

EFFECT *SIDA PAKISTANICA* S. ABEDIN AND *SENNA HOLOSERICEA* FRESEN ON GROWTH AND ROOT ROT DISEASES OF OKRA AND MASH BEAN

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Abstract

Leaves and stem extract and powder of *Sida pakistanica* S. Abedin and *Senna holosericea* Fresen were used as seed treatment, soil drenching and soil amendment for the control of root rot diseases of okra and mash bean. Results showed that plant growth parameter enhanced and reduced the infection of *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina* on mash bean and okra. Seed treatment with leaves and stem extract of *S. pakistanica* and *S. holosericea* used @ 25, 50 and 100 % w/v showed control of root rot fungi on mash bean and okra, and significantly increased the plant growth parameter in terms of shoot weight and root weight. Soil drenching with *S. holosericea* leaf and stem extracts were more effective in the control of *Fusarium* spp., *R. solani* and *M. phaseolina* on mash bean and okra plant followed by the soil and seed treatment with *S. pakistanica* leaf and stem extracts. *Fusarium* spp., was controlled by stem extract of *S. holosericea* @ 50 and 100% w/v. Soil amendment with leaves and stem powder of *S. pakistanica* and *S. holosericea* used @ 0.1 and 1% w/w showed reduction in infection of *R. solani* and *M. phaseolina* on okra and mash bean and significantly enhanced plant weight of mash bean. *S. pakistanica* stem powder and *S. holosericea* leaf powder @ 0.1% w/w were more effective on growth of okra and mash bean whereas *S. pakistanica* leaf powder @ 0.1 and 1% w/w were more effective in the control of root rot fungi on mash bean and okra.

Introduction

Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms (Gordan & David, 2001). Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents that together combine to give the plant its therapeutic value. Consequently, it is believed that the whole plant has more effective healing properties than its isolated constituents. Any part of the plant may contain active components (Nair & Chanda, 2004). *S. pakistanica* (Family-Malvaceae) is found popularly in the desert areas of Pakistan and India. Among 12 species of *Sida* examined, seed extracts of *S. acuta* and *S. rhombhofolia* were found to contain significant amounts of ecdysteroids, seed extracts of *S. filicaulis* contained only moderate levels, whilst the remaining species showed no detectable levels of ecdysteroids (Dinan *et al.*, 2001). The shrub *Senna holosericea* commonly found in Karachi hilly areas and its vicinity were studied for the plant water status and proline contents during rainy and dry periods. Leaves and seeds of *S. holosericea* were used to treat constipation and stomach cramps whereas pods contain anthraquinones and glycosides, sennosides C and B, which are the glycosides of heterodanthrones (Ghazanfar, 1994). Plants of the genus *Senna* that contain anthranoides derivatives are frequently used as cathartics (Nadal *et al.*, 2003).

Present study was carried out to examine the effect of *S. pakistanica* and *S. holosericea* on seed germination and control of root rot fungi viz., *Fusarium* spp., *R.*

solani, *M. phaseolina* on mash bean [*Vigna mungo* (L.) Hepper] and okra [*Abelmoschus esculentus* (L.) Moench] used as test plants.

Materials and Methods

Collection of plant: Leaves and stems of *Sida pakistanica* S. Abedin and *Senna holosericea* Fresen., were collected and dried under shade. After drying all plant parts were separately ground and their powder was used for further studies.

Preparation of plant extract: Ten g dried powder of *S. pakistanica* and *S. holosericea* leaves and stems were soaked separately in 100 ml (stock solution) of sterilized distilled water in flask and left for over night. After 24 hours the extract was filtered with Whatman's filter paper. This gave 100% stock solution which were further diluted to make 50 and 25% w/v concentrations.

Preparation of pots: Soil used for seed treatment and soil drenching was obtained from experimental plot of Botany Department, University of Karachi. The soil was sandy loam (sand, silt, clay, 60, 22 & 18%), pH ranged from 7.1-7.5 with moisture holding capacity (MHC) of 29% (Keen & Raczkowski, 1922), total nitrogen 0.077-0.099% (Mackenzie & Wallace, 1954), 3-4 sclerotia/g of *M. phaseolina* as found by wet sieving technique (Sheikh & Ghaffar, 1975), 5-10% of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and *Fusarium* spp., 3500 cfu g⁻¹ as assessed by soil dilution technique (Nash & Synder, 1962).

Seed treatment: Seeds of mash bean and okra were surface sterilized with 1% Ca (OCl)₂ for 3 minutes, rinsed thoroughly in running tap water and dried aseptically. Seeds were treated with 25, 50 and 100% w/v leaf and stem extracts of *S. pakistanica* and *S. holosericea* for 5 minutes. Five treated seeds were sown in 8cm diam., plastic pots, each containing 300 g soil. Seeds treated with sterile distilled water served as control. Treatments were replicated three times.

Soil drenching: A 20 ml aqueous leaf and stem extract of *S. pakistanica* and *S. holosericea* used at 25, 50 and 100% w/v were drenched in 8 cm diam., plastic pots and 5 seeds of mash bean [*Vigna mungo* (L.) Hepper] and okra [*Abelmoschus esculentus* (L.) Moench] served as test plant were sown in each pot, each containing 300 g soil. Pots treated with sterile distilled water served as control. Treatments were replicated three times.

Soil amendment: Soil was amended with dried leaves and stems powder of *S. pakistanica* and *S. holosericea* @ 0.1 and 1% w/w and kept in 8cm diam., pots, each containing 300 g soil. The soil was watered daily to allow decomposition of the material. After 10 days of amendment, 5 seeds of mash bean and okra were sown in each pot. Non amended soil served as control. Each treatment was replicated three times.

Isolation of fungi from infected roots: To determine the incidence of fungi, one cm long root pieces after washing in running tap water were surface sterilized with 1% Ca(OCl)₂ and transferred on PDA plates supplemented with penicillin at 200mg/l and

streptomycin at 200mg/l @ 5 pieces per plate, Petri dishes were incubated at room temperature (28°C) and after one week, infection of roots by root infecting fungi was recorded.

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 statistical software according to Sokal & Rohlf (1995).

Results and Discussion

Result showed significant increase in shoot weight ($p < 0.01$) and root weight ($p < 0.001$) of mash bean plants when seeds were treated with 25, 50 and 100% w/v leaf and stem extracts of *S. pakistanica* and *S. holosericea* (Table 1). 25% w/v leaf extract of *S. holosericea* and 50% stem extract of *S. pakistanica* were more effective in increasing the shoot length and root length of mash bean. There was significant reduction in infection of *Fusarium* spp. ($p < 0.05$), *R. solani* ($p < 0.05$) and *M. phaseolina* ($p < 0.05$) on mash bean plants (Table 2). *S. holosericea* leaf extract (50%) and stem extract (25%) were more effective in the control of *Fusarium* spp., *R. solani* and *M. phaseolina* on mash bean plants. *S. holosericea* (50%) stem extract completely controlled the infection of *Fusarium* spp., *R. solani* and *M. phaseolina* whereas significant reduction in the infection of *M. phaseolina* ($p < 0.01$) @ 100 % w/w was observed on okra (Table 2). Stem extract of *S. holosericea* was more effective in the control of *Fusarium* spp., *R. solani* and *M. phaseolina* followed by 50 and 100% w/v leaf extract of *S. holosericea* on okra. Singh *et al.*, (1993) reported the antifungal activities of leaf extracts of some medicinal plants such as *Calotropis procera*, *Vitex negundo*, *Lantana camara*, *Azadirachta indica*, *Ficus religiosa*, *Thuja orientalis*, *Argemone mexicana*, *Achyranthes aspera*, *Datura fastuosa* and *Ricinus communis* against *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Helminthosporium spiciferum*, *Curvularia lunata*, *Aspergillus flavus* and *Trichothecium roseum* and observed good control against these pathogens.

There was significant increase in shoot length ($p < 0.001$), shoot weight ($p < 0.01$), root length ($p < 0.001$) and root weight ($p < 0.001$) of mash bean (Table 1) where soil was drenched with 25, 50 and 100% w/v leaf and stem extracts of *S. pakistanica* and *S. holosericea*. *S. holosericea* @ 50 and 100% w/v stem extract and *S. pakistanica* @ 25 and 50% w/v leaf extracts were more effective on the growth parameter of mash bean. There was significant reduction in the infection of *Fusarium* spp., ($p < 0.05$), *R. solani* ($p < 0.01$) and *M. phaseolina* ($p < 0.01$) on mash bean (Table 5). Stem extract of *S. holosericea* @ 25% w/v completely controlled the *Fusarium* spp., *R. solani* and *M. phaseolina* followed by the *S. holosericea* leaf extract (50%). *S. holosericea* was found to be more effective on growth parameter of okra plants. *S. holosericea* stem extract significantly reduced the infection of *Fusarium* spp., ($p < 0.05$) on okra (Table 2). *S. holosericea* leaf extract and *S. holosericea* stem extract were found to be more effective followed by the *S. pakistanica* leaf extracts in the control of *Fusarium* spp., *R. solani* and *M. phaseolina* on mash bean and okra. *S. holosericea* leaf and stem extracts @ 100% w/v were more effective in the control of *Fusarium* spp., *R. solani* and *M. phaseolina* followed by the leaf and stem extract of *S. pakistanica*.

Table 1. Effect of seed treatment, soil drenching with leaf , stem extracts and soil amendment with leaf, stem powder of *Sida pakistanica* and *Senna holosericea* on growth parameter of mash bean and okra.

Treatments	Mash bean				Okra			
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Seed treatment								
Control	20.1 ab	1.53 a	10.45 a	0.40 a	11.9 abc	1.4 a	5.04 ab	0.30 ab
25% <i>S. pakistanica</i> leaves	21.86 ab	1.42 a	11.02 a	0.38 ab	16.5 ab	1.28 a	5.7 ab	0.13 abc
50% <i>S. pakistanica</i> leaves	22.9 a	1.16 abcd	9.5 a	0.23 bc	16.8 ab	1.72 a	6.16 ab	0.32 a
100% <i>S. pakistanica</i> leaves	24.2 a	1.39 ab	9.7 a	0.22 bc	18.6 ab	1.42 a	5.75 ab	0.12 abc
25% <i>S. pakistanica</i> stem	23 a	1.37 ab	10.7 a	0.19 cd	16.15 ab	1.03 a	4.10 ab	0.21 abc
50% <i>S. pakistanica</i> stem	25.1 a	1.35 ab	8.1 a	0.11 cd	23.4 a	1.34 a	5.66 ab	0.28 ab
100% <i>S. pakistanica</i> stem	23.6 a	1.25 abc	9.03 a	0.17 cd	13.5 abc	0.74 a	3.1 ab	0.06 bc
25% <i>S. holosericea</i> leaves	25.6 a	1.12 abcd	11.08 a	0.17 cd	19.46 ab	1.19 a	7.2 a	0.12 abc
50% <i>S. holosericea</i> leaves	15.16 a	0.47 de	5.6 a	0.06 cd	15.7 ab	1.3 a	6.2 ab	0.08 abc
100% <i>S. holosericea</i> leaves	15.06 a	0.65 bcde	6.1 a	0.09 cd	18.91ab	1.06 a	7.5 a	0.07 abc
25% <i>S. holosericea</i> stem	8 b	0.163 e	4.3 a	0.02 cd	12.76 abc	1.04 a	2.6 ab	0.08 abc
50% <i>S. holosericea</i> stem	13.7 ab	0.3 e	4.5 a	0.03 d	0 c	0 a	0 b	0 c
100% <i>S. holosericea</i> stem	22.2 a	0.58 cde	7.6 a	0.06 cd	6.94 bc	0.89 a	2.4 ab	0.08 abc
L S D 0.05=	12.57	0.665	5.835	0.160	12.67	1.694	5.408	0.217

Table 1. (Cont'd.).

Treatments	Mash bean			Okra		
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Shoot weight (g)	Shoot length (cm)	Root weight (g)
Soil drenching						
Control	27.4 ab	2.45 ab	10.85 c	0.52 bc	20.8 bc	2.48 abc
25% <i>S. pakistanica</i> leaves	29.2 ab	2.99 a	12.1 c	0.98 a	23.6 abc	2.72 ab
50% <i>S. pakistanica</i> leaves	26.3 ab	2.38 ab	12.3 c	0.90 a	30.6 ab	3.80 a
100% <i>S. pakistanica</i> leaves	27 ab	2.21 ab	12.3 c	0.48 bc	24.3 abc	2.39 abc
25% <i>S. pakistanica</i> stem	30.7 ab	2.31 ab	15.8 bc	0.59 abc	18.5 bc	1.32 bc
50% <i>S. pakistanica</i> stem	29.2 ab	2.28 ab	11.9 c	0.35 c	15.5 bc	1.29 bc
100% <i>S. pakistanica</i> stem	30.4 ab	2.33 ab	13.4 bc	0.43 bc	32.1 ab	2.67 ab
25% <i>S. holosericea</i> leaves	20.8 b	1.53 b	11.7 c	0.39 c	17.5 bc	0.72 bc
50% <i>S. holosericea</i> leaves	30.1 ab	2.25 ab	22.9 a	0.92 a	45.1 a	2.10 abc
100% <i>S. holosericea</i> leaves	31.3 ab	1.98 ab	19.5 ab	0.80 ab	16.4 bc	0.89 bc
25% <i>S. holosericea</i> stem	0 c	0 c	0 d	0 d	31.4 ab	2.96 ab
50% <i>S. holosericea</i> stem	33.3 a	1.79 ab	13.3 bc	0.42 bc	14.96 bc	2.39 abc
100% <i>S. holosericea</i> stem	36.9 a	2.14 ab	14.9 bc	0.41 bc	5 c	0.17 c
L S D 0.05=	10.52	1.0707	6.2478	0.3499	20.93	2.1530
						0.366

Table 1. (Cont'd.).

Treatments	Mash bean			Okra		
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)
Soil amendment						
Control	16.58 a	0.32 d	5.83 a	0.04 b	14.5 a	1.32 a
0.1% <i>S. pakistanica</i> leaves	17.83 a	0.62 cd	6.86 a	0.09 b	14.8 a	1.33 a
1% <i>S. pakistanica</i> leaves	23.60 a	1.09 bc	8.27 a	0.14 ab	17.4 a	0.91 a
0.1% <i>S. pakistanica</i> stem	31.01 a	1.5 ab	11.26 a	0.27 a	12.3 a	0.56 a
1% <i>S. pakistanica</i> stem	27.5 a	1.6 ab	8.75 a	0.16 ab	11.38 a	1.02 a
0.1% <i>S. holosericea</i> leaves	29.46 a	2.01 ab	10.82 a	0.18 ab	13.11 a	0.78 a
1% <i>S. holosericea</i> leaves	26.58 a	1.26 abc	8.1a	0.11ab	5.26 a	0.67 a
0.1% <i>S. holosericea</i> stem	29.5 a	1.84 ab	10.54 a	0.2 ab	24.1 a	1.92 a
1% <i>S. holosericea</i> stem	27.6 a	1.52 ab	8.59 a	0.18ab	14.46 a	1.20 a
L S D 0.05 =	13.65	0.68	6.035	0.1415	16.90	1.57

Same letters in each column are not significantly different at p<0.05 according to DMRT

Table 2. Effect of seed treatment, soil drenching with leaf, stem extracts and soil amendment with leaf, stem powder of *Sida pakistanica* and *Senna holosericea* in the control of root rot fungi on mash bean and okra.

Treatments	Mash bean			Okra		
	<i>Fusarium</i> spp.	<i>R. solani</i>	<i>M. phaseolina</i>	<i>Fusarium</i> spp.	<i>R. solani</i>	<i>M. phaseolina</i>
Seed treatment						
Control	100 a	100 a	78 a	89 a	100 a	89 a
25% <i>S. pakistanica</i> leaves	67 abc	87 ab	67 ab	56 abcd	56 abc	44 bc
50% <i>S. pakistanica</i> leaves	44 abc	67 abc	56 abc	78 ab	56 abc	11 c
100% <i>S. pakistanica</i> leaves	44 abc	56 abcd	56 abc	33 abcd	56 abc	67 ab
25% <i>S. pakistanica</i> stem	33 bc	78 abc	22 bcd	56 abcd	44 bc	22 bc
50% <i>S. pakistanica</i> stem	67 abc	100 a	33 abcd	67 abc	56 abc	22 bc
100% <i>S. pakistanica</i> stem	89 ab	56 abcd	22 bcd	33 abcd	22 bc	11 c
25% <i>S. holosericea</i> leaves	33 bc	67 abc	33 abcd	22 bcd	67 ab	22 bc
50% <i>S. holosericea</i> leaves	11 c	33 cd	0 d	22 bcd	56 abc	11 c
100% <i>S. holosericea</i> leaves	44 abc	56 abcd	44 abcd	22 bcd	44 bc	0 c
25% <i>S. holosericea</i> stem	11 c	11 d	11 cd	44 abcd	33 bc	22 bc
50% <i>S. holosericea</i> stem	33 bc	44 bcd	22 bcd	0 d	0 c	0 c
100% <i>S. holosericea</i> stem	44 abc	56 abcd	22 bcd	11 cd	33 bc	11 c
L S D 0.05=	51.85	44.12	42.15	53.57	48.28	40.05

Table 2. (Cont'd.).

Treatments	Mash bean			Okra		
	<i>Fusarium</i> spp.	<i>R. solani</i>	<i>M. phaseolina</i>	<i>Fusarium</i> spp.	<i>R. solani</i>	<i>M. phaseolina</i>
Seed drenching						
Control	100 a	100 a	89 a	78 a	78 a	78 a
25% <i>S. pakistanica</i> leaves	89 a	100 a	22 cde	56 abc	67 a	33 b
50% <i>S. pakistanica</i> leaves	89 a	89 a	89 a	56 abc	56 a	22 b
100% <i>S. pakistanica</i> leaves	89 a	100 a	67 abc	67 ab	67 a	44 ab
25% <i>S. pakistanica</i> stem	78 a	89 a	67 abc	33 abcd	33 a	11 b
50% <i>S. pakistanica</i> stem	89 a	67 a	44 abcde	33 abcd	33 a	33 b
100% <i>S. pakistanica</i> stem	79 a	89 a	79 ab	44 abcd	56 a	33 b
25% <i>S. holosericea</i> leaves	67 a	56 a	22 cde	22 bcd	22 a	22 b
50% <i>S. holosericea</i> leaves	89 a	89 a	11 de	33 abcd	22 a	11 b
100% <i>S. holosericea</i> leaves	89 a	78 a	56 abcd	11 cd	22 a	33 b
25% <i>S. holosericea</i> stem	0 b	0 b	0 e	78 a	67 a	22 b
50% <i>S. holosericea</i> stem	89 a	89 a	56 abcd	56 abc	56 a	22 b
100% <i>S. holosericea</i> stem	67 a	67 a	33 bcde	0 d	22 a	22 b
L S D 0.05=	45.38	41.72	41.95	45.98	47.92	39.08

Table 2. (Cont'd.).

Treatments	Mash bean			Okra		
	<i>Fusarium</i> spp.	<i>R. solani</i>	<i>M. phaseolina</i>	<i>Fusarium</i> spp.	<i>R. solani</i>	<i>M. phaseolina</i>
Soil amendment						
Control	100 a	89 a	89 a	89 a	78 a	78 a
0.1% <i>S. pakistanica</i> leaves	67 a	22 cd	22 b	33 ab	44 a	0 b
1% <i>S. pakistanica</i> leaves	89 a	56 abc	11 b	22 b	33 a	11 b
0.1% <i>S. pakistanica</i> stem	78 a	11 d	33 b	0 b	33 a	11 b
1% <i>S. pakistanica</i> stem	89 a	11 d	56 ab	44 ab	22 a	11 b
0.1% <i>S. holosericea</i> leaves	78 a	78 ab	22 b	22 b	33 a	11 b
1% <i>S. holosericea</i> leaves	78 a	44 bcd	22 b	11 b	22 a	11 b
0.1% <i>S. holosericea</i> stem	100 a	56 abc	0 b	56 ab	44 a	22 b
1% <i>S. holosericea</i> stem	100 a	78 ab	33 b	44 ab	56 a	33 b
L S D 0.05 =	35.78	37.64	50.25	57.14	63.00	40.01

Same letters in each column are not significantly different at p<0.05 according to DMRT

A significant ($p < 0.01$) increase in shoot weight was observed in mash bean where soil was amended with leaf and stem powder used @ 0.1 and 1% w/w (Table 1). *S. holosericea* stem powder and leaf powder @ 0.1% w/w and *S. pakistanica* stem powder @ 0.1% w/w were effective which enhanced the shoot length, shoot weight, root length and root weight of mash bean plants. There was a significant reduction in infection of *R. solani* ($p < 0.01$) on mash bean plants (Table 1). *S. holosericea* stem powder, *S. pakistanica* stem and leaf powder were more effective in the control of root rot fungi on mash bean plants. *S. holosericea* @ 0.1% w/w stem powder was effective which enhanced the shoot length and shoot weight (Table 1) on okra whereas significant reduction in infection of *M. phaseolina* ($p < 0.05$) was observed on okra plants (Table 2) and *S. pakistanica* @ 0.1% w/w stem powder completely controlled *Fusarium* spp., and *S. pakistanica* leaf powder @ 0.1% w/w completely controlled *M. phaseolina* on okra. *S. pakistanica* stem and leaf powder and *S. holosericea* leaf powder were more effective in the control of *Fusarium* spp., *R. solani* and *M. phaseolina* on okra. *S. holosericea* leaf and stem powder and *S. pakistanica* stem powder were more effective on growth parameter of mash bean and okra and in the control of root rot fungi. Similarly Tariq *et al.*, (2008) reported that mangrove plant parts powder used @ 1 and 5% w/w increased all growth parameters of potato plants. Plants produce a large variety of secondary metabolites like phenols, tannins, terpenoids, alkaloids, polyacetylenes, fatty acids and steroids, which have an allelopathic effect on the growth and development of the same plant or neighboring plants. Present research on *S. holosericea* and *S. pakistanica* showed control of root disease of mash bean and okra which can increase economy of country.

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