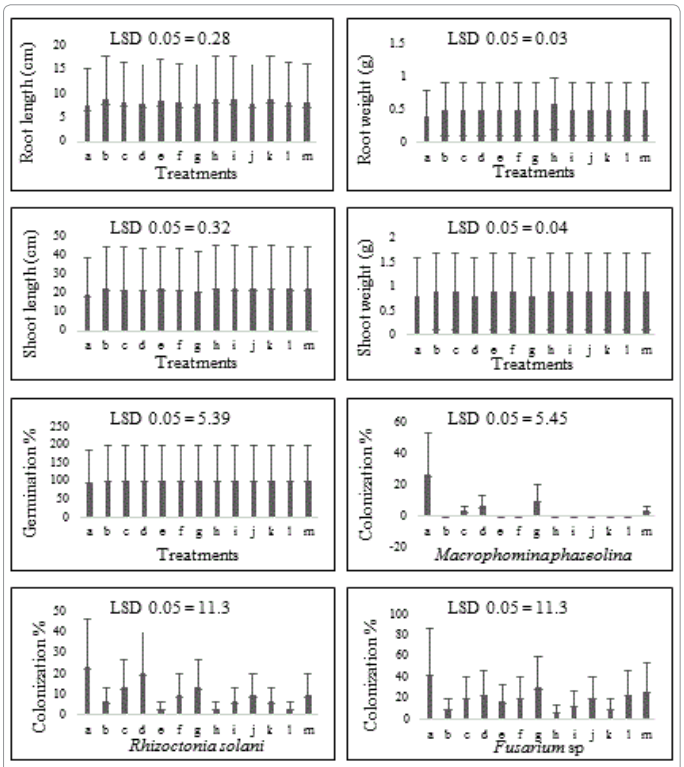

Figure 4: Evaluation of effective doses for bio-priming of sunflower seeds with microbial antagonists and plant ectracts in the management of root rot fungi and promotion of plants.

a. Control
b. *A. nilotica* @100 %
c. *A. nilotica* @ 50 %
d. *A. nilotica* @ 25 %
e. *S. mukorossi* @ 100 %
f. *S. mukorossi* @ 50 %
g. *S. mukorossi* @ 25%
h. *T. harzianum* @100 %
i. *T. harzianum* @ 75 %
j. *T. harzianum* @ 25@
k. *R. meliloti* @ 100 %
l. *R. meliloti* @ 75 %
m. *R. meliloti* @ 25 %

of methanolic extracts and aqueous extract of *A. nilotica* with percentage inhibition ranging from 34.27 ± 1.45 to 93.35 ± 1.99. Present Investigation suggested that bio-priming of seeds with 100 % concentration of *A. nilotica*, and *S. mukorossi* leaves extracts and *T. harzianum* conidal suspension doses @ 100 % was found to be most effective for suppression of root infecting pathogenic fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp as well as for the promotion of crop plants such as peanut, chickpea, sunflower and okra. Thus, these bio-priming methods can contribute to minimizing the risks and hazards of toxic fungicides especially on crop plants, which are produced for fresh consumption.

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