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EFFICACY OF *PARKINSONIA ACULEATA* AND *CASSIA FISTULA* WITH SELECTED MICROBIAL ANTAGONISTS FOR THE IMPROVEMENT OF GROWTH AND REDUCTION IN ROOT DISEASES

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ABSTRACT

Combination of soil drenching with microbial antagonists (*Bacillus subtilis, Pseudomonas fluorescens, Paecilomyces variotii* and *Trichoderma harzianum*) and seed treatment with 75 and 100% leaves extracts of medicinal plants like *Parkinsonia aculeata* and *Cassia fistula* were used to control the pathogenic fungi. These root pathogenic fungi produce rot disease in roots of crop plants resulting in stunted growth and death of plants. Results showed that soil drenching with *T. harzianum* and *P. fluorescens* in combination with seeds treated with 100% extract of leaves of *P. aculeata* showed highest control of pathogenic fungal colonization and improved the growth in both barley and okra as compared to *C. fistula* leaves extract.

Key words: Fungal colonization, microbial antagonists, root pathogenic fungi, seed treatment, soil drenching.

INTRODUCTION

Cassia fistula L. commonly known as amaltase belongs to family *fabacea*e, found mostly in Asia, South Africa, China, West Indies and Brazil (Gupta, 2010). *Cassia fistula* showed antifungal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (Duraipandiyan and Ignacimuthu, 2007). Compounds isolated from *C. fistula* showed antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Aspergillus niger* and *Fusarium oxysporum* (Yadav *et al.*, 2013**)**.

Control of soil borne infecting fungi is mainly dependent on fungicidal applications, but such types of applications are hazardous for environment and also for human health. Biological control is the inhibition of reproduction, infection and growth of plant pathogens through antagonistic fungal and bacterial strains (Cook, 1993; Baker, 1987). Antagonistic fungal and bacterial strains possess the ability to protect plants and reduce the population density of plant pathogens. Such anatagonists acts as biofertilizers or biofungicides in agriculture area. *Trichoderma* spp. secretes secondary metabolites and cell wall degrading enzymes and enhanced the growth of the plants (Mukherjee *et al*, 2013). *Paecilomyces* spp. also enhanced the growth and reduce infecting pathogens. *P. fluorscens* secreates hydrogens cyanide and siderophores (Hass and Defago, 2005).

Bacillus strains secretes lipoproteins which are involved in the enhancement of plant growth parameters (Shafi, 2017).

Main purpose of the present study was to evaluate *C. fistula* and *P. aculeata* to control the root infecting fungi on different crop plants.

MATERIALS AND METHODS

Cassia fistula and *Parkinsonia aculeata* were collected from University of Karachi campus while antagonists cultures were obtained from Karachi University Culture Collection (KUCC). Leaves of *C. fistula* and *P. aculeata* plants after collection, dried and powdered by a mechanical grinder and stored in air tight bottles separately. For the preparation of stock solution of plants, 10 g powder of the tested sample were mixed in sterilized distilled water (90 mL) and leave it for 24 h. After that, filter it and the extract obtained regarded as stock solution which was further diluted with sterilized distilled water for the preparation of 75% concentration.

Soil (300g /pot) was drenched with 7 days old cultures of *Paecilomyces variotti* ($23x10^{-3}$ cells/mL) *Trichoderma harzianum* ($165x10^{-4}$ cells/mL) prepared in PDA medium and 4 days old culture of *Bacillus subtilis* prepared in ($65x10^{-7}$cells/mL) and *Pseudomonas fluorescens* ($156x10^{-7}$cells /mL) prepared in nutrient media and KB medium (King's B), respectively. After drenching of soil with antagonists, five seeds of okra and barley treated with leaves extracts (75 and 100%) of both plants were sown in each pot, separately and watered regularly to maintained sufficient moisture required for the growth of plants. Untreated seeds served as control. After 30 days of germination, growth parameters and colonization percentage of root rot fungi were also recorded. Roots of each treatment cut into 5 pieces and surface sterilized with 1% sodium hypochlorite for 5 minutes and plated on PDA poured petri-plates containing antibiotics (penicillin and streptomycin) and incubate plates for the observation of infection in roots. The data obtained were subjected for analysis of variance.

RESULTS

Okra

Growth parameters of roots and shoot of okra were enhanced when soil was drenched with *T. harzianum* in addition to seeds treated with 75% extracts of *P. aculeata* and *C.fistula* leaves separately. Maximum enhancement in length and weight of shoots were recorded when soil was drenched with *P. variotii* and *B.subtilis* in addition to seeds treated with 100 and 75% extracts of *P.aculeata* and *C. fistula* leaves, respectively. Significant ($P<0.01$) enhancement of shoot length of okra were recorded when *P. flourescens* was drenching in soil in combination with okra seed treated with 75% extract of *C. fistula* and *P. aculeata* leaves, separately (Fig.1). Okra seeds treated with 75 and 100% extract of *P. aculeata* in addition with soil drenching with *T. harzianum* and *B. subtilis* were found better to reduce the colonization of *M. phaseolina* and *R. solani* followed by *Fusarium* spp., separately. However, 100% extract of *C. fistula* leaves in addition to soil drenched with *T.harzianum* inhibited the colonization of *M. phaseolina* followed by *R.solani* and *Fusarium* spp. Significant inhibition of *M. phaseolina* was recorded when soil was drenched with *P. flourescens* and *P. variotii* in combination with seeds treated with 75 and 100% extracts of *C. fistula* followed by *P. aculeata* leaves (Fig.2).

Barley

Root length and weight of barley crop enhanced when soil was drenched with *T. harzianum* was used in addition to seeds treated with 75 and 100% (w/v) extract of *C. fistula* and *P. aculeata* leaves. Similar results were recorded for *P. variotii* in combination with seeds treated with 100% extract of both plants. Shoot and root length of barley enhanced when *B. subtilis* was drenched in soil in addition to seeds treated with 75 and 100% extract of both plants ($P<0.001$). Root length and weight of barley enhanced with *P. flourscenc* with seeds treated with 100 and 75% (w/v) extract of *C. fistula* and *P. aculeate* (Fig.1).

Overall results showed that soil drenched with *P. flourescens* and *T. harzianum* in addition with seeds treated with 100% extracts of *P. aculeata* and *C. fistula* gave maximum growth of barley and okra and also controlled the root rot fungi (Fig.2).

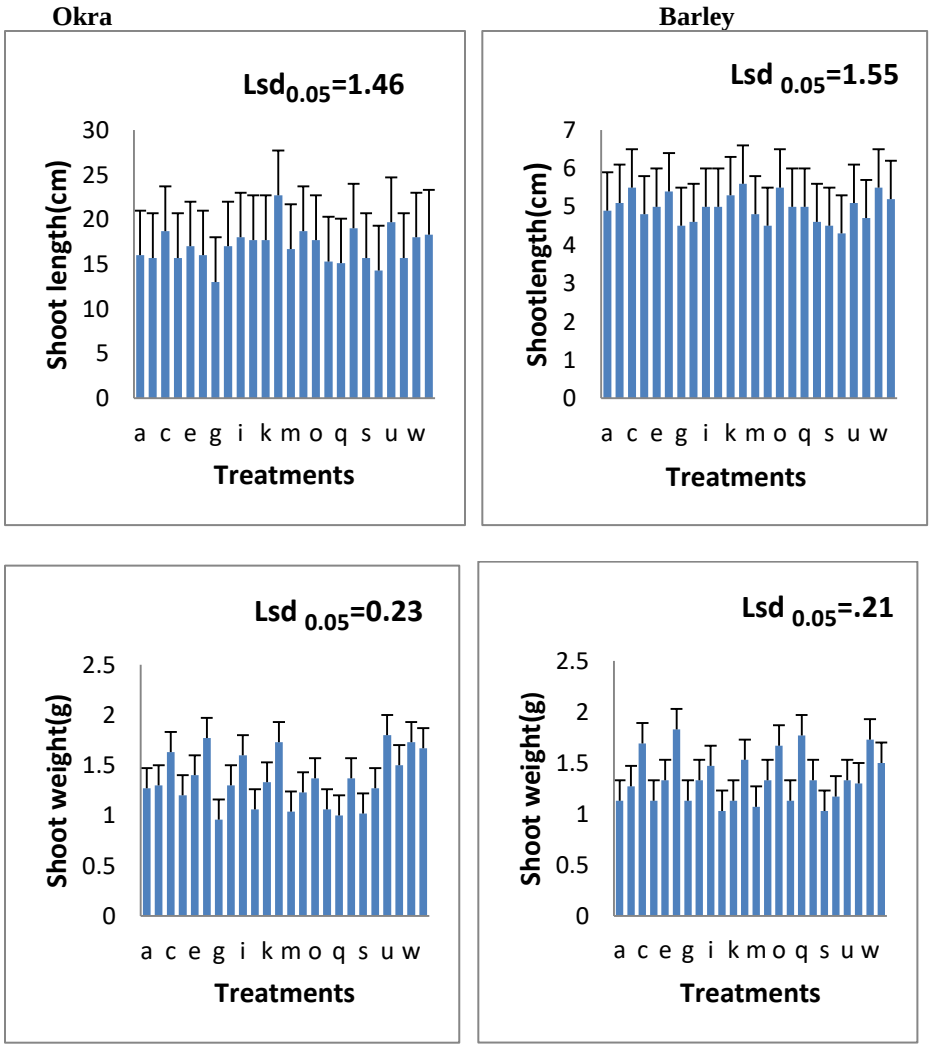


Figure 1 continued

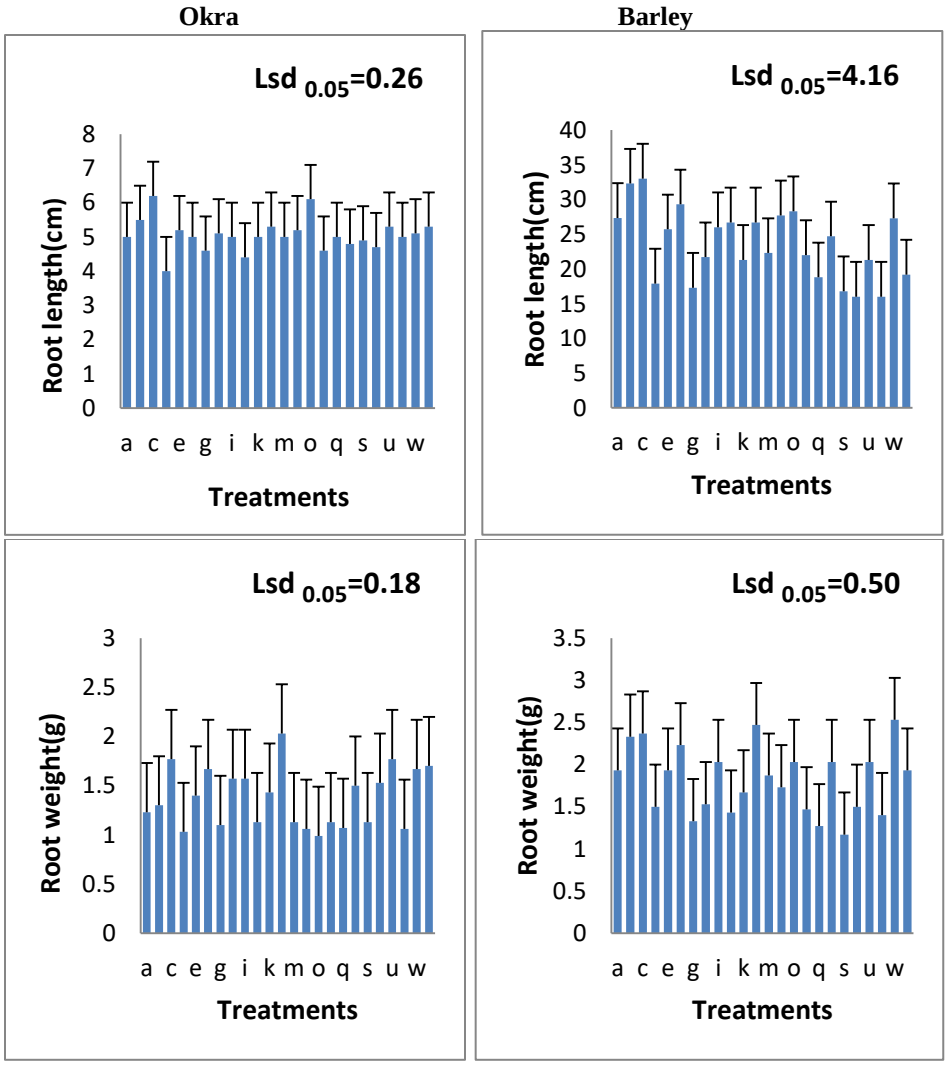


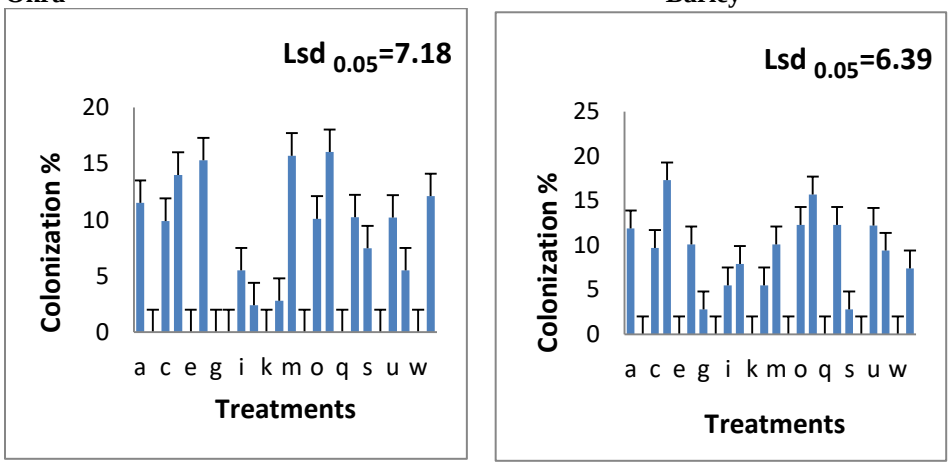
Fig.1. Combined effect of medicinal plants and microbial antagonists on plant growth of okra and barley. **Where, S.T=Seed treatment; S.D=Soil drenching**

a=Control of *T.harzianum*, **b**=100% S.T by *C.fistula* leaves and S.D *T.harzianum*, **c**=75% S.T by *C.fistula* leaves and S.D *T.harzianum*, **d**=Control *P.variotii*, **e**=100% S.T by *C.fistula* leaves and S.D *P.variotii*, **f**= S.T by *C.fistula* leaves and S.D 75% *P.variotii*, **g**=*B.subtilis* control, **h**=100% S.T by *C.fistula* leaves and S.D *B.subtilis* **i**=75% S.T by *C.fistula* leaves and S.D B.subtilis , **j**=Control *P.flourescens*, **k**=100% S.T by *C.fistula* leaves and S.D with *P.flourescens* **l**=75% S.T by *C.fistula* leaves and S.D *P.flourescens* ,**m**=Control of *T.harzianum*, **n**=100% S.t *by P.aculeata* leaves and S.D *T.harzianum*, **o**=75% S.T by *P.aculeata* leaves and S.D *T.harzianum*, **p**=Control *P.variotii***, q**=100% S.T by *P.aculeata* leaves and S.D *P.variotii*, **r**= S.T *by P.aculeata* leaves and S.D 75*% P.variotii***, s**=*B.subtilis* control, **t**=100% S.T by *P.aculeata* leaves and S.D *B.subtilis* **u**=75% S.T by *P.aculeata* leaves and S.D *B.subtilis* , **v**=Control *P.flourescens*, w=100% S.T by *P.aculeata* leaves and S.D with *P.flourescens* **x**=75% S.T by *P.aculeata* leaves and S.D *P.flourescens.*

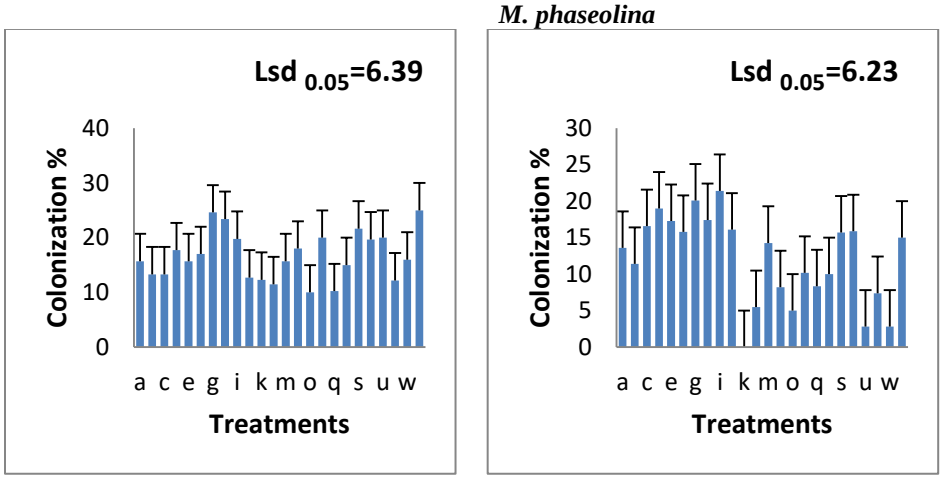
DISCUSSION

Bio-control agents control diseases of plants has been found effective and it is a cheapest way to control measure. In the past, extensive work has been done by different researchers. The present study describes the effect of different fungal and bacterial antagonists (*T.harzianum, P.variotii, B.subtilis* and *P.fluorescens)* in addition with barley and okra seeds treated with 75 and 100% extracts of *C. fistula* and *P. aculeata* and gave maximum enhancement of growth parameters of roots and shoots of okra and barley crop but also, reduced the colonization of *M. phaseolina* ,*R. solani* and *Fusarium* spp. Growth parameters of roots and shoots of okra enhanced when soil was drenched with *T.harzianum* in addition to seeds treated with 75% extracts of *P. aculeata* leaves and *C.fistula,* separately. *Trichoderma* spp. acts as antagonistic to many soils borne and plant pathogenic fungi (Prasad *et al*., 2002; Ramanujam *et al*., 2005; Suleman *et al*., 2008). *Trichoderma* spp. are commercially marketed as bio-fungicide, bio-fertilizers and soil amendments (Harman, 2000; Harman *et al*., 2004; Lorito *et al*., 2004).

Okra **Barley**

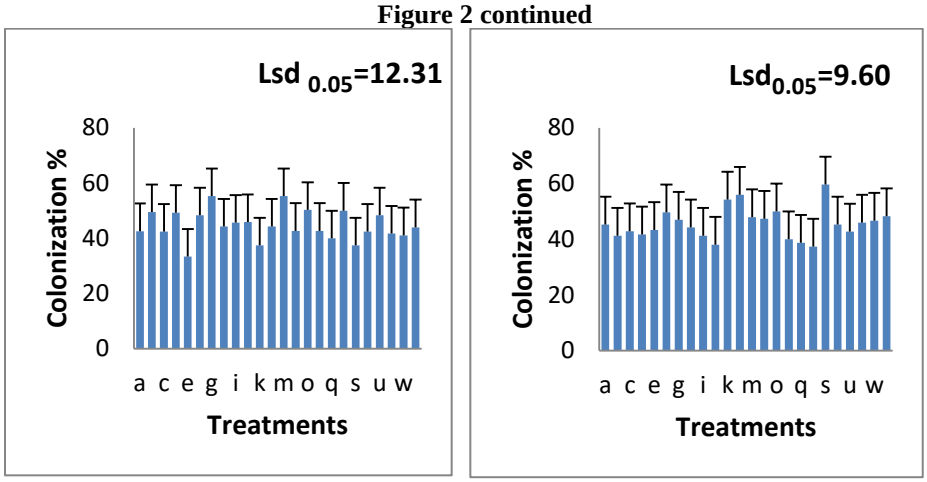


M. phaseolina



R. solani

Figure 2 continued



F. solani

Fig.2. Combined effect of medicinal plants and microbial antagonists on colonization of root rot fungi on okra and barley. **Where, S.T=Seed treatment; S.D=Soil drenching**
a=Control of *T.harzianum*, **b**=100% S.t by *C.fistula* leaves and S.D *T.harzianum*, **c**=75% S.T by *C.fistula* leaves and S.D *T.harzianum*, **d**=Control *P.variotii*, **e**=100% S.T by *C.fistula* leaves and S.D *P.variotii*, **f**= S.T by *C.fistula* leaves and S.D 75% *P.variotii*, **g**=*B.subtilis* control, **h**=100% S.T by *C.fistula* leaves and S.D *B.subtilis* **i**=75% S.T by *C.fistula* leaves and S.D B.subtilis , **j**=Control *P.flourescens*, **k**=100% S.T by *C.fistula* leaves and S.D with *P.flourescens* **l**=75% S.T by *C.fistula* leaves and S.D *P.flourescens* ,**m**=Control of *T.harzianum*, **n**=100% S.t *by P.aculeata* leaves and S.D *T.harzianum*, **o**=75% S.T by *P.aculeata* leaves and S.D *T.harzianum*, **p**=Control *P.variotii***,** **q**=100% S.T by *P.aculeata* leaves and S.D *P.variotii*, **r**= S.T *by P.aculeata* leaves and S.D 75% *P.variotii***,** **s**=*B.subtilis* control, **t**=100% S.T by *P.aculeata* leaves and S.D *B.subtilis* **u**=75% S.T by *P.aculeata* leaves and S.D *B.subtilis* , **v**=Control *P.flourescens*, w=100% S.T by *P.aculeata* leaves and S.D with *P.flourescens* **x**=75% S.T by *P.aculeata* leaves and S.D *P.flourescens.*

In the present studies seeds treated with 75 and 100% extracts of *P.aculeata* in addition with soil drenching with *T.harzianum* were found good to stop the colonization of *M.phaseolina* and *R.solani* followed by *Fusarium* spp. separately. According to Elebrarian (2006), *T. harzianum* is effective in the control of *M. phaseolina* that causes charcoal stem rot in melon. It is believed that hydrolytic enzymes secrete by *T.harzianum* at a constitutive level play a significant role in the improvement of the plant health and stimulation of root growth and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation (Harman *et al*., 2004; Lorito *et al*., 2006; Woo and Lorito, 2007). Bacterial antagonists were used and found more effective and showed maximum inhibition in the colonization of *M.phaseolina*. *P.variotii* possesses parasitic nature against plant pathogens (Mehdi and Dawar, 2008). Another study reveals that tested seeds treated with *P.variotii* alone or in addition with soil drenching with *D. alba* and *C. dactylon* significantly suppressed root rot fungi viz., *Fusarium* spp., *R. solani* and *M. phaseolina* (Dawar *et al*., 2010). *B. subtilis* in addition with okra and barley seeds treated by 100 and 75% extracts of *P.aculeata* reduced the colonization of *M.phaseolina* and *R.solani* followed by *Fusarium* spp. Similar results were obtained when seeds treated by 100% extract of *C. fistula* leaves. Seeds of tomato treated with *B. subtilis* used to reduce the disease severity and development caused by *R.solani* and *F. oxysporum* and improved the plant growth

parameters as well as quality of fruits were also increased significantly due to seed treatment as compared to untreated seeds (Chen *et al*., 2013).

There are several reports that Rhizobacteria (*P. fluorescens* and *B. subtilis*) have promoted the growth of cereals, ornamentals and vegetables (Vessey, 2003; Lugtenberg and Kamilova, 2009; Mahalakshmi and Reetha, 2009; Mishra *et al*., 2010; Egamberdieva *et al*., 2011).Complete inhibition of *M. phaseolina* and reduced colonization of root infecting fungi were recorded when soil was drenched with *P. fluorescens* in combination seeds treated with 75 and 100% extracts of *C. fistula* and *P. aculeata* leaves. According to Gupta *et al*., (2002), *P. fluorescens* control *M. phaseolina* that causes charcoal rot of peanut.

Cassia fistula and *Parkinsonia aculeata* were helpful in the control of root rot fungi and promote the growth of barley and okra crops. Using plant sources for its anti-fungal activity are beneficial in the agricultural field. Due to its cheaper and friendly bases, more improvement in crop yield can easily be done.

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