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COMPARATIVE EFFECT OF PLANT EXTRACT OF *DATURA ALBA* NEES AND *CYNODON DACTYLON* (L.) PERS., ALONE OR IN COMBINATION WITH MICROBIAL ANTAGONISTS FOR THE CONTROL OF ROOT ROT DISEASE OF COWPEA AND OKRA

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Abstract

To investigate the effect of *Cynodon dactylon* (L.) Pers. and *Datura alba* Nees extracts used as soil drenching and seed of cowpea (*Vigna unguiculata* L.) and okra (*Abelmoschus esculentus* L.) were treated with different antagonists like *Trichoderma harzianum, Rhizobium meliloti* and *Paecilomyces variotii* under screen house conditions. Results showed that *Macrophomina phaseolina* (Tassi) Goid and *Rhizoctonia solani* Kühn were completely suppressed when *D. alba* extract was used as soil drenching and seeds of okra and cowpea were coated with *P. variotii* while *Fusarium* spp., was suppressed when *C. dactylon* extract was used in combination with *P. variotii* as seed treatment. Growth parameters were maximum when soil was drenched with *D. alba* and *C. dactylon* extracts and seeds were treated with *P. variotii.*

Introduction

The presence of antifungal compounds, in higher plants, has long been recognized as an important factor in disease resistance (Madhadevan, 1982). Such compounds, being biodegradable and selective in their toxicity are considered valuable for controlling some plant diseases eg., *Sclerotium rolfsii* root rot on barley (Singh & Dwivedi, 1987). *Cynodon dactylon* Pers. (Poaceae) is a hardy perennial grass. Although it is a problem for farmers but is also valuable herbal and medicinal plant and used as first aid for minor injuries (Oudhia, 1999 a, b). Farmers traditionally apply crushed leaves to minor wounds to stop bleeding (Oudhia & Pal, 2000). Traditionally *Cynodon* is used for eye disorder and weak vision and it is advised to walk bare foot on dew spread over *Cynodon* plant each morning.

Datura is known as Jimsonweed, Thorn apple, Devil's trumpet. Among its 15 species, *D. alba* observes an important drug plants which is distributed through out the warmer portions of the world. The whole plant has medicinal value, but leaves and seeds alone are recognized as official. This annual plant is bushy, smooth, fetid, 2 or 3 feet in height also attaining 6 ft or more in rich soils (Sasthri, 1952). *Datura* has been used extensively in medicine, as an anaesthetic for setting bones, treating bruises and wounds, skin ulcers, hemorrhoids, asthma, rheumatism, whooping cough, muscle spasm, sciatica etc., (Satyavati *et al*., 1976).

Plant diseases causing organisms like fungi, actinomycetes, bacteria and nematodes produce extensive damage to crop plants and adversely affect the agricultural economy of Pakistan (Hafeez, 1986). These soil borne plant pathogens, which infect roots of plant, resulting in death of the plants. Since damage to plants by soil borne pathogens results below ground infection, losses to crop plants from such diseases are underestimated and

generally go unnoticed (Baker & Cook, 1974). The damage caused by plants parasitic pathogens root infecting fungi considered as worldwide and have extensive host range. Due to this they cause potentially serious constrains to crop productivity. The fungus *Macrophomina phaseolina* (Tassi) Goid., is a soil borne pathogen distributed worldwide with a host range of more than 500 plant species (Mihail & Taylor, 1995). *M. phaseolina* in a sun flower seed reduce the germination and vigor besides producing pre and post emergence seedling blight and charcoal disease (Fakir *et al.*, 1976). *Rhizoctonia solani* (Kühn.) has been reported to cause dieback of leaves and young stem also root rot disease in capsicum (Szirmai, 1941). *R. solani* exists as active mycelium in the soil, attacks over 2000 species of plants (Parmeter, 1970) and *Fusarium* species (Booth, 1971) are known to attack a wide range of host plants in different parts of the world.

Keeping in view the medicinal importance of plants, the present research was conducted to observe the effect of seed treatment with microbial antagonists and soil drenching with *C. dactylon* and *D. alba* extract in the control of root rot fungi on cowpea (*Vigna unguiculata* L.) and okra (*Abelmoschus esculentus* L.).

Materials and Methods

Collection of plants: Leaves of *Datura alba* (Nees) and lawn grass *Cynodon dactylon* (L.) Pers., were collected separately from the Department of Botany, University of Karachi. The leaves of plant were washed with distilled water to remove dirt, air dried, then powdered in an electric grinder.

Preparation of Plant extract: For the extraction of plant, 50 g powdered leaves were soaked in 250 ml sterilized distilled water for 24 hrs, filtered with Whatman's No 1 filter paper and stored at 4ºC in sterilized flasks. To avoid contamination and prospective chemical alterations, the extracts were used within 3-4 days.

Microbial cultures: Culture of *R. meliloti* (R-5) obtained from the root nodules of *Melilotus alba* were grown on Yeast Extract Mannitol Agar medium for 1-2 days. *Paecilomyces variotii* Bain (Pv-17) and *Trichoderma harzianum* (Th-65) were obtained from the Karachi University Culture Collection (KUCC). The cultures were grown on PDA medium supplemented with antibiotics (Penicillin @ 1000,000 units/L and Streptomycin @ 0.2 g/L and the plates were incubated for 7 days at 30±2˚C.

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 & Raczkowski, 1922), total nitrogen 1.5% (Mackenzie & 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 & 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 & Snyder, 1962).

Seed treatment and soil drenching: Seeds of cowpea (*Vigna unguiculata* L.) and okra (*Abelmoschus esculentus* L.) were surface sterilized with 1% $Ca(OCl)_2$ for three minutes,

rinsed thoroughly in running tap water and dried aseptically. The seeds were treated with fungal and bacterial antagonists viz., *T. harzianum*, *R. meliloti* and *P. variotii* separately using gum arabic as a sticker for 4-5 minutes and air dried in laminar flow. The soil was drenched with 20ml extract of *C. dactylon* and *D. alba*, 5 treated seeds of cowpea and okra with antagonists were sown in each pot separately. Untreated seeds and soil served as control. There were three replicates of each treatment and pots were randomized on a screen house bench.

Isolation of fungi from infected root: After 30 days, plants were uprooted to observe growth parameters in terms of shoot length, shoot weight, root length and root weight. To determine the incidence of fungi, roots were cut into small segments (5 mm) and after surface sterilization in 1% $Ca(OCl)_2$ for 3 min, five such segments were plated onto potato dextrose agar (PDA, MERCK) plates supplemented with benzyl penicillin potassium salt (0.1 g/l) and streptomycin sulfate (0.2 g/l). The plates were incubated at 28˚C for 1 week and infection of fungi from each root segment was determined.

Statistical analysis: Data were subjected to analyses using statistica software according to Sokal & Rohlf (1995).

Results

Application of *C. dactylon* extract as soil drenching showed that germination % of both cowpea and okra were significantly ($p<0.001$) increased as compared to *D. alba* extract but shoot length, shoot weight of okra ($p<0.05$) and root length of cowpea were significantly ($p<0.01$) increased when *D. alba* extract was drenched in soil (Fig. 1). Similarly germination % was increased when seeds of cowpea were treated with *P. variotii* followed by *T. harzianum* and *R. meliloti*. All root infecting fungi viz., *R. solani*, *M. phaseolina* and *Fusarium* spp., were significantly ($p<0.001$) reduced on okra and cowpea when soil was drenched with *C. dactylon* (Fig. 2).

When soil was drenched with *C. dactylon* and seeds of okra and cowpea were treated with *P. variotii*, it was observed that the germination % was significantly ($p<0.001$) increased and gave 100 % results while shoot length and root weight in cowpea and okra were significantly ($p<0.01$) increased when *D. alba* extract was used for soil drenching and seed were treated with *P. variotii* followed by seed treatment with *T. harzianum* and *R. meliloti* (Fig. 1). Increased plant weight and root length were observed when soil was drenched with *D. alba* and *C. dactylon* extracts and seed of okra were treated with *R. meliloti* (Fig. 1).

When *C. dactylon* applied as soil drenching and seeds of okra and cowpea were treated with *P. variotii* showed reduction in infection of *M. phaseolina*, *Fusarium* spp., and *R. solani*. Complete reduction in infection of *R. solani* and *M. phaseolina* on okra and cowpea was observed when *D. alba* was drenched in combination with *P. variotii* treated seed ($p<0.001$) (Fig. 2). Infection of *Fusarium* spp., was completely controlled when cowpea seeds were treated with *P. variotii* and soil was drenched with *C. dactylon* extract ($p<0.05$) (Fig. 2). *C. dactylon* and *D. alba* extracts gave satisfactory results when used as soil drenching and seeds were treated with *P. variotii* followed by *R. meliloti* and *T. harzianum* (Fig. 2).

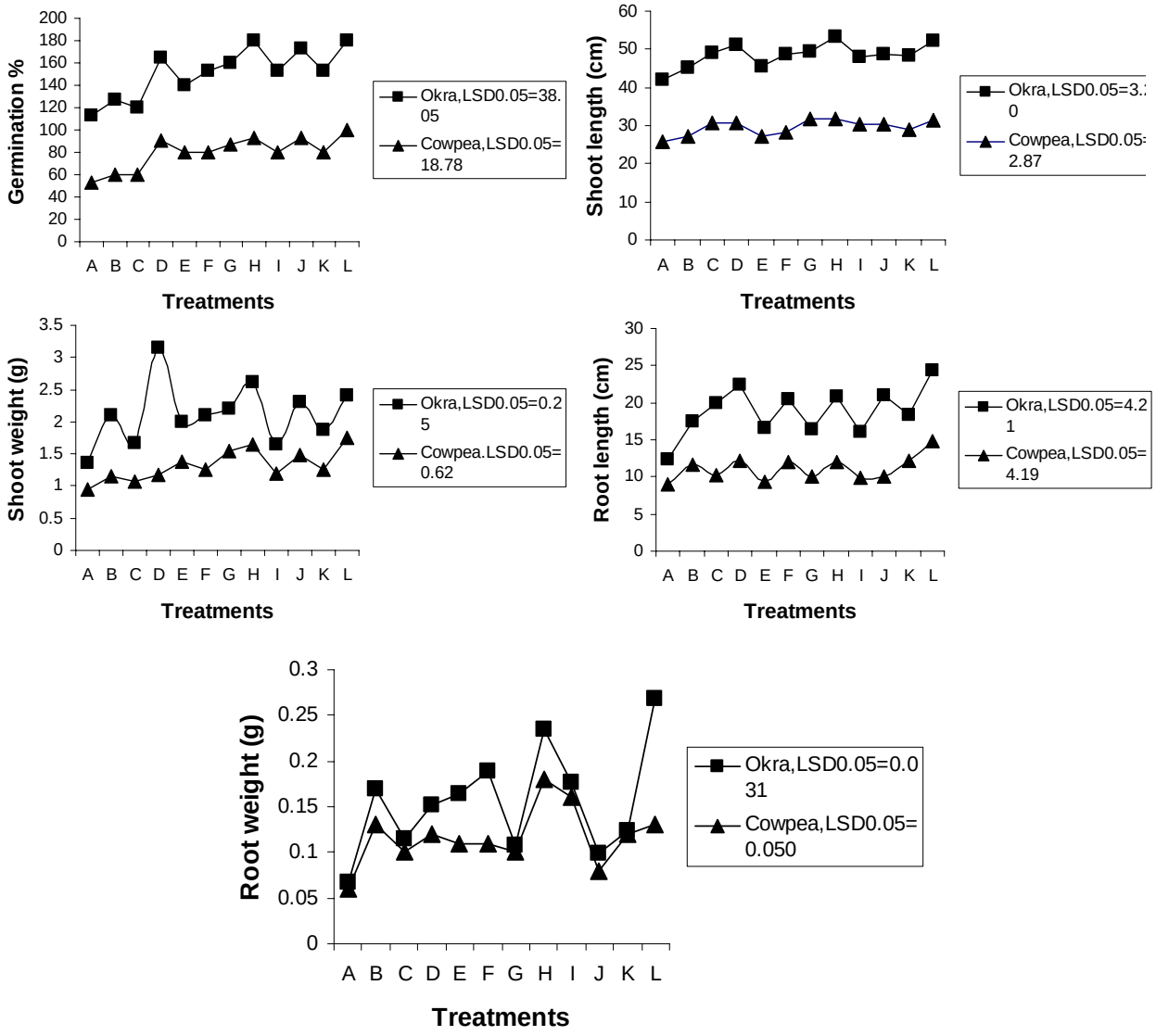

Fig. 1. Effect of plant extract alone and in combination with microbial antagonists on growth parameters of cowpea and okra.
A= Control, B = Soil drenching with *D. alba*, C = seed treated with *R. meliloti*, D = B+C, E = seed treatment with *T. harzianum*, F = B+E, G = seed treatment with *P. variotii*, H= G+B, I = soil drenching with *C. dactylon*, J = I+C, K = I+E, L = I+G

Discussion

Present results showed that soil drenching with *D. alba* and *C. dactylon* alone or in combination with seeds treated with *P. variotii* significantly suppressed root rot fungi viz., *Fusarium* spp., *R. solani* and *M. phaseolina*. It was reported that *Mangifera indica* have antimicrobial and antifungal activity against other pathogens (Grand & Lee, 1989). The presence of antifungal compounds, in higher plants, has long been recognized as an important factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Singh & Dwivedi, 1987). Results showed that *D. alba* used as soil drenching and *P. variotii* treated seeds of okra and cowpea completely reduced *R. solani* and *M. phaseolina* in okra and cowpea plants. *Datura fastuosa* have been reported to contain compounds Tigloidine (3*B*-tigloyloxytropane), 6*B*-tigloxytropane-a-ol, tropine (3a-hydroxy tropane), apoatropine, hyoscyamine, scopolamine (Shahwar *et al.*, 1995)

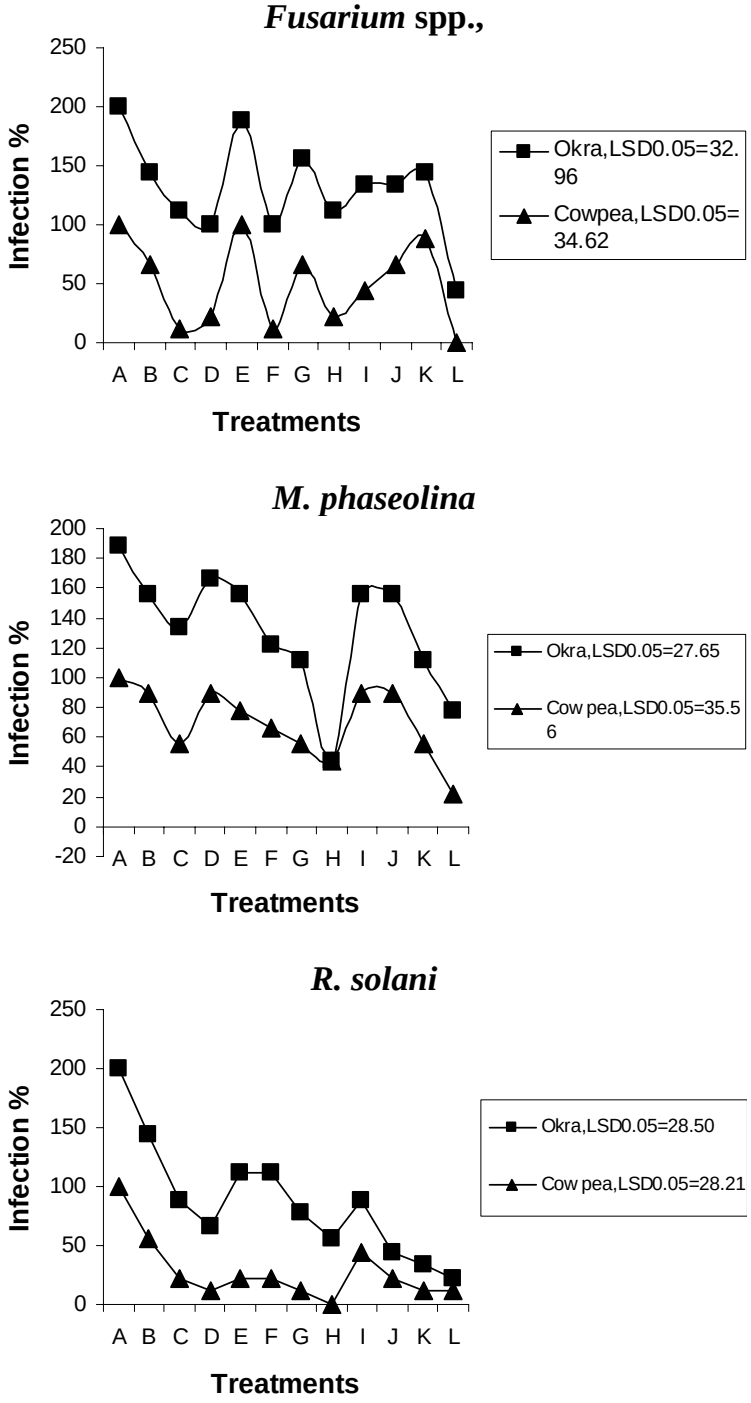

Fig. 2. Effect of plant extract alone and in combination with microbial antagonists on root infecting fungi of cowpea and okra.
A= Control, B = Soil drenching with *D. alba*, C = seed treated with *R. meliloti*, D = B+C, E = seed treatment with *T. harzianum*, F = B+E, G = seed treatment with *P. variotii*, H= G+B, I = soil drenching with *C. dactylon*, J = I+C, K = I+E, L = I+G

which have nematicidal activity. Results from the present study suggested that *D. alba* and *C. dactylon* leaf extracts with different antagonists used as a potential approach for the improvement of plant growth and in the control of root rot diseases. Ghorbany & Salary (2005) examined *Trachyspermum capticum* and *Rheum ribes* which have the highest inhibitory effect on incidence of cumin disease. Mehdi & Dawar (2008) reported that *P. variotii* was more frequent in suppression of root infecting fungi followed by *T.*

harzianum due to its parasitic nature. Amendment of soil with leaves or aqueous extract of *Azadirachta indica* L., reduced soil borne fungal pathogens and other phytopathogens (Locke, 1995). The present observation clearly suggested that plants and different microbial antagonists used as biocontrol agents can release many different compounds into their surrounding environment, which results in suppression of disease causing organisms and better growth of plants.

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