USE OF SOLANACEOUS LEAVES EXTRACTS ALONG WITH MICROBIAL ANTAGONISTS AGAINST ROOT ROT DISEASES

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ABSTRACT

Current research objective was to controlled root pathogens by using solanaceous leaves extract in addition with microbial antagonists. Tested seeds (okra and cowpea) were treated with *W. somnifera, S. nigrum* and *D. alba* leaves extracts (100% w/v concentration), while soil was drenched with microbial antagonists namely, *P. lilacinus, T. harzianum, R. meliloti* and *P. aeruginosa* separately. Improvement of growth parameters (length and weight of root/shoot) were recorded in both tested plants as well as suppressed the colonization of *Fusarium* spp., *R. solani* and *M. phaesolina* in combination with microbial antagonists as compared to alone treatments. Highest number of nodules was noticed in cowpea plants when seeds treated with extracts of *D. alba* leaves along with soil drenched with *P. aeruginosa.* Best control of root rot colonization was noted in both hosts when seeds were treated with *W. somnifera* and *D. alba* leaves extract in addition with soil drenched with *T. harzianum* and *P. aeruginosa*, respectively.

Keywords: Solanaceous leaves extracts, microbial antagonists and root pathogenic fungi.

INTRODUCTION

Plant infection caused by pathogenic fungi produces serious affect in agricultural field (Rattink, 1992) especially root pathogenic fungi such as *Fusarium* spp. (produces wilt, root and stem rot diseases), *R. solani* (produces damping off of seedling) and *M. phaseolina* (causes charcoal rot and root rot diseases) which reduced the yield of crops but also agricultural economy (Wheeler and Rush, 2001; Tanina *et al*., 2004).

Medicinal plant extracts possess anti-microbial activity (Talibi *et al.*, 2012). Therapeutic effects of medicinal plants attract the attention of researchers as substitute technique for controlling diseases and prevent environment from the application of hazardous fungicides (Monte, 2001). Bioactive compounds in plants used in medicines which show potent effect in the health of human and also against microbial diseases (Ateş and Erdoğrul, 2003). Medicinal plants include solanaceace family which have four thousand species present in mild and humid areas (Knapp *et al*., 2004). Important species includes *S. nigrum, W. somnifera* and *D. alba* (Abramowics, 1990) popularly known for medicinal significance due to the presence of alkaloids which includes scopolamine, atropine, steroidal glycoalkaloids, withanolides and hyoscyamine compounds (Ansari, 2005; Mahesh and Satish, 2008). Compounds isolated from these plants used to cure many types of disease infections (Kone *et al*., 2004).

Several formulations have been used against root pathogens like medicinal plant extracts (Babu *et al.,* 2008; Dawar *et al.,* 2020), bacterial suspensions or fungal spores as microbial antagonists to inhibit the population of root rot fungi in plants (Harman *et al*., 1980; Saleem *et al*., 2000; Hanif and Dawar, 2015). Antagonists act as a bio-control agent to suppress the pathogenic population, in which fungal antagonists (*Trichoderma* spp. and *Paecilomyces* spp.) and bacterial antagonists species (*Rhizobium* sp., *Pseudomonas* sp. and *Bacillus* sp.) applied as soil drenching or seed treatment methods to control the root pathogens (Bari, 2001; Bajwa *et al.,* 2003; Abdel-Monaim, 2014) by the production of chitinolytic enzymes secreted by microbial antagonists which killed the mycelium of pathogens to recover the plant health (Cook, 2000; Gilreath, 2002). Chitinase enzymes produced by microbial antagonists break the fungal cell wall which is made up of β-glucan, chitin and polysaccharides (Haram *et al*., 1996; Howell, 2003) protecting roots from the invasion of fungal attack in plants (Weller, 1988).

Therefore, the main purpose of this study was to evaluate the controlled of root rot pathogenic fungi by the combined effect of solanaceous plants and microbial antagonists for the improvement of growth parameters of crops.

MATERIALS AND METHODS

I) Collection/preparation of leaves extract and culture of microbial antagonists

Healthy leaves of *W. somnifera, S. nigrum* and *D. alba* were collected from various Departments of University of Karachi. Leaves were dried and made fine powder. Powder of each tested plant was kept in the separate jar. Tested leaves powder (10g) was soaked in 90mL SDW (sterilized distilled water) separately. After 24 h filtrate the

extract, respectively. Culture of microbial antagonists was collected from Plant Pathology Laboratory, KU. Fungal antagonists such as *Paecilomyces lilacinus* (Strain #12), *Trichoderma harzianum* (Strain #60) were grown on PDA (Potato Dextrose Agar) medium for one week. While, bacterial antagonists such as *Rhizobium meliloti* (Strain #-19) and *Pseudomonas aeruginosa* (Strain #-20) were grown on YEMA (yeast extract mannitol agar) and King's B medium respectively, were kept at room temperature (28-30^{0}C) for 48 hours depending upon population growth.

II) *In Vivo* experiment

Sandy loam soil collected from Department of Botany (KU) contains ≥7.8 pH and moisture content 40% (Keen and Raczkowski, 1992). Natural infestation isolated from soil contains *R. solani* 15% by baiting technique (Wilhelm, 1955), 9-11 sclerotia/g of *M. phaseolina* by wet sieving technique (Sheikh and Ghaffar, 1975) and 2700 CFU/g *Fusarium* spp. by serial dilution technique (Nash and Synder, 1962). Cowpea and okra seeds were treated with pure concentration of leaves extracts of *W. somnifera, S. nigrum* and *D. alba*, respectively. Five treated seeds of both hosts were sown in each plastic pots (300g) individually along with each microbial antagonist suspensions of *P. lilacinus* (82×10^{-6}conida/mL), *T. harzianum* (96×10^{-5}conida/mL), *R. meliloti* (77×10^{-5}cells/mL) and *P. aeruginosa* (68×10^{-5}cells/mL) were drenched (20mL) individually in soil and replicated thrice. Untreated seeds and undrenched soil was taken as control. These pots were kept in the screen house for one month and regularly watered the plants.

III) Collection of data

Uprooted plants after one month and record the growth parameters (shoot/root length and weight and number of nodules). Each root after surface sterilization with 1.0% calcium hypochloride was cut in to five pieces and each piece was placed on poured plates of (PDA). Plates were incubated at 30-32^{0}C for six days and after incubation period, percentage of root rot pathogens colonization from each root pieces were recorded. Data were analysed by one way of (ANOVA) and note down the least significant difference (LSD) at P=0.05 (Gomez and Gomez, 1984).

RESULTS

1) *Abelmoschus esculentus*

Treated seeds with 100% w/v leaves extract of *W. somnifera, S. nigrum* and *D. alba* along with fungal/bacterial antagonists (*P. lilacinus, T. harzianum, R. meliloti* and *P. aeruginosa*) were drenched in soil separately that enhanced the growth of shoot length/weight and root length/weight followed by individual treatments ($P < 0.001$) . Maximum plant length and weight was observed when seeds treated with *D. alba* and *W. somnifera* leaves extracts in combination with microbial antagonists of *T. harzianum* and *P. aeruginosa*, respectively as compared to other treatments. Weight of root improved significantly ($P < 0.001$) when treated with *W. somnifera* leaves extract in addition with soil drenched with fungal conidia of *T. harzianum* but also showed greater inhibition of root pathogenic fungi colonization. Colonization of *R. solani* was completely suppressed by the leaves extract of W. *somnifera* and *D. alba* along with *T. harzianum* and *P. aeruginosa* drenched in soil, respectively followed by *P. lilacinus* and *R. meliloti* (Table 1).

2) *Vigna unguiculata*

Soil drenched with fungal and bacterial antagonists respectively along with solanaceous leaves extract not only improved the growth of cowpea plant but also controlled the fungal colonization of root rot pathogens (*Fusarium* spp., *R. solani* and *M. phaseolina)* as compared to alone treatments. Significant ($P< 0.001$) increased in growth parameters were noticed when tested leaves extract used along with microbial antagonists. Weight/length of shoot and root significantly ($P<0.01$) recorded highest with leaves extracts of *W. somnifera* and *D. alba* along with soil drenched with *T. harzianum* and *P. aeruginosa,* respectively as well as significantly ($P<0.01$) showed suppression against root rot pathogens as compared to untreated plant (control).. When soil was drenched with *P. aeruginosa* and *R. meliloti* separately and cowpea seeds were treated with leaves extract of *D. alba* increased the number of nodules ($P<0.01$) as compared to other treatments but also observed complete suppressed of mycelial growth of *R. solani* (Table 2).

On the whole, better result of controlling root infecting fungi on both crops (okra and cowpea) was showed by the seeds treated with 100% leaves extract of *W. somnifera* and *D. alba* along with soil drenched with microbial antagonist (*T. harzianum* and *P. aeruginosa*), respectively. Microbial antagonists in combination with antifungal compounds of leaves extract of solanaceous plants played vital role in the management of root rot fungi in contrast of using agrochemical (fungicidal) which controlled the plant pathogens but in the cost of disturbing soil ecosystem.

Table 1. Effect of seed treatment with solanaceous leaves extract along with soil drenched with microbial antagonists in the controlled of root rot fungi and on the growth of okra plants.

Treatments	Growth parameters				Root rot fungi colonization (%)		
	Shoot length (cm)± SD	Shoot weight (g) ± SD	Root length (cm) ± SD	Root weight (g) ± SD	*Fusarium* spp. ± SD	*R. solani* ± SD	*M. phaseolina* ± SD
Control (untreated)	14.77±0.83	0.53±0.07	3.63±0.59	0.07±0.02	31.11±15.39	26.66±6.66	19.99±6.66
Seed treatment with *W. somnifera*@100%	17.94±1.10	0.57±0.22	3.99±0.76	0.09±0.02	13.30±6.71	13.33±11.54	4.44±7.69
Seed treatment with *S. nigrum* @ 100%	23.66±1.85	0.7±0.52	6.65±0.98	0.14±0.01	22.22±16.77	8.89±3.84	17.78±19.24
Seed treatment with *D. alba* @ 100%	20.38±0.94	0.59±0.07	4.90±1.24	0.08±0.02	13.33±6.66	15.55±13.87	8.89±10.18
Soil drenching with *P. lilacinus*	18.77±1.22	0.58±0.06	4.49±0.88	0.05±0.00	22.22±10.18	4.44±3.85	6.66±6.66
Soil drenching with *T. harzianum*	19.33±1.09	0.53±0.01	4.66±1.5	0.06±0.01	28.89±10.18	4.44±3.85	6.66±6.66
Soil drenching with *R. melilati*	20.10±0.50	0.81±0.02	4.83±0.33	0.06±0.01	31.11±3.84	11.11±7.69	2.22±3.85
Soil drenching with *P. aeruginosa*	18.78±2.54	0.75±0.21	5.44±1.25	0.06±0.02	11.11±7.69	15.55±10.18	0.00±0.00
S.D with *P. lilacinus* + S.T with *W. somnifera* @ 100%	12.16±1.73	0.59±0.05	4.5±2.17	0.090±0.00	20.0±20.0	2.22±3.85	6.66±11.54
S.D with *T. harzianum*+ S.T with *W. somnifera* @ 100%	14.71±0.91	0.53±0.11	7.0±2.17	0.11±0.02	33.33±6.66	0.00±0.00	0.00±0.00
S.D with *R. melilati*+ S.T with *W. somnifera* @ 100%	14.83±0.60	0.63±0.06	4.66±1.87	0.08±0.01	17.77±13.87	0.00±0.00	0.66±1.15
S.D with *P. aeruginosa* + S.T with *W. somnifera* @ 100%	17.66±1.30	0.55±0.05	3.99±2.04	0.07±0.01	24.44±10.18	8.88±7.69	0.00±0.00
S.D with *P. lilacinus* + S.T with *S. nigrum* @ 100%	14.49±1.75	0.55±0.03	3.77±0.91	0.07±0.00	4.44±3.85	0.00±0.00	0.00±0.00
S.D with *T. harzianum* +S.T with *S. nigrum* @ 100%	15.66±2.18	0.69±0.12	4.33±0.72	0.08±0.01	15.55±7.69	4.44±7.69	8.89±10.18
S.D with *R. melilati*+S.T with *S. nigrum* @ 100%	14.22±0.69	0.67±0.12	6.27±1.92	0.09±0.01	20.0±6.67	0.00±0.00	11.11±13.87
S.D with *P. aeruginosa* + S.T with *S. nigrum* @ 100%	12.38±2.26	0.61±0.13	5.22±1.10	0.08±0.04	20.0±13.33	0.00±0.00	8.89±3.84
S.D with *P. lilacinus* + S.T with *D.alba*@ 100%	16.77±1.18	0.56±0.05	4.71±0.96	0.07±0.02	17.77±30.79	11.11±13.87	2.22±3.85
S.D with *T. harzianum*+ S.T with *D.alba* @ 100%	17.72±1.51	0.62±0.05	5.10±0.94	0.07±0.01	11.11±7.69	4.44±7.69	0.00±0.00
S.D with *R. melilati*+ S.T with *D.alba* @ 100%	16.05±0.94	0.65±0.04	5.77±0.50	0.1±0.020	17.77±3.85	11.11±3.84	6.66±6.66
S.D with *P. aeruginosa* + S.T with *D.alba* @ 100%	14.55±1.42	0.59±0.15	3.88±0.76	0.06±0.00	26.66±29.05	20.0±17.63	2.22±3.85
$LSD_{0.05}$=	2.38	0.25	2.16	0.30	22.72	13.24	12.78

Table 2. Effect of seed treatment with solanaceous leaves extract along with soil drenched with microbial antagonists in the control of root rot fungi and on the growth of cowpea plants.

Treatments	Growth parameters					Root rot fungi colonization (%)		
	Shoot length (cm)± SD	Shoot weight (g) ± SD	Root length (cm) ± SD	Root weight (g) ± SD	Number of nodules ± SD	*Fusarium* spp. ± SD	*R. solani* ± SD	*M. phaseolina* ± SD
Control (untreated)	16.89±7.29	0.78±0.56	7.99±2.08	0.12±0.05	3.11±1.01	60±17.63	17.77±3.85	28.88±7.69
Seed treatment with *W. somnifera*@100%	18.55±2.34	1.01±0.62	8.33±3.17	0.16±0.16	3.33±0.33	37.77±13.87	4.44±7.69	2.22±3.85
Seed treatment with *S. nigrum* @ 100%	25.49±4.93	1.19±0.14	8.99±1.47	0.17±0.04	5.21±2.21	46.66±17.63	4.44±7.69	2.22±3.85
Seed treatment with *D. alba* @ 100%	22.36±0.82	1.0±0.03	10.10±3.07	0.31±0.11	5.66±3.51	17.77±16.77	15.55±7.69	4.44±7.69
Soil drenching with *P. lilacinus*	17.61±0.67	0.57±0.00	6.77±0.66	0.16±0.0	3.44±0.38	0.00±0.00	2.22±3.85	4.44±3.85
Soil drenching with *T. harzianum*	18.22±2.03	0.85±0.05	7.05±0.50	0.2±0.12	4.44±0.69	35.55±3.85	0.00±0.00	2.22±3.85
Soil drenching with *R. melilati*	20.32±1.60	1.19±0.27	6.16±1.04	0.19±0.05	2.77±0.50	17.77±13.87	0.00±0.00	11.11±3.84
Soil drenching with *P. aeruginosa*	23.55±1.63	1.26±0.38	9.66±3.37	0.16±0.0	3.44±1.07	19.99±11.54	6.66±6.66	22.22±7.70
S.D with *P. lilacinus* + S.T with *W. somnifera* @ 100%	20.05±0.92	1.28±0.17	9.77±1.10	0.27±0.01	2.44±0.38	37.77±20.36	2.22±3.85	2.22±3.85
S.D with *T. harzianum*+ S.T with *W. somnifera* @ 100%	21.55±0.47	1.08±0.13	9.94±2.93	0.31±0.01	4.10±0.69	17.77±3.85	0.00±0.00	0.00±0.00
S.D with *R. melilati*+ S.T with *W. somnifera* @ 100%	17.82±3.05	0.84±0.21	8.38±0.66	0.23±0.06	2.33±0.67	24.44±20.36	0.00±0.00	0.00±0.00
S.D with *P. aeruginosa* + S.T with *W. somnifera* @ 100%	21.0±2.64	0.93±0.15	16.72±3.91	0.29±0.0	2.33±0.57	24.44±3.85	0.00±0.00	4.44±7.69
S.D with *P. lilacinus* + S.T with *S. nigrum* @ 100%	18.99±1.75	1.20±0.03	9.94±2.41	0.25±0.02	2.33±0.33	33.33±0.00	11.11±3.84	15.55±7.69
S.D with *T. harzianum* +S.T with *S. nigrum* @ 100%	19.60±4.05	1.10±0.25	9.05±0.50	0.20±0.01	2.88±0.50	33.33±0.00	2.22±3.85	11.11±10.18
S.D with *R. melilati*+S.T with *S. nigrum* @ 100%	22.22±1.16	0.76±0.00	10.77±1.54	0.21±0.06	2.88±0.83	35.55±10.18	0.00±0.00	24.44±7.69
S.D with *P. aeruginosa* + S.T with *S. nigrum* @ 100%	17.16±2.60	0.77±0.44	6.83±1.09	0.27±0.02	2.77±0.38	33.33±0.00	0.00±0.00	6.66±11.54
S.D with *P. lilacinus* + S.T with *D.alba*@ 100%	18.55±0.53	0.87±0.11	9.94±1.34	0.27±0.02	3.22±0.69	20.0±6.67	6.66±6.66	31.11±10.18
S.D with *T. harzianum*+ S.T with *D.alba* @ 100%	22.38±1.35	1.40±0.17	11.99±5.13	0.24±0.06	2.33±0.88	11.11±10.18	0.00±0.00	8.89±3.84
S.D with *R. melilati*+ S.T with *D.alba* @ 100%	17.49±1.85	0.80±0.37	6.55±0.53	0.19±0.00	3.55±0.19	24.44±7.69	6.66±6.66	15.55±7.69
S.D with *P. aeruginosa* + S.T with *D.alba* @ 100%	19.60±4.85	1.9±0.44	10.83±1.32	0.36±0.13	4.33±0.67	24.44±10.18	0.00±0.00	0.00±0.00
$LSD_{0.05}$=	4.78	0.48	3.77	0.11	1.83	19.21	7.24	10.81

DISCUSSION

Family Solanaceae is important due to possessing antifungal compounds which contain alkaloid compounds including scopolamine, atropine and hyoscyamine which make this medicinally important (Ansari, 2005). In our present research, the main motive was to control root fungal pathogens and improved the growth of cowpea and okra plants by using inexpensive and biological method. Cowpea and okra seeds treated with leaves extract (100% concentration) of *W. somnifera,* S. *nigrum* and *D. alba* along and with soil drenched with *T. harzianum* and *P. aeruginosa* respectively, ameliorate the parameters of growth of both tested hosts but also suppressed the infection of pathogenic fungi. Similar result was also reported by Hanif and Dawar (2015) when tested seeds (okra, sunflower, mung and mash beans) were treated with *A. montana* and *T. occidentalis* (homeopathic drugs) at 75% concentration along with soil drenched with microbial antagonists enhanced the growth of tested plants but also reduced the colonization of root pathogens as compared to alone treatments. Furthermore, similar result was also observed when seeds treated with leaves extracts of *S. pakistanica* and *S. holosericea* at 100% concentration inhibit the colonization of *Fusarium* spp, *R. solani* and *M. phaseolina* (Emmanual *et al*., 2010). Leaves extracts of *A. nilotica* and *S. mukorossi* also suppressed the pathogenic fungi but also improved the growth of crops (Rafi *et al*., 2015). Similar observation was also recorded by Abdel Kader *et al*., (2012) when *T. harzianum* and *Pseudomonas* spp., used single or in combination showed significant suppression of soil borne pathogens followed by *B. subtilis.* Leaves extract of *P. juliflora* at 100% w/v showed significant reduction of root rot diseases when drenched in soil (Ikram and Dawar, 2014).Same researchers also used in combination with microbial antagonists as soil drenching along with wild plant leaves extract as seed treatment which not only improved the growth of plant but also suppressed the root rot fungi (Ikram and Dawar, 2015). Alkhali (2005) reported that *A. sativum, C. proxins, C. carvi* and *A. indica* extract possess excellent antifungal activity against *F. oxysporum, B. cinereal* and *R. solani*. Seed treatment suppressed the residing fungi either on surface or inside the seed but also protect from the pathogen that are inhabitant in the soil which caused different seed borne diseases (Martha *et al.,* 2003).

Application of fungal/bacterial antagonists used as drenching in soil showed healthier plant growth but also suppressed fungal pathogens (Ehtesham-ul-Haque *et al*., 1990; Shahzad and Ghaffar 1992). Application of fungal and bacterial antagonists of such genera including *Aspergillus, Trichoderma* and *Rhizobium* have been reported to be efficient in reducing root rot disease (Rajesh *et al*., 2007; Ullah, 2011). Used of halophytic plant extracts either alone or in combination with *P. lilacinus* gave better result against root rot fungi (Mehdi *et al*., 2000). Seedling infection can be controlled by the application of *Trichoderma* spp. (Bankole and Adebanjo, 1998). *Trichoderma* species gained considerable success against pathogenic fungi as it protect the root system against fungal infection reported on a number of crops (Siddiqui *et al*., 2001; Siddiqui and Shaukat, 2004; Lokesha and Benagi, 2007). *Trichoderma* species have the potential to colonize on the rhizosphere of plant roots but also suppressed the root pathogens of plant by producing antibiosis which enhanced the growth of plants (Harman *et al*., 2004; Ramezani, 2008). *Rhizobacteria* increased plant growth by producing growth regulators which increased the yield of plants (Weller *at al.,* 2002) but also controlled root pathogens (Seuk Bae *et al.,* 2000). Rhizobial suspension when drenched in soil alone or in combination had been reported to inhibit fungal diseases and enhanced plant growth (Mazen *et al*., 2008). *R. meliloti* was found effective in controlling *M. phaseolina* colonization (Arora *et al*., 2001). *Rhizobia* bacteria also reported that it suppressed the *M. phaseolina* and *Pythium* spp. (Bardin *et al*., 2004). Marscher (1995) reported that increased in nitrogen level of root exudation by the stimulation of higher population caused by *Pseudomonas aeruginosa* and *Rhizobium* spp. around roots which reduced the *M. phaseolina* population in soil.

Microbial antagonist increases the mineral nutrients in the plants by improving the plant nitrogen fixation due to secondary metabolites produces by friendly bacteria which also improve the plant health by controlling plant pathogens (Sturz and Christie, 2003; Moeinzadeh *et al*., 2010). Interactions of microbial antagonist with root pathogens considered as important mechanisms for biological control of various plant diseases (Khara and Hadwan, 1990; Shalini *et al.,* 2006). Application of medicinal plant extracts in the controlling of root diseases in plants are non hazardous and do not disturb the soil environment (Elad, 2000). Use of solanaceous plants extracts along with microbial antagonists showed positive result in the growth promotion of okra and cowpea plants but also controlled the root infecting pathogens which is strongly recommend to use in agricultural fields.

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(*Accepted for publication June 2020*)