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TOXICITY OF AQUEOUS EXTRACTS OF KAMINI (*GUAICUM OFFICINALE* L.) ON GERMINATION AND SEEDLING GROWTH OF WHEAT (*TRITICUM AESTIVUM* L. VAR. KIRAN)

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ABSTRACT

Aqueous extracts from various plant / litter components of *Guaicum officinale* L. significantly inhibited germination of wheat (*Triticum aestivum* L.) except bark which had very mild inhibitory effect. The degree of germination inhibition was proportional to the concentration of the extracts. Bark extract was the least inhibitory. Suppression of germination under influence of the extracts employed was in the following order of toxicity as - Pericarp > Flowers > Yellow leaves > Green leaves > Green fruits > Bark.

Seedling growth in terms of plumular elongation, number of seminal roots, average length of seminal root in a seedling, total length of seminal roots per seedling and the biomass accumulated over three days of growth in seedlings per Petri plate indicated significant reduction in seedling performance under influence of the extracts of plant components such as abscised yellow leaves, abscised floral parts, pericarp of the dehisced fruits, and green leaves. The abscised yellow leaves and floral parts were the highly toxic litter components. Bark, on the other hand had no inhibitory effect on seedling growth – rather it stimulated the plumular growth significantly. Extract of green fruit had very mild inhibitory effects on wheat seedlings.

Key words: *Guaicum officinale* L., Aqueous extracts of plant (litter) components; *Triticum aestivum* L.; Phytotoxicity

INTRODUCTION

Guaicum officinale L. (Kamini, lignum vitae) is commonly grown as ornamental tree in Karachi along roadsides or in gardens for its close growing foliage and showy flowers produced copiously from March to October (Ghafoor, 1974).

A few Kamini trees were grown along the borders of the lawn in Government National College, Karachi around 1985. It was observed that as a result of copious litter fall from these trees through years the soil underneath these trees became gradually unsupportive to lawn grass, *Cynodon dactylon*. The grass disappeared in patches in correspondence with the canopies of the trees. The growth of other plants (*Phyllanthus niruri, Vernonia cinera, Euphorbia hirta*, etc.), which invaded the open spaces created below the canopies of these trees was also differentially suppressed. It was hypothesized that besides some other unknown reasons, the accumulation of litter from *Guaicum trees* should be phytotoxic in nature and leachates from the litter on irrigation should have entered the soil and inhibited the growth of plants under its canopy. This paper describes the results of a preliminary base line *in vitro* investigations undertaken to look for phytotoxicity of various plant parts constituting the litter of *G. officinale* against wheat (*Triticum aestivum* L.) - a common test species employed in growth bioassays.

MATERIALS AND METHODS

The fresh litter from underneath *G. officinale* trees grown along the borders of the lawn in Government National College, Karachi was collected in October, 2007 and dried at room temperature in shade. It was sorted in different components viz., bark, green leaves, green fruits, yellow leaves, floral parts (including, pedicel, petals, ovaries etc.) and pericarp of the dehisced fruits.

Aqueous extracts of various plant components were prepared by soaking (not crushing) 10 g dry plant material in 200 ml distilled water for 24 h. The filtrates were taken as stock from which dilutions (25, and 50%) were prepared. The toxicity of these extracts was tested against *Triticum aestivum* var. Kiran, supplied by PARC, Karachi.

Twenty surface sterilized (2% sodium hypochlorite for 5 min.) caryopses of wheat were placed on Whatman No. 1 filter paper in 9 cm diameter sterile petri plates and 5 ml of an extract was added to each. Controls received glass-distilled water. Treatments and controls were replicated thrice and the petri plates were kept under 14 h illumination of 4000 Lux. Germination counts were made daily. A seed was considered germinated if its radicle or plumule protruded out of the seed and attained a length of not less than 1.5 mm (Taylor, 1942). The length of plumule, number of seminal roots and their length were recorded at 72h of growth. Dry weight data on seedling biomass was expressed as biomass accumulated by

seedlings on per plate basis. The biomass included all seminal roots and the plumules of all seedlings in a plate sans depleted caryopses. The data was analyzed statistically.

RESULTS

A. Germination

Aqueous extracts from various litter components significantly inhibited germination of wheat caryopses except bark which had very mild inhibitory effect. The degree of germination inhibition was proportional to the concentration of the extracts. Bark extract was the least inhibitory and pericarp extract was highly inhibitory to the germinability of wheat caryopses (Fig. 1). Suppression of germination under influence of the extracts employed was in the following order of toxicity as - Pericarp > Flowers > Yellow leaves > Green leaves > Green fruits > Bark.

B. Seedling Growth

Plumule

The plumular growth was differentially influenced by the extracts of various plant components of *Guaicum*. Bark extract had stimulatory effect on plumule which, with slight irregularity, was the direct function of the extract concentration. Extract of green fruits had no effect on plumule and the extract of the abscised flowers was the most toxic followed by the extract of the abscised leaves. Extract of green leaves had moderate inhibitory effect. The extract of pericarp of abscised fruits inhibited plumular growth at 50%S and 100%S of the extract. Extracts of abscised leaves and floral components were the most highly inhibitory to the plumular growth (Table 1).

Number of seminal roots

The number of seminal roots remained almost unaffected by the extracts of the green leaves and marginally increased in bark and green fruits extracts. It was significantly reduced in extracts of abscised flowers and leaves. The number of seminal roots decreased significantly in 25%S and 50%S of the pericarp extract but slightly increased in 100%S (Table 1).

Average length of seminal roots per seedling (ALSR)

ALSR remained more or less unaffected by the extract of bark but reduced greatly under the influence of extracts of abscised flowers and leaves. It, moderately but progressively, declined with the concentration of green leaves extract. ALSR was significantly declined only in higher concentration of green fruit extract (50%S and 100%S). Extracts of the abscised fruits reduced the root length also significantly as a direct function of the extract concentration. Extract of the floral parts was the most inhibitory to root growth (Table 1).

Total length of seminal roots per seedlings (TLSR)

Like other seedling growth parameters, TLSR was not affected by any concentration of bark extract investigated and up till 25%S of extract of green fruits. In higher green fruit extract TLSR reduced significantly. TLSR was highly inhibited by the extracts of abscised floral parts and yellow abscised leaves to the extent that growth of seminal roots was completely inhibited in stock extract. Extracts of green leaves and pericarps were more or less equally and significantly inhibitory to TLSR (Table 1).

Taken together the behaviour of various seedling growth parameters under the influence of the extracts, it was obvious that extracts of abscised floral parts were the most toxic followed by abscised yellow leaves, pericarp of the dehisced fruits, green leaves and green fruits. Bark had no effect on seedling performance.

Seedling Biomass

Extracts of various plant parts of *Guaicum* except bark reduced the biomass accumulated over 72 h of seedlings growth under their influence (Fig. 2). Extracts of the abscised floral parts was highly inhibitory. Green fruits' extract was lesser inhibitory than that of other litter components.

ANOVA of the biomass data indicated that both extract concentrations ($P < 0.001$) and plant litter components ($p < 0.001$) had significant negative effects on seedling biomass and interacted significantly ($p < 0.039$) with each other (Fig. 2). ANOVA separated the extract sources into two groups. Group I composed of non-inhibitory plant components such as bark and green fruits and group II composed of inhibitory components like green leaves and yellow leaves, floral parts and pericarp. Yellow leaves and the abscised floral parts were highly toxic litter components.

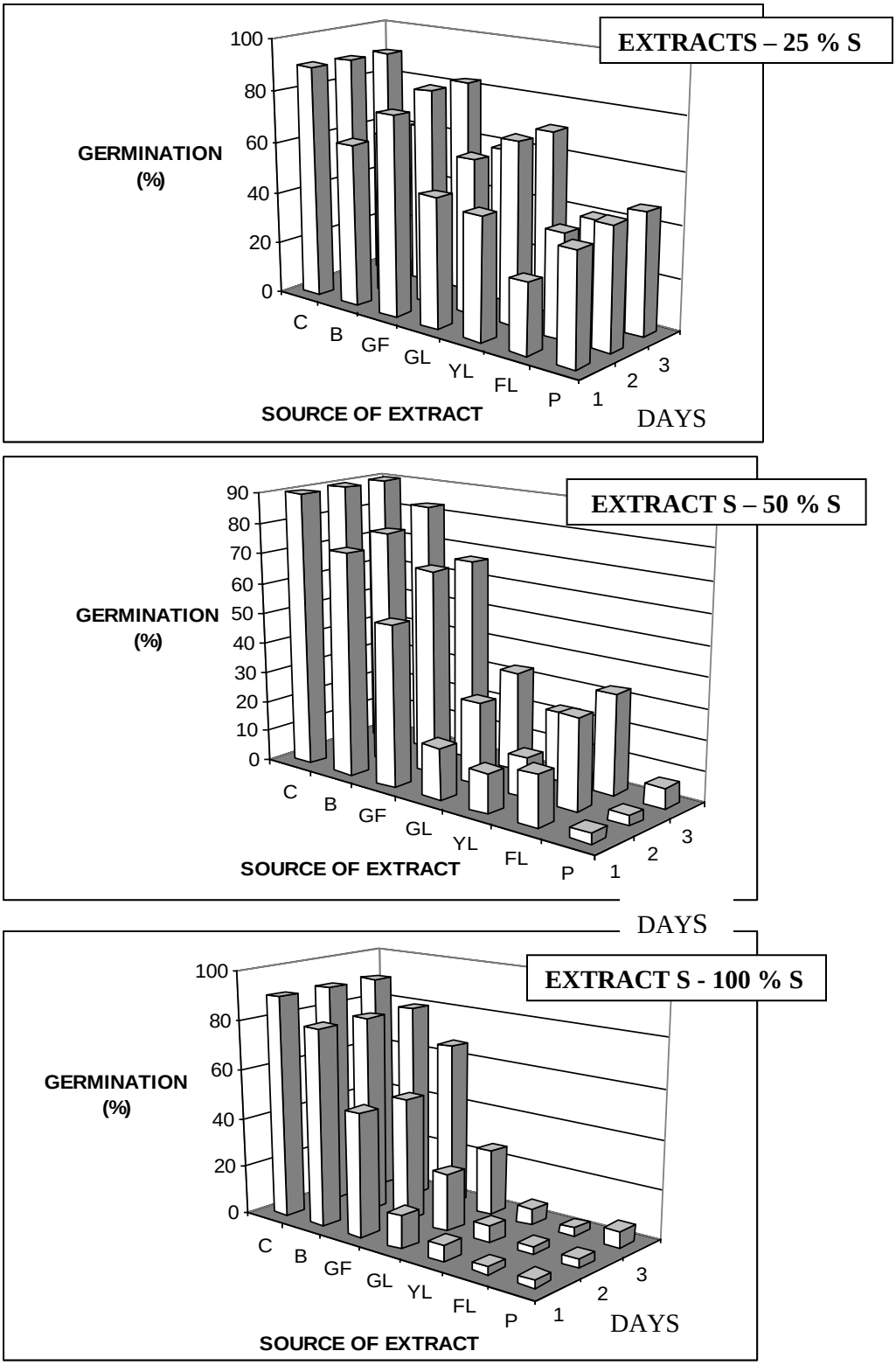

Fig. 1. Germination of *Triticum aestivum* var. *Kiran* under the influence of extracts from various plant parts / litter of *Guaicum officinale*. C, Control; B, Bark; GF, Green fruits, GL, Green leaves; YL, Yellow abscised leaves; FL, Flowers, P, Pericarp.

Table 1. Effects of aqueous extracts of various morphological parts of *Guaicum officinale* on seedling growth of *Triticum aestivum* var. Kiran.

GREEN LEAVES				
Treatments	**Control**	**25%S**	**50%S**	**100%S**
Plumule (cm)	2.53 ± 0.16 33.2	1.17 ± 0.29 71.9*	1.79 ± 0.36 64.2	1.60 ± 0.24 43.2
Seminal Roots	3.93 ± 0.16 22.2	3.44 ± 0.32 39.7	3.50 ± 0.31 27.8	3.71 ± 0.39 29.9
ALSR (cm)	3.76 ± 0.21 62.7	2.77 ± 0.26 55.4	2.38 ± 0.26 64.0	1.21 ± 0.11 49.5
TLSR / seedling (cm)	14.90 ± 4.50 161.9	8.75 ± 0.76 71.2	8.52 ± 1.96 72.8	4.24 ± 0.68 45.0

ABSCIZED LEAVES				
Plumule (cm)	2.53 ± 0.16 33.2	1.64 ± 0.18 50.8	0.51 ± 0.08 47.4	1.50
Seminal Roots	3.93 ± 0.16 22.2	3.56 ± 0.31 40.0	1.14 ± 0.48 128.4	Zero
ALSR (cm)	3.76 ± 0.21 62.7	1.56 ± 0.12 64.8	0.79 ± 0.16 81.5	Zero
TLSR / seedling (cm)	14.90 ± 4.50 161.9	5.60 ± 0.82 67.7	1.45 ± 0.65 135.4	Zero

ABSCIZED FLOWER PARTS				
Plumule (cm)	2.53 ± 0.16 33.2	1.77 ± 0.31 60.2	1.53 ± 0.21 20.9	Zero
Seminal Roots	3.93 ± 0.16 22.2	3.08 ± 0.43 48.9	3.70 ± 0.34 28.6	Zero
ALSR (cm) **	3.76 ± 0.21 62.7	2.08 ± 0.23 69.2	1.48 ± 0.20 81.0	Zero
TLSR / *** seedling (cm)	14.90 ± 4.50 161.9	6.78 ± 1.45 74.1	5.46 ± 1.34 77.4	Zero

BARK				
Plumule (cm)	2.53 ± 0.16 33.2	2.03 ± 0.27 58.6	4.66 ± 1.30 139.7	3.50 ± 0.20 28.4
Seminal Roots	3.93 ± 0.16 22.2	3.77 ± 0.30 33.4	4.33 ± 0.17 20.0	4.04 ± 0.22 27.8
ALSR (cm)	3.76 ± 0.21 62.7	3.22 ± 0.31 82.5	3.51 ± 0.17 51.1	3.91 ± 0.33 84.6
TLSR / seedling (cm)	14.90 ± 4.50 161.9	11.15 ± 2.49 77.3	15.35 ± 0.87 27.9	14.51 ± 1.32 45.4

GREEN FRUITS				
Plumule (cm)	2.53 ± 0.16 33.2	2.74 ± 0.37 38.5	2.76 ± 0.21 33.2	2.67 ± 0.33 50.54
Seminal Roots	3.93 ± 0.16 22.2	3.86 ± 0.66 48.3	4.53 ± 0.21 19.9	4.23 ± 0.24 22.9
ALSR (cm)	3.76 ± 0.21 62.7	3.80 ± 0.34 50.2	2.74 ± 0.16 53.8	2.34 ± 0.16 46.9
TLSR / seedling (cm)	14.90 ± 4.50 161.9	15.20 ± 2.68 49.9	12.44 ± 1.26 44.3	9.95 ± 1.13

PERICARP OF DEHISCED FRUITS				
Plumule (cm)	2.53 ± 0.16 33.2	2.48 ± 0.32 50.2	0.88 ± 0.31 100.5	1.20 ± 0.70
Seminal Roots	3.93 ± 0.16 22.2	3.50 ± 0.53 59.0	2.66 ± 0.53 56.6	5.0 ± 0
ALSR (cm)	3.76 ± 0.21 62.7	2.44 ± 0.17 52.8	1.02 ± 0.22 86.8	0.72 ± 0.095
TLSR / seedling (cm)	14.90 ± 4.50 161.9	9.54 ± 1.47 59.7	2.72 ± 1.69 152.2	3.60

*, CV (%); **, Average length of a seminal root; ***, Total length of seminal roots per seedling.

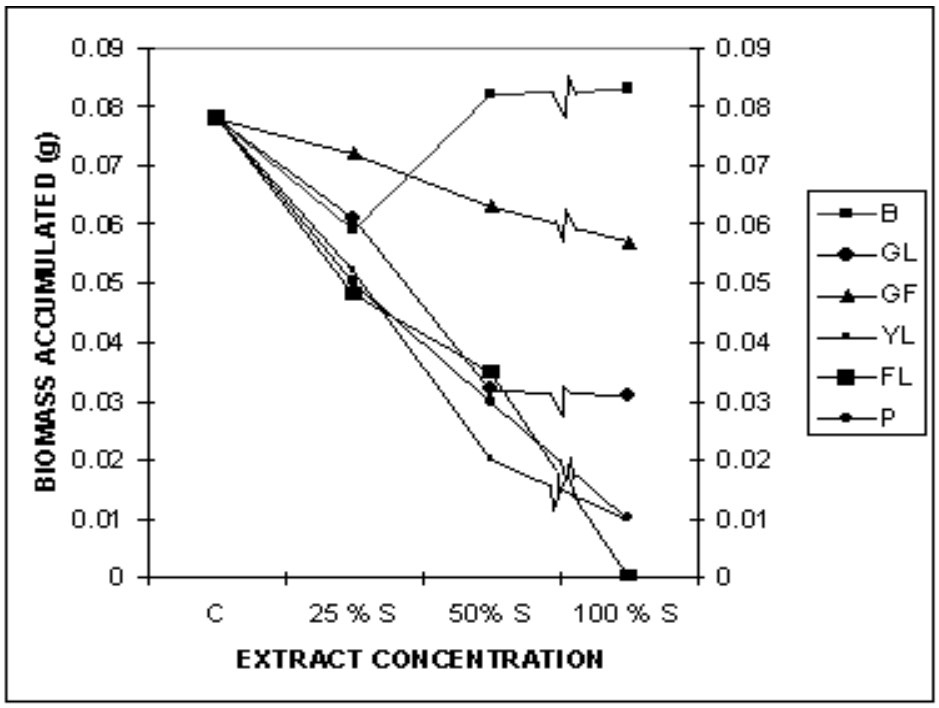

Fig. 3. Biomass accumulation in seedlings of *T. aestivum* cv. *Kiran* grown under influence of various concentrations of aqueous extracts of different morphological parts of *Guaicum Officinale*. Key – C, Control; B, Bark; GL, Green Leaves; GF, Green Fruits; YL, Yellow leaves; FL, Flowers; P, Pericarp of dehisced fruits. Dry weight data expressed on per plate basis (seminal roots + plumule sans depleted caryopsis).

Table 2. ANOVA for biomass accumulation in *T. aestivum* seedlings as influenced by the extract of various plant (or litter) components of *G. officinale*.

Source	SS	df	MS	F	p
Extract Concentration	0.02033	3	0.006776	24.28	0.001
Plant component	0.01362	5	0.002724	9.828	0.001
Interaction (Ext. Conc. X Plant component	0.011529	15	0.0007686	2.773179	0.039
Error	0.0133037	48	0.00027716	-	-
Total	0.0587803	72	-	-	-

DMRT and LSD calculation

Extract concentration			Plant (or litter) component		
Rank	Treatment	Mean	Rank	Treatment	Mean
1	Control	0.07721 a	*1*	Bark	0.07453 a
2	25%S	0.05679 b	*2*	Green fruit	0.06741 a
3	50%S	0.04374 c	3	Green leaves	0.05058 b
4	100%S	0.03192 d	4	Pericarp of abscised fruits	0.04195 b
$LSD_{0.05}$ = 0.01115758 N (each treatment) =18			5	Abscised flowers	0.04004 b
			6	Abscised leaves (yellow)	0.03996 b
			$LSD_{0.05}$ = 0.013666 N (each treatment) = 12		

DISCUSSION

The examination of the influence of the aqueous extracts of various plant / litter components of *G. Officinale* on germination of wheat caryopses indicated that extract of pericarp of dehisced fruits was highly inhibitory to the process of germination. The suppression of germination was in the order - Pericarp of dehisced fruits > abscised flowers > Yellow leaves > Green leaves > Green fruits > Bark. The aqueous extracts of many species are known to inhibit seed germination (Naqvi and Muller, 1975; Shaukat *et al*., 1983, 1985, 2003 a and b; Prati and Bossdorf, 2004, Khan and Shaukat, 2006 a and b). The inhibitory effect on germination of wheat by the extracts of *Guaicum* may presumably be due to the presence of phenolic compounds in them. Phenolic compounds are reported to occur widely in plants and inhibit germination of seeds (Evenari, 1961; Rice, 1974; Lodhi, 1979; Shaukat *et al*., 2003 a and b; Chon and Boo, 2005). Besides phenolic compounds, the extracts, owing to their copious persistent froth forming nature especially those of abscised yellow leaves, pericarp of dehisced fruits, floral parts (petals) and green fruits, may also be suspected to contain saponin (s) in varying amount or number. Saponins have considerable impact in agriculture because of their growth inhibiting properties (Khan and Shaukat, 2006 a and b). They are highly hydrophilic in nature, may leach out easily and interfere with the permeability of seed coat (Mircham *et. al.*, 1975) and thus the depleted oxygen availability causes inhibition of germination and lag in vegetative growth (Mirchaim *et al*. 1974, 1975). They affect the growth of soil microorganisms, especially fungi (Fons *et al*., 2003). Saponins thus modify the soil characteristics. The extracts of abscised floral parts, yellow leaves, pericarp and green leaves significantly inhibited the seedling growth of wheat. In all cases, the degree of inhibition was directly related to the extracts' concentration. The instances of inhibitory effects of one plant on the other are numerous (Karachi and Pieper, 1987; Gilani *et. al,* 2002; Morgan and Overholt, 2005; Khan and Shaukat., 2006 a and b, 2007). It appears that phytotoxic principles are, presumably, larger in amount or greater in number in these active plant parts. Bark and green fruits, on the other hand, have, presumably, no inhibitors in them or in very low concentration. The stimulation of plumular growth by bark may probably be due to stimulatory action of inhibitors in low concentrations. Such a non-linear response (hormesis) in allelopathic dose-response data is fairly common (An *et. al.,* 2005).

From the foregoing discussion *G. officinale* appears to be a fairly phytotoxic plant and contain highly water-soluble phytotoxins, which may leach out in moist conditions and enter soil. The effects of aqueous extracts of *Guaicum* on germination, growth and development of wheat seedlings indicate to its possible allelopathic potential. It is known that bioactive concentrations of allelochemicals are determined through their sorption, fixation, leaching, and chemical and microbial degradation (Blum, 1999; Inderjit *et; al.,* 1999). Chemical, physical and biological characteristics of the soil are to a great extent the determiner of detoxification or further enhancement of allelopathic activities of plant leachates (Cheng, 1995; Schmidt and Ley, 1999). It is, however, imperative to undertake further research and perform bioassays in the presence of soil to demonstrate allelopathy in its ecological relevance. Further work on allelopathic potential of this plant i.e., characterization of its phytotoxins, their accumulation and degradation in soil, and their activity against the associate species is underway.

REFERENCES

An, M., J.E. Pratley, T. Haig and D.L. Liu (2005). Whole-range assessment: A simple method for analyzing allelopathic dose-response data. Nonlinearity in Biology. *Toxicology & Medicine,* 3: 245 – 260.

Blum, U. (1999). Designing laboratory plant-debris soil bioassays: some reflections. In: *Principles Practices of Plant Ecology* (Inderjit, K.M.M. Dakshini and C.L. Fay, eds.) CRC Press, Boca Raton, Florida. Pp. 17 – 23.

Cheng, H.H. (1995). Characterization of the mechanism of allelopathy: modeling and experimental approaches. In: *Allelopathy: Organisms, Processes and Applications*. (Inderjit, K.M.M. Dakshini and F.A. Einhellig, eds.). Am. Chemical Society, Washington,pp. 132 – 141.

Chon, S.-U. and H.-O. Boo (2005). Difference in allelopathic potential as influenced by root periderm colour of sweet potato (*Ipomoea batatas)*. *J. Agron & crop Sci*., 191 (1): 75 – 80.

Evenari, M. (1961). Chemical influences of plants (Allelopathy). In: *Hand Buch der Pflanzen Physiologie*. Ed. W. Ruhland. Springer-Verlag.

Fons, F., N. Amella, C. Leyval, N. saint-Martin and M. Henry (2003). Effects of *Gypsophila* saponins on bacterial growth kinetics and on selection of subterranean clover rhizosphere bacteria. *Can. J. Microbiol.* 49(6): 367-373.

Ghafoor, A. (1974). *Zygophyllaceae*. Flora of West Pakistan (E. Nasir & S.I. Ali, eds.). No. 76.

Gillani, S.S., S.M. Chughtai, M.A. Khan, and I.K. Wazir (2002). Study of the allelopathic potential of *Eucalyptus microtheca* F. Muell. *Hamdard Medicus*, XLV (2): 25 – 30.

Inderjit, H.H. Cheng abd H. Nishimura (1999). Plant phenolics and terpenoids transformation, degradation and potential for allelopathic interactions. In: *Principles and Practices of Palnt Ecology*. (Inderjit, K.M.M. Dakshini and C.L. Foy; eds.), CRC Press, Boca Ratton, Florida, pp. 255 – 266.

Karachi, M. and R.D. Pieper (1987). Allelopathic effects of *Kochia* on blue grama. *J. Range Management*. 40 (4): 380 – 381.

Khan, D. and S.S. Shaukat (2006a). Phytotoxic potential of *Achyranthes aspera* L. – A tropical medicinal weed of Pakistan. *Intern .J. Biol & Biotech*., 3 (1): 57 –71.

Khan, D. and S.S. Shaukat (2006b). Phytotoxic effects of *Prosopis juliflora* Swartz. D.C. against some of its field associates and the cultivated species. *Intern. J. Biol & Biotech.,* 3 (2): 353 –366.

Khan, D. and S.S. Shauakt (2007). Preliminary obserevations on auto- and reciprocal toxicities in *Achyranthes aspera* L and *Peristrophe bicalyculata* (Retz.) Nees. *Intern J. Biol. & Biotech.,* 4 (1): 65 – 70.

Lodhi, M.A.K. (1979). Germination and decreased growth of Kochia scoparia in relation to its autoallelopathy. *Can. J. Bot*., 57: 1083 – 1088.

Mirchaim, U., Y. Birk, A. Dovat and T. Berman (1972). Lucerne saponins as inhibitors of cotton seed germination: their effect on diffusion of oxygen through the seed coat. *J. Exp. Bot.,* 23: 302 – 309.

Mirchaim, U., E,. werker and W.D.E. Thomas (1974). Changes in the anatomy of cotton seeds coats caused by Lucerne saponins. *Bot. Gaz.,* 135: 139 – 146.

Mirchaim, U., Y. Birk, A. Dovat and T. Berman (1975). Kinetics of the inhibition of cotton seeds germination by Lucerne saponins. *Plant & Cell Physiol*., 16: 857 – 864.

Morgan, E.C. and W.A. Overholt (2005). Potential allelopathic effects of Brazilean pepper (Schinus terebinthifolius Raddi., Anacardiaceae) aqueous extract on germination and growth of selected Florida native plants. *J. Torrey Bot. Soc*., 132 (1): 11 – 15.

Naqvi, H.H. and C.H. Muller (1975). Biochemical inhibition (allelopathy) exhibited by Italian ryegrass (*Lolium multiflorum* L.). *Pak. J. Bot.,* 7: 139 – 147.

Prati, D. and O. Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *Am. J. Bot.* 91: 285 – 288.

Rice, E.L. (1974). *Allelopathy*. Academic Press, London.

Shaukat, S.S., G. perveen, D. Khan and M. Ahmad (1985). Phytotoxic effects of *Citrullus colocynthis* (L.) Schrad on certain crop plants. *Pak. J. Bot*., 17(2): 235 – 246.

Shaukat, S.S, D. Khan and S.T. Ali (1993). Suppression of herbs by *Inula grantioides* Boiss. In the Sind desert, Pakistan. *Pak. J. Bot*., 15(1): 43-67.

Shaukat, S.S., N. Munir, ands I.A. Siddiqui (2003a). Allelopathic responses of *Conyza Canadensis* (L.) Cronquist: A cosmopolitan weed. *Asian J. Pt. Sci*., 2 (14): 1034 – 1039.

Shaukat, S.S., Z. Tajuddin, and I.A. Siddiqui (2003b). Allelopathic potential of *Launaea procumbens* (Roxb.) Rammaya & Rajgopal: a tropical weed. *Pak. J. Biol. Sci.,* 6(3): 225-230.

Schmidt, S. K. and R. Ley (1999). Microbial competition and soil structure limit expression of allelochemicals in nature. In: *Principles and Practices in Plant Ecology : Allelochemical Interactions*. (Inderjit, K.M.M. Dakshini and C.L. Foy, eds.). CRC Press, Boca Raton, Florida, pp. 339 – 351.

Taylor, I. (1942). Influence of oxygen tension on respiration, fermentation and cadmium in wheat and rice. *Am. J. Bot.,* 29: 721 – 738.

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