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PHYTOTOXIC EFFECTS OF *PROSOPIS JULIFLORA* SWARTZ. DC. AGAINST SOME OF ITS FIELD ASSOCIATES AND THE CULTIVATED SPECIES

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ABSTRACT

Bioassays with *Prosopis juliflora (*Swartz) DC. demonstrated that aqueous root, shoot and fruit extracts against *Lactuca sativa*, a cultivated species and two field associates viz. *Chloris barbata* and *Cassia holosericea* impeded or reduced germination of test species. The fruit extract was more inhibitory than shoot or root extract. The suppression of germination and seedling growth was in the order: *Cassia holosericea* < *C. barbata* < *L. sativa.* Reduction in seedling growth was species specific. In general, inhibition was a function of the extract concentration. Decaying *P. juliflora* shoot was relatively more toxic to germination and seedling growth of *T. aestivum*. Artificial rain-drip arrested lettuce seedling growth at higher concentration only. Root and shoot extracts were autotoxic to *P. juliflora* seedling growth at higher concentrations only. Fruit extract was, however, more autotoxic to germination and seedling growth. Phytotoxic principles of *P. juliflora* were thermostable or thermo-convertible to secondary inhibitors. The physico-chemical nature of soil and the soil microorganisms provided limited protection to lettuce seedling growth against toxicity of the extracts. Coleoptile bioassay of ether fraction of aqueous extracts indicated some phenolic inhibitors in the extracts. Radicle growth bioassay of *Brassica compestris* with crude saponins, extracted by precipitation method using lead acetate, indicated some active saponins /glycosides in the extracts. The toxic effects of *P. juliflora* extracts further facilitated explanation to the simplicity of sociological organization in *P. juliflora* dominated stands.

Key Words: *Prosopis juliflora*, phytotoxicity, biochemical assays for phenolics & saponins / glycosides.

INTRODUCTION

Allelopathy has attracted the attention of ecologists in interpreting community structure and distribution pattern of several plant populations (Einhellig, 1995; Hegazy, 1999; El-Khatib, 1998, 2000; El-Khatib *et al*. 2004; Chen *et al*., 2005; Khan and Shaukat, 2006). This phenomenon is characterized with reduction in emergence or growth of some target species in the community and rendering it simple in structure and organization with greater degree of monopolization by the dominating species.

Prosopis juliflora (Swartz.) DC, an invasive species, is highly predominating in marine tropical coastlands of Pakistan - forming highly dense thickets of growth (Khan, 1980, 1987). The communities dominated by *P. juliflora* are simple in composition, structure and organization (low diversity and high dominance situation) and exhibit geometric pattern of abundance among species - stressful situation characterized with low number of associated species. Moreover, the conspicuousness or importance of *P. juliflora* in its stands is reported to relate negatively with the number of harboring species – a trend to lead to dense and essentially monospecific growth of *P. juliflora* with time in its invaded areas. This species has also been suspected to be allelopathic. Noor *et al.* (1995) reported phytotoxicity of *P. juliflora* against some cultivated species such as *Zea mays*, *Triticum aestivum*, and *Albizia lebbeck.* Al-Humaid and Warrag (1998) have reported phytotoxic effects of *P. juliflora* foliage to seed germination and seedling growth of *Cynodon dactylon* (Bermuda grass) – also a cultivated species. Sen and Chawan (1970) have reported suppression of radicle growth of *Euphorbia caducifolia*, a naturally growing species in arid areas of Rajasthan [also Sindh and Las Bela, Balochsitan]. Since most of the phytotoxic studies of *P. juliflora* have been carried out against cultivated species, the present studies focused on investigating its phytotoxicity against itself and two of its neighbouring species viz. *Chloris barbata* and *Cassia holosericea*, by using its aqueous extracts with the hypothesis that water-soluble principles of *P. juliflora* may be released from the plant tissue and may exert phytotoxic effects on the neighboring species. Such information should further elucidate ecology of this highly invasive species ranked as pest and even as devil tree in some parts of the world.

MATERIALS AND METHODS

The vigorously growing plants of *P. juliflora*, around 1m in height, were collected from its population in Karachi University Campus and dried at room temperature in shade and used for extract preparation. The seeds of species tested for auto-toxicity were also collected from the same locality. Fruits were collected from the mature trees.

Phytotoxicity of aqueous extracts of *P. juliflora* against a cultivated species and two of its field associates: Aqueous extracts of shoot, root and fruit materials of *P. juliflora* were prepared by soaking 10 g dry material in 200 ml distilled water for 24 h. The filtrates were taken as stock from which dilutions (25, 50, and 75%) were prepared. The toxicity of these extracts was tested against *Lactuca sativa* cv. Grand Rapids and its common herbaceous field associates - *Chloris barbata,* and *Cassia holosericea.*

Twenty surface sterilized (2% sodium hypochlorite for 5 min.) seeds of a test species were placed on Whatman No. 1 filter paper in 9 cm diameter sterile petriplates and 5 ml of shoot, root or fruit extract was added. Controls received glass-distilled water. The seeds of *C. holosericea* were mechanically scarified with the help of glass paper No. 1.5 before placing them in petri plates (Khan *et al*., 1984). Treatments and controls were replicated thrice and the petriplates were kept under 14 h illumination of 4000 Lux. Germination counts were made daily and length of roots and shoots were recorded at 96 h of growth.

Phytotoxicity of decaying *P. juliflora*: The shoot and root of *P. juliflora* were crushed separately and mixed thoroughly with sandy loam (76.1% sand, 15.3% silt and 17.6 % clay) at the rate of 5, 10, or 20 g root or shoot material per 400 g soil. These were placed in 8 cm diameter plastic pots, sprinkled with some water and kept for one week to allow microbial activity. Control pots contained the sandy loam soil only. Subsequently 10 seeds of *T. aestivum* were sown in each pot. Each treatment and control was replicated three times. The pots were kept at 25 ± 2 ^{o}C. The light intensity at the top of pots was 4000 Lux for 14 h day-length. The Emergence counts were made daily and root and shoot lengths of seedlings were measured at 10th day and dried at 80 ^{o}C for dry weight measurement.

Auto-toxicity: The response of *P. juliflora* was tested against the root, shoot and fruit extracts of its own for any possible auto-toxicity. To accelerate germination the seeds were slightly abraded (Khan *et al.*, 1984) before placing them on filter paper moistened with the extract

Artificial rain-drip and leaching of phytotoxins: The technique employed for this experiment was essentially the same as described by Naqvi and Muller (1975). Air-dried leaves of *P. juliflora* were chopped into small fragments and placed in a large funnel attached to a conical flask. Five Hundred milliliter of deionized distilled water was sprayed on the plant material and the leachate was collected. The spraying was done slowly and gradually and lasted for 1 h. The leachate (or the artificial rain-drip) was filtered and a portion of it was reduced to one-fourth in a rotary vacuum evaporator. The phytotoxicity of the leachate was then assayed using *Lactuca sativa* cv. Grand Rapids in petriplates as given above.

Exudation of phytotoxins from germinating seeds: Surface sterilized slightly abraded seeds of *P. juliflora* were kept on moist Whatman No. 1 filter paper and incubated at 26 ± 1 ^{o}C for 24 h. The seeds were removed now and 20 seeds of *Lactuca sativa* v. Grand Rapids or *C. barbata* were sown on the same filter paper. Plates were kept at 26 ± 1 ^{o}C under light intensity of 4000 Lux at the top of plates for 14 h day length. Controls received distilled water only.

Activity of soil and soil-micro-organisms against phytotoxins: To check the activity of soil or soil microbes against the *P. juliflora* phytotoxins, the method of Quarterman (1973) was employed. Soil collected from Karachi University Campus was passed through 2 mm screen and was used as germination substrate; a portion of the soil was autoclaved at 120 ^{o}C for 2 h. Two grams of non-autoclaved or autoclaved soil was placed in sterilized plate and covered with a layer of Whatman No. 1 filter paper. A portion of stock aqueous extracts was autoclaved at 120 ^{o}C for 1h. Germination substrate made of either non-autoclaved or autoclaved soil and filter paper layering was moistened with 15 ml of either non-autoclaved or autoclaved aqueous extract of root or shoot. In one treatment germination substrate was filter paper only. Controls received distilled water only. Surface sterilized seeds of *L. sativa* v. Grand Rapids were sown at the rate of 20 seeds per plate and incubated at 25 ^{o}C under light of 4000 Lux for 14 h day length. Germination counts and root and shoot measurement were made after 96 h of growth.

Partial characterization of phytotoxins:

Wheat coleoptile bioassay: Ten g air-dried material of root, shoot or fruits of *P. juliflora* was crushed in 200 ml distilled water, homogenate centrifuged and adjusted to 3 with 0.5N H_2SO_4. The homogenate was then extracted three times with per-oxidase free ether and pooled ether fraction was evaporated to dryness over $CaCl_2$ in a desiccator. To the dry material, 2 ml of absolute ethanol was added and streaked on Whatman no.1 filter paper.

Duplicate 10 cm wide chromatograms were developed by descending chromatography in iso-propanol: Ammonia: Water (10:1:1, v/v/v). When solvent had moved 30 cm from the origin, the chromatograms were taken out, dried and 10 equal width strips were cut and assayed for growth regulators using wheat coleoptile straight growth test (Nitsch and Nitsch, 1956). After discarding the upper3 mm coleoptile segments, 5 mm segments of 3-day old dark grown wheat (*T. aestivum* cv Z76) were excised and floated in distilled water for 1 h. Ten coleoptile segments were placed between two strips, of the same Rf-value cut from the duplicate chromatograms and kept in 11.5 cm diameter petriplates over two layers of tissue paper moistened with 4 ml of 0.02M citrate-phosphate buffer (pH 4.8). After 48 h of growth in dark at 22 ± 2 ^{o}C, the length of the coleoptile segments was measured.

Extraction and biochemical activity of saponins: Saponins from **t**he aqueous extracts of *P. juliflora* were extracted by precipitation method (Wasi ur Rahman, 1957). Aqueous extracts were treated with 10% lead acetate to precipitate neutral saponins. The brown precipitate so obtained was dissolved in absolute ethanol. The solution contained lead salt of saponin (s) through which H_2S gas was passed to remove lead in form of lead sulphide and to liberate the saponins into ethanol. The solution was then warmed to remove H_2S and was filtered. Residue was discarded and filtrate was collected which contained neutral saponins in ethanol.

The filtrate obtained after treatment of extracts with 10% lead acetate, was basified by adding liquid ammonia to precipitate acidic saponins. The yellow precipitate obtained was poured into absolute ethanol and H_2S gas was passed through this solution, warmed and filtered. The filtrate contained acidic saponins. The two samples of saponins were pooled and evaporated to dryness over $CaCl_2$ in a desiccator. Crude saponins were dissolved in 2 ml absolute ethanol to streak on Whatman No. 1 filter paper. Saponins are polar compounds and are easily separated by paper chromatography (Harborne, 1973). Duplicate 10 cm wide chromatograms were developed by descending chromatography in Chloroform: acetone (4:1, v/v). When solvent had moved 30 cm from the origin, the chromatograms were removed, dried and cut into 10 equal width strips and assayed for their biochemical activity using *Brassica compestris* seeds. Ten surface sterilized seeds were placed between the two strips of the same Rf value, cut from the duplicate chromatograms and kept in 11.5 cm diameter plates over two layers of tissue paper moistened with distilled water (4 ml). The plates were kept in light of intensity of 4000 Lux and temperature around 26-28 ^{o}C. After 48 h of incubation, germination counts and radicle-length measurements were made.

The presence of alkaloids in methanolic extract of *P. juliflora* was tested by Dragondorff reagent.

RESULTS

Phytotoxicity of aqueous Extracts: Germination of *L. sativa* was drastically reduced by shoot as well as root extract of *P. juliflora*. Inhibitory effect increased with the increase of extract concentration (**Fig.1**). Fruit extract was the inhibitoriest, which inhibited lettuce seed germination completely at 50 (%S) concentration. Compared to lettuce, seed germination of *C. barbata* and *C. holosericea* was relatively less susceptible process to aqueous extracts of *P. juliflora*. In both of the species, however, fruit extract was more detrimental than shoot or root extract (**Fig. 2 and 3**).

Both root as well as shoot growth of lettuce seedlings progressively declined with increasing concentration of root, shoot or fruit extract - root elongation more sharply reduced than shoot's (**Fig.4**). Seedling growth was completely inhibited at stock concentration of shoot and root extracts. Fruit extract was highly toxic to seedling growth. Lettuce seedlings couldn't grow in fruit extract beyond 25 (%S) concentration. Shoot growth of *C. barbata* was significantly stimulated ($p < 0.05$) at 25 (% S) concentration of shoot extract and at 25 (%S) and 50 (%S) concentrations of fruit extract (**Fig. 5**). In higher concentrations of the extracts seedling growth, however, declined regularly – root growth being more reduced than shoot's and fruit extract being more toxic than others. Shoot and root growth of *C. holosericea* remained unaffected by the presence of the shoot extract of *Prosopis* (**Fig. 6**). The seedling growth parameters were significantly suppressed ($p < 0.05$) at 50 (% S) and 100 (%S) concentrations of root extract. Compared to root growth, shoot growth was more depressed in root and fruit extract. As usual fruit extract was more suppressive to seedling growth.

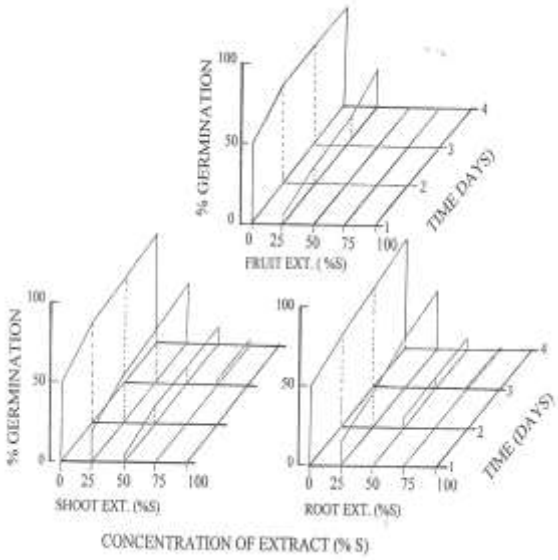

Fig.1. Effects of aqueous extracts of *Prosopis juliflora* on germination of *Lactuca sativa* cv. Grands Rapids.

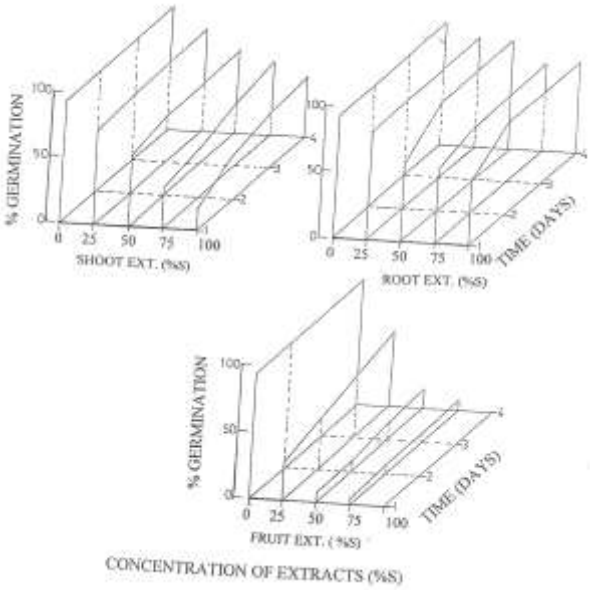

Fig.2. Effects of aqueous extracst of *P. juliflora* on Germination of *Chloris barbata*.

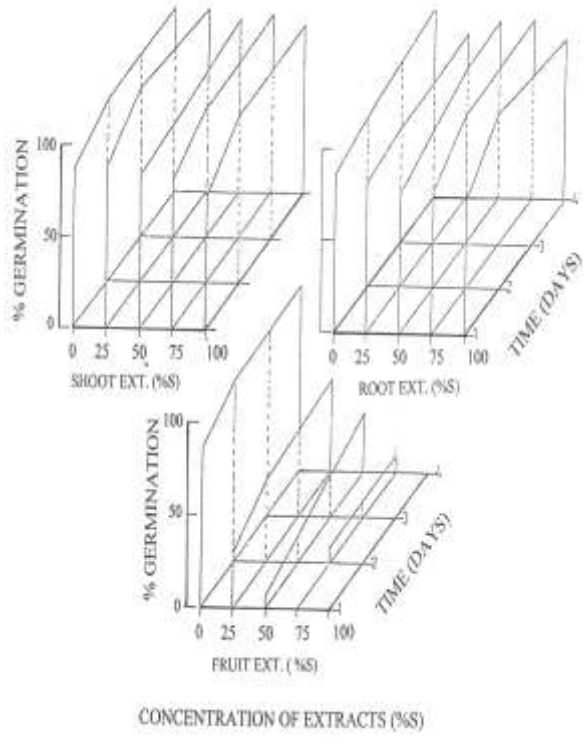

Fig.3. Effects of aqueous extracts of *Prosopis juliflora* on germination *Cassia holosericea*.

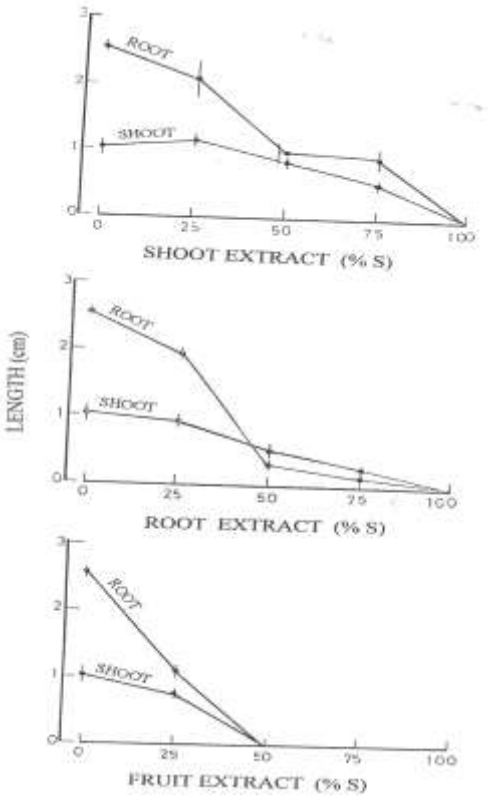

Fig.4. Effects of aqueous extracts of *Prosopis juliflora* on seedling growth of *Lactuca sativa* cv. Grand Rapids.

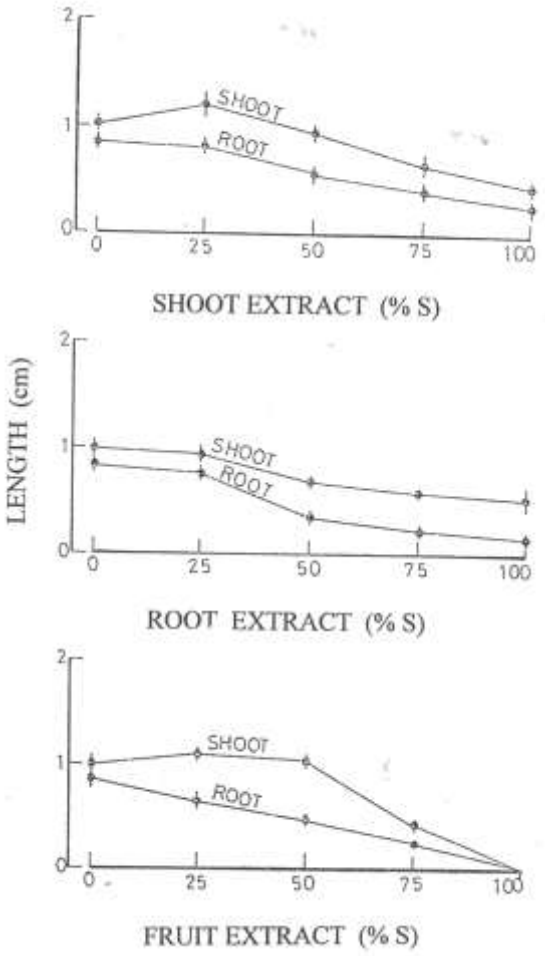

Fig.5. Effects of aqueous extracts of *Prosopis juliflora* on seedling Growth of *Cloris barbata*.

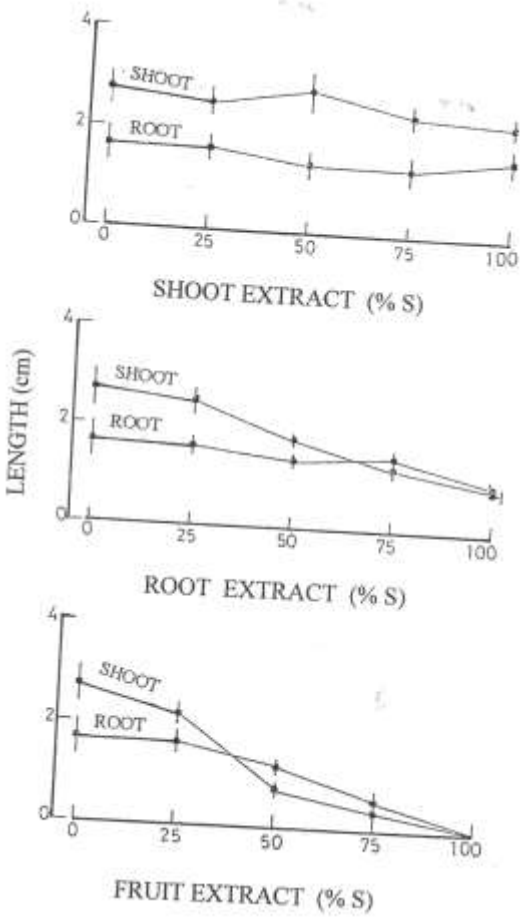

Fig.5. Effects of aqueous extracts of *Prosopis juliflora* on Seedling growth of *Cassia holosericea*.

Phytotoxicity of decaying *P. juliflora*: The cumulative percentage and rate of germination of wheat were significantly reduced ($P < 0.01$) in soil containing the decaying shoot of *Prosopis*. Germination inhibition increased with the increase in amount of decaying shoot material (Fig 7). Decaying root material didn't affect the final germination percentage, though rate of germination was impeded. Wheat's root and shoot lengths and their dry weights were generally stimulated ($p < 0.05$) in soils incorporated with 5 g decaying Prosopis material, however, substantial reduction in these parameters took place in soils incorporated with increased amount of shoot or root material – 20 g shoot or root / 400 g soil (Fig. 8).

Autotoxicity: Final germination of *P. juliflora* under influence of its own shoot and root extracts was not altered at any concentration of the extracts. The rate of germination was slightly impeded at higher concentration of root extract and more so in 100 (%S) concentration. A significant decrease ($p < 0.01$) in germination took place at 75 (%S) of the fruit extract (Fig. 9). Root growth of *Prosopis* was significantly stimulated ($p< 0.05$) at 25 (%S) concentration of root and shoot extracts but no such stimulation was observed in shoot growth (Fig. 10). The root and shoot lengths were significantly suppressed ($p < 0.05$) only at 75 (%S) of shoot extract and at 100 (%%S) of root extract ($p < 0.01$). A slight enhancement of shoot growth appeared ($P < 0.05$) at 50 (%S) concentration of fruit extract but the root growth was drastically suppressed ($p < 0.01$) at this concentration (Fig. 10). Shoot growth remained unaffected with fruit extract.

Artificial rain-drip and leaching of phytotoxins: The original 1X or 4X concentration of the leachate collected from *P. juliflora* did not significantly alter the seed germinability and seedling growth of *L. sativa*. Only 4X concentration of leachate significantly suppressed ($p < 0.01$) shoot and root growth of lettuce (Table 1).

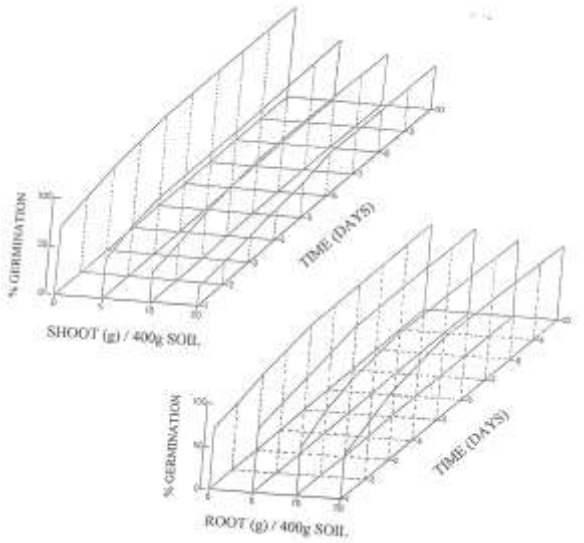

Fig.7. Germination of *Tricum aestivum* cv. Z.76 in soils incorporated With decaying shoot or root materials of *P. juliflora*.

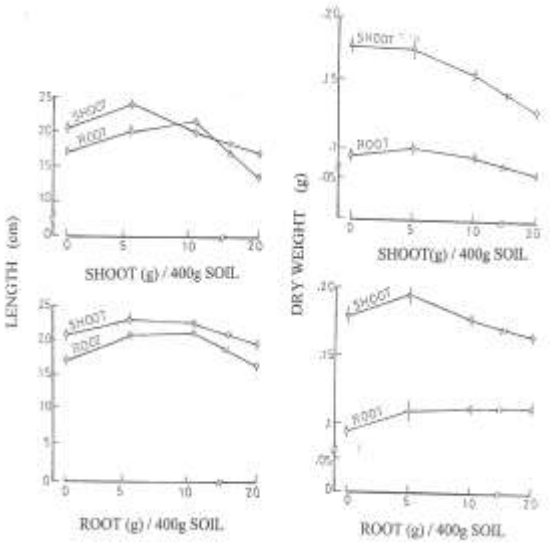

Fig.8. Seedling growth of *Triticum aestivum* cv.Z.76 in soils Incorporated with decaying shoot root materials of *P. juliflora.*

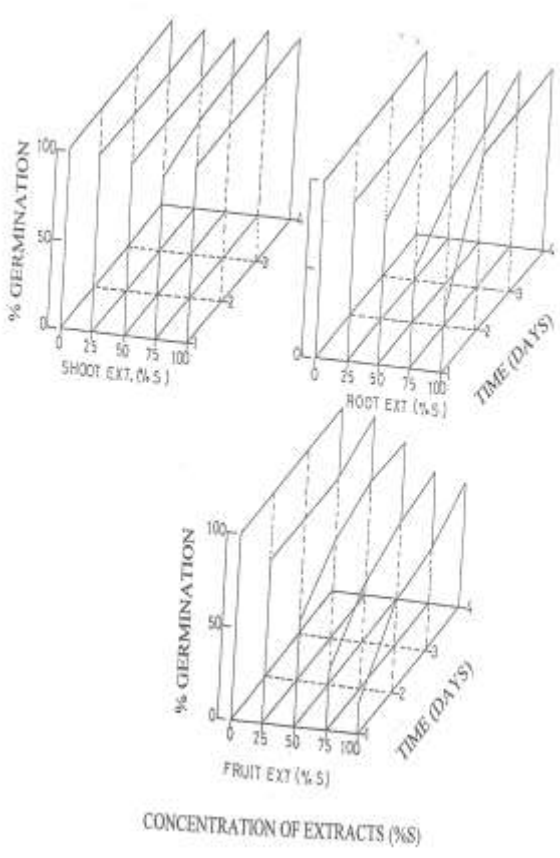

Fig.9. Germination behaviour of *P. juliflora* seeds under influence of its own aqueous shoot, root and fruit extract.

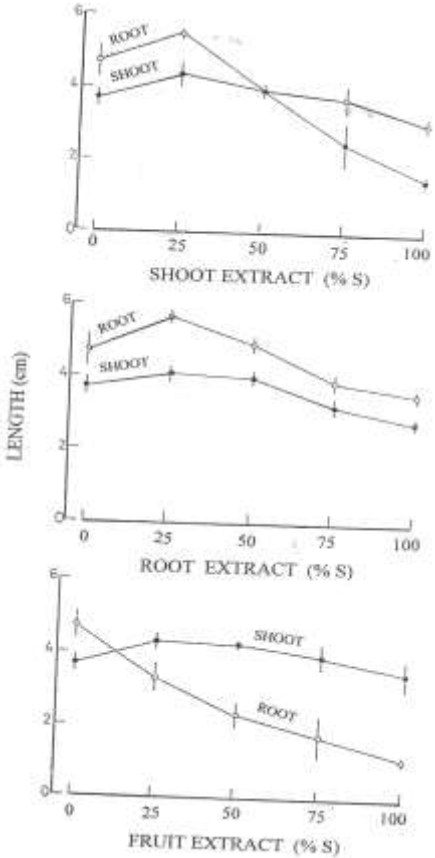

Fig.10. Seedling growth of *P. juliflora* under influemce of its own aqueous extracts.

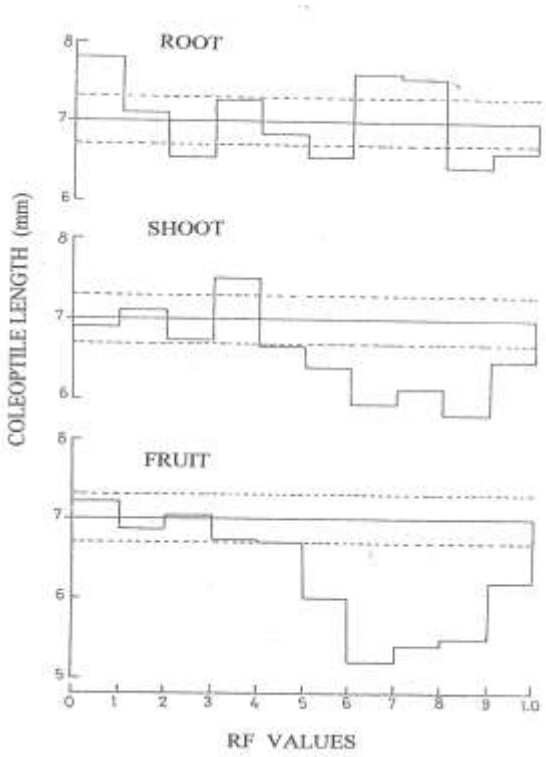

Fig.11. Histograms of ether fraction of extracts of *P. juliflora* Chromatographed on Whatman filter paper and developed in Solvent, iso-prppanol:Ammonia:Water (10:1:1, v/v/v). Dotted line represents 95% confidence interval.

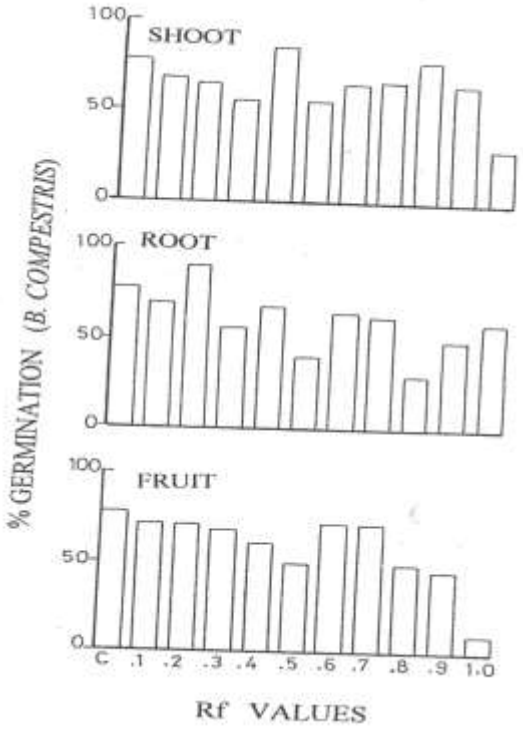

Fig.12. Germination behaviour of *Brassica* compestris at Rf values of chromatogram of crude saponins / glycosides as extacted from *P. juliflora* root, shoot and fruit by precipitatiobn method with lead acetate and run on whatman filter paper in solvent chloroform:Aceton (4:1, v/v).

Table 1. Effects of artificial rain drip from *P. juliflora* on germination and early seedling growth of *Lactuca sativa* var. Grand Rapids.

Leachate Concentration	Germination (%)	Shoot Length (cm)	Root Length (cm)
Control	100 ± 0	1.75 ± 0.03	5.62 ± 0.07
I x	100 ± 0 ns	1.93± 0010 ns	5.32 ± 0.02 ns
4 x	96.6 ± 6.66 ns	1.23 ± 0.09 **	5.05 ± 0.04 **

*, $p < 0.05$; **, $p < 0.01$, ns, non-significant as given by t-test.

Table 2. Effects of *P. juliflora* seed exudates on early seedling growth of *Lactuca sativa* var. Grand Rapids and *C. barbata*.

Test Species	Treatment	Shoot Length (cm)	Radicle Length (cm)
L. sativa	Control	1.75 ± 0.033	5.53 ± 0.079
	Test	1.33 ± 0.036 ***	2.82 ± 0.162 ***
C. barbata	Control	0.52 ± 0.054	1.34 ± 0.051
	Test	0.63 ± 0.065 ns	0.14 ± 0.013 ***

***, $p < 0.001$ as given by t-test.

Exudation of phytotoxins from germinating seeds: Exudates of germinating seeds of *P. juliflora* significantly inhibited both root ($p < 0.001$) and shoot growth ($p < 0.001$) of lettuce. The exudates could, however, significantly reduce radicle growth only in case of *C. barbata* (Table 2).

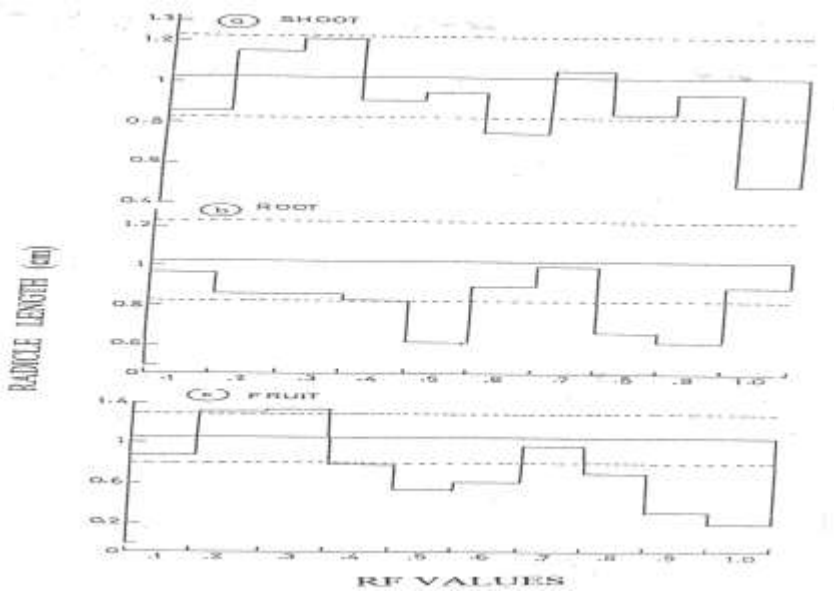

Fig.13. Histograms of radicle length of *Brassica conpestris* at Rf values of chromatogram of crude saponins/ glycosides extracted from *P. Juliflora* root, shoot and fruit by precipitation method with lead acetate and run on whatman filter paper in solvent chloroform: Acetone (4L1, v/v). Dotted line represents 95% confidence interval.

Table 3. Effects of soil and soil microorganisms on inhibition of germination and seedling growth of *L. sativa* var. Grand Rapids by *P. juliflora* extracts. Each datum is a mean of three replicates (20 seeds / plate).

Treatment	Germination (%)	Shoot Length (cm)	Root Length (cm)	Germination (%)	Shoot Length (cm)	Root Length (cm)
US + H2O + Filter paper (No Extract)	100 ± 0 a	2.41 ± 0.06 a	4.92 ± 0.11 a	96.66 ± 3.33 a	2.40 ± 0.06 a	4.92 ± 0.11 a
SS + H2O + Filter paper (No Extract)	100 ± 0 a	2.42 ± 0.01 a	5.06 ± 0.13 a	100.0 ± 0 a	2.42 ± 0.06 a	5.06 ± 0.13 a
US Ext. + Filter paper	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
S Ext. + Filter paper	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
US + US Ext. + Filter paper	40.66 ± 6.66 c	2.30 ± 0.21 a	3.01 ± 0.42 c	36.33 ± 3.33 c	2.05 ± 0.13 c	3.86 ± 0.25 c
US + S Ext. + Filter paper	35.00 ± 10.57 c	1.09 ± 0.03 c	3.00 ± 0.58 c	26.66 ± 6.66 c	1.13 ± 0.07 d	1.29 ± 0.10 d
S S+ US Ext. + Filter paper	36.33 ± 3.33 c	1.31 ± 0.05 d	2.03 ± 0.05 c	33.33 ± 3.33 c	1.25 ± 0.09 d	1.38 ± 0.08 d
SS + S Ext. + Filter paper	26.66 ± 6.66 c	0.92 ± 0.08 e	1.20 ± 0.03 d	23.66 ± 3.33 c	0.82 ± 0.03 e	1.00 ± 0.08 e

US = Unsterilized soil; SS= sterilized soil; US Ext.= Unsterilized Extract; S Ext. = Sterilized Extract. Figures not sharing the same letter within a single character are significantly different at least at $p < 0.05$ as given by t-test.

Activity of soil and soil microorganisms against phytotoxins: The root and shoot extracts either sterilized or unsterilized inhibited lettuce seed germination completely. Significant promotion in seed germination took place under influence of these extracts when they were supplemented with sterilized or unstetrilized soils. Seedling growth was significantly low in the unstrerilized root extract supplemented with unsterilized soil; however, shoot growth under this treatment was not statistically different from the control (Table 3). Moreover, root and shoot growth of lettuce were significantly higher in unsterilized root and shoot extracts of *Prosopis* supplemented with unsterilized soil compared to their growth in sterilized soil. Shoot extract was found to be more toxic. Seedling growth was significantly enhanced in sterilized root and shoot extracts supplemented with sterilized soils as compared to their growth in sterilized root and shoot extracts supplemented with filter paper only. The results indicated that soil and its micro flora provided some protection to lettuce growth against toxicity of root and shoot extracts of *P. juliflora* and the phytotoxins present in the extracts were thermostable or thermo-convertible to other phytotoxins.

Partial characterization of phytotoxins:

i. Wheat Coleoptile Bioassay: The bioassay of ether fraction of root extract disclosed significant amount of growth inhibitors at Rf values 0.2 – 0.3, 0.5 – 0.6, 0.8 – 0.9 and 0.9 – 1.0 The assay also showed promotion at Rf value 0.0 – 0.1, 0.3 – 0.4, 0.6 – 0.7 and 0.7- 0.8. The shoot extract showed inhibitors at Rf values 0.2 -.3, 0.5 – 0.6, 0.6 – 0.7, 0.7 –0.8, 0.8- 0.9 and 0.9 – 1.0 and promontory effect at 0.3 – 0.4. The fruit exhibited inhibition at Rf 0.5 – 0.6, 0.6 – 0.7, 0.7 – 0.8, 0.8 – 0.9 and 0.9 – 1.0 (Fig. 11).

ii. Germination and radicle growth bioassay of *Brassica compestris*: The crude saponins extracted by precipitation method using lead acetate were tested for their biochemical activity against *B. compestris* (Fig. 12). On the chromatogram of the shoot extract of *P. juliflora* the germination of *Brassica* was reduced significantly at Rf values 0.2-0.3, 0.4 – 0.5 and 0.9 – 1.0 by a quantum of 37.5, 37.5, and 62.5%, respectively (Fig. 12). In the root extract the germination was inhibited at Rf values 0.2 – 0.3, 0.4 – 0.5 and 0.7 – 0.8 by 33.3, 50, and 62.5 %, respectively. The glycosides of the fruit extract reduced the germination at Rf values 0.4 – 0.5, 0.7 – 0.8, 0.8 – 0.9 and 0.9 – 1.0 by 37.5, 37.5, 42.25, and 87.5%, respectively. Inhibition of radicle growth of B. compestris due to saponins (or related glycosides) was exhibited at Rf values 0.5 – 0.6 and 0.9 – 1.0 in shoot, at Rf values 0.4 – 0.5, 0.7 – 0.8 and 0.8 – 0.9 in root and at Rf values 0.4 – 0.5, 0.5 – 0.6, 0.8 – 0.9, and 0.9 – 1.0 in fruit extract (Fig. 13).

DISCUSSION

The examination of the influence of the aqueous extracts of root, shoot and fruit of *P. juliflora* on germination of some associated herbs in field and cultivated species disclosed that the fruit extract was more inhibitory to germination in comparison to the root and shoot extracts. The suppression of germination in either extracts was in the order: *C. holosericea* < *C. barbata* < *L. sativa*. The aqueous extracts of many species are known to inhibit seed germination (Quarterman, 1973; Datta and Sinha-Roy, 1975; Naqvi and Muller, 1975; Mubarak and Hussain, 1978, Burhan and Shaukat, 1999; Padhy *et al*., 2000; Rabaz *et al*., 2001; Shaukat *et al*., 1983, 1985, 2003 a and b; Tajuddin *et al*., 2002; Prati and Bossdorf, 2004). The inhibitory effect of extracts on germination was presumably due to the presence of phenolic and some other water-soluble compounds in the extracts. Phenolic compounds in the extract were demonstrated in the bioassay of ether fraction of aqueous extracts where as the presence of saponins was demonstrated by radicle growth bioassay of the crude saponins precipitated by lead acetate method when *Brassica* was the test species. Phenolic compounds have widely been reported to inhibit germination of seeds (Evenari, 1961; Naqvi, 1976; Burhan and Shaukat, 1999; Shaukat *et al*., 2003 a and b; Chon and Boo. 2005; JayaKumar and Manikandan, 2005; Chen *et al*., 2005). Inhibition of germination of cotton seeds by saponins of alfalfa has been reported by Mirchaim *et al*. (1970, 1972, 1974). The saponins seem to interfere the permeability of seed coat as the immersion of cotton seeds in Lucerne saponins caused structural changes in the membranes showing increased swelling of fringe and cell walls affecting their permeability to O_2 (Mirchaim *et al*., 1975).

The fruit extract of *P. juliflora* was more phytotoxic to seedling development compared to the root or shoot extract. Some degree of stimulation in lower concentration of shoot extract was only seen in case of *C. barbata*. Such a non-linear response (hormesis) in allelopathic dose-response data is fairly common (An *et al.,* 2005). Suppression of seedling growth in test species was of the same order as in case of germination i.e., *C. holosericea* < *C. barbata* < *L. sativa*. Radicle growth was more sharply reduced in our experiments. It is in agreement with Sen and Chawan (1970) who found suppression of radicle growth of *E. caducifolia* more drastic with aqueous extracts of stem, leaf, and immature fruits of *P. juliflora*. They reported leaf to bear more phytotoxic effects than other parts of the plant. In our study greater toxicity was found in extract of ripe fruits of this species. The difference of activity

may be due to immaturity of fruit used by them or the specificity of test species involved. The effects of the aqueous extracts on germination and early seedling growth of the test species in our studies were fairly specific. Such a species-specificity of phytotoxins has been demonstrated by Datta and Sinha-Roy (1975), Friedman *et al.* (1977), Mubarak and Hussain (1978), Burhan and Shaukat (1999), Shaukat *et al*. (2003 a and b) for extracts of *Croton bonplandianum*, *Artemisia herba-alba*, *Datura innoxia*, *Conyza canadensis*, and *Launaea procumbens*, respectively. This phenomenon is undoubtedly due to inherent differences in physiological and to a certain extent morphological characteristics of the various species involved.

Only decaying shoots of *P. juliflora* were found to be pernicious to germination and seedling growth of wheat at higher concentration only. Decaying roots being less effective. The reports on the toxicity of decaying materials of plants are contradictory. Le Tourneau and Haggenes (1957) found no evidence of phytotoxicity even after incorporation of 20g material of various spurges in the soil. Wilson and Rice (1968) reported both stimulatory and inhibitory effects with as little as 1g of decaying sunflower leaves in 454g soil (2/3 garden soil + 1/3 sand). Datta and Sinha Roy (1975) obtained significant reduction in germination with 5 and 10 g decaying *Croton bonplandianum* leaves per 250g soil in 13 out of 15 species tested. Similarly, Burhan and Shaukat (1999) and Shaukat *et al* (1983, 1985, 2001, 2003) found decaying material of several species to be inhibitory for germination and seedling growth of test species like *Pennisetum americanum*, and *T. aestivum*. In such experiments the texture of soil used is of considerable importance as the phytotoxins are more effective in coarse textured soils than in the fine-textured soil where they may get irreversibly adsorbed on colloidal particles that form the high proportion of such soils. The significance of phytotoxins as habitat variable is greater in desert regions where the process of leaching is restricted owing to scanty rainfall.

The artificial rain-drip and leachate from mesquite foliage and germinating seeds were found to be phytotoxic. Phytotoxicity of leachate from germinating seeds of *C. holosericea* has been reported by Khan and Shaukat (1990). The inhibitory nature of the artificial rain drip and leachate from the germinating seeds indicates that phytotoxins present in *P. juliflora* are not only water soluble but also it is very likely that they, under natural conditions, may be washed out from the leaves by rain, fog, or dew into the soil and may exert their effect on germination and growth of neighboring plants if accumulated in physiologically active concentrations. Recently, aqueous leachate of mesquite foliage has been shown to contain syringin and (-) –lariciresinol (Nakano *et al*., 2002) and –tryptophan (Nakano *et al.*, 2003). These substances have been suggested by them to be possible allelochemicals from *P. juliflora* due to their strong inhibitory activity against root growth of burnyard grass, *Echinocloa crus-galli* L. The contents of syringin and (-) – lariciresinol in the rhizosphere of *Prosopis* were, however, found to be in sub-physiological concentrations – substantially lower than that required to inhibit growth.

Our experiment related to the activity soil and soil microorganisms are suggestive to this fact that aqueous extracts containing phytotoxic principles did not completely lose the potential to inhibit germination or seedling growth of lettuce when incorporated in the soil. The phytotoxic principles of *P. juliflora* were thermostable or thermo-convertible to secondary inhibitors, as there occurred no change in the activity of the extracts on autoclave-sterilization. The physico-chemical nature of the soil and the soil microorganisms provided limited protection to the seedling growth against the toxicity of the extracts, which indicate that in spite of the adsorption of the phytotoxins on to the soil particles and their microbial breakdown, the phytotoxins were fairly stable in the soil. These results are similar to those reported for *Achyranthes aspera* by Khan and Shaukat (2006). Quarterman (1973) while working with the extracts of *Sedum pulchellum* (L.) Scop. found the extracts to be stable against microorganisms and it significantly inhibited the germination of various species such as *Arenaria petula* Michx., *Leavenworthia stylosa* and *Talium calcaricum* Ware. Only slight protection was afforded to *L. stylosa* by the soil itself. Bajwa *et al.* (2001) have also reported antifungal activity of extracts of three asteraceous species against *Aspergillii*. Aqueous extract of *Parthenium hysterophorus* is found to be antifungal against some pathogenic fungi such as *Drechslera tetramera*, *Aspergillus niger*, and *Phoma glomerata* (Bajwa *et al*., 2003). Indeed many phenolic inhibitors have been isolated from soil (Guenzi and Mc Calla, 1966; Glass, 1976; Patrick, 1971; Chou and Muller, 1972; Burhan and Shaukat, 1999; Rabaz *et al*., 2001; Shaukat *et al*., 2003 a and b; Tajuddin *et al.*, 2002) and to be fairly stable in the soil (Minderman, 1968). If prevented from leaching, and accumulated in substantial amounts, they may determine the composition and dynamics of other species (Abdul Wahab and Rice, 1967; Muller and Chou, 1972; Lodhi, 1975) and may affect crops establishment and productivity (Rice, 1974) as they may influence soil nutrient and microbial ecology (Inderjit and Asakawa (1998).

Our studies of ether fraction of aqueous extract indicated a few phenolic inhibitors in the shoot, root, and fruit of *P. juliflora*. Moreover, radicle growth bioassay of *B. compestris* indicated the presence of growth-inhibiting saponins in shoot and root. Saponins are glycosides of both triterpenes and sterols and have been detected in over 70 families of plants (Basu and Rastogi, 1967). It has been reported that lucerne saponins although had no direct effect on cotton embryo but in the presence of saponins the fibrils of cellulose of cotton seeds swell up hindering in the

free passage and diffusion of O_2. The depleted oxygen availability likely causes inhibition of germination and lag in vegetative growth may bring suppression of root and shoot (Mirchaim *et al*. 1974, 1975). Saponins have considerable impact in agriculture because of their allelopathic effects (Fons *et al*, 2003). Saponins affect the growth of soil microorganisms, especially fungi. *Gypsophila.* Saponins are reported to increase the lag phase of bacterial growth. With the additions of saponins, the populations of *Chryseomonas* and *Acinetobacter*, the two dominant culturable genera of control clover were no longer detectable or were significantly decreased (Fons *et al*., 2003). Waller *et al.* (1995, 1996) have, however, reported both inhibitory as well as stimulatory activity of partially purified mungbean (*Vigna radiata*) saponins over mungbean seedling growth itself – extract from leaves enhanced the growth, extract from stem inhibited the growth and extract from root slightly promoted the radicle elongation. In lettuce bioassay mungbean stem saponins were inhibitory at 2 and 4 weeks growth but slightly promontory at 7 and 10 weeks.

Dragondorff reagent indicated the presence of alkaloid (s) in ethanolic extracts of *P. juliflora* foliage. Viqaruddin Ahmad *et al.* (1978) have isolated three alkaloids namely juliflorine, julifloricine (in gum) and julifloridine from *P. juliflora*. Julifloricine is reported to be toxic against several microbes (Aqeel *et al*., 1989). Nakano *et al.* (2004) have recently isolated alkaloids namely 3-oxo-juliprosopine, secojuliprosopine, 3 - oxo-juliprosine and 3′-oxo-juliprosine from *P. juliflora* which are inhibitory to root and shoot growth of *Lepidium sativum*.

P. juliflora is a species of wide ecological amplitude. It is adapted to virtually all frost-free semi-arid climatic regions of the world (CAB International, 2000). It is fast growing, produces large number of highly fertile seeds, tolerates drought, salinity and fire, and is unpalatable to local grazing animals (Khan *et al*. 1984; 1986; 1989; Pasiecznik *et al*., 2001; Nadaf and Madkaikar, 2002; Daehler, 2005), which may be thought to attribute to its great invasive potential (cf. Chen *et al*., 2005; Raghubanshi *et al*., 2005). *P. juliflora* is a fairly phytotoxic plant to *E. caducifolia* (Sen and Chawan, 1970), *Cyanodon dactylon* (Al-Humaid, and Warrag. 1998), *Z. mays, T. aestivum* and *A. lebbeck* (Noor, *et al.*, 1995). Suppressive effects of its aqueous extracts on germination, growth and development of its herbaceous field associates viz. *C. barbata* and *C. holosericea* and cultivated species such as *L. sativa* and *T. aestivum* and *B. compestris* as suggested by our present studies indicate that this species bears allelopathic potential to a wide range of species. It appears that *P. juliflora* may be a significant factor in structuring its populations and its phytotoxicity may be one of the factors contributing to its ecological success. Our studies suggest that allelopathy may also be involved in invasiveness of *P. juliflora* as been suggested for some invasive species (Hierro and Callaway, 2003; Vila and Weiner, 2004; Morgan and Overholt, 2005; Chen *et al*. 2005). Coder and Warnell (1999) have indeed included *P. juliflora* amongst strongly allelopathic plants. However, the competitive interactions among *P. juliflora* and its field associates, particularly arboreal ones, in our arid ecosystems still remain unexplored. In arid or semi-arid areas the competition for water and nutrients is intense, allelopathic interaction would presumably create further deleterious conditions for the species growing in the vicinity of *P. juliflora*.

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