

PHYTOTOXIC POTENTIAL OF *ACHYRANTHES ASPERA* L. - A TROPICAL MEDICINAL WEED OF PAKISTAN

D. Khan¹ and S. Shahid Shaukat²

¹Department of Botany, Govt. National College, Karachi, Pakistan

²Department of Botany, University of Karachi, Karachi, Pakistan

ABSTRACT

Bioassays with *Achyranthes aspera* L. demonstrated that aqueous root and shoot extracts against *Triticum aestivum*, a cultivated species and four field associates viz. *Cenchrus pennisetiformis*, *C. setigerus*, *Chloris barbata*, and *Peristrophe bicalyculata* impeded or reduced germination of test species. The suppression of germination in shoot extract was in the order: *C. barbata* < *T. aestivum* < *C. setigerus* < *C. pennisetiformis* < *P. bicalyculata* and root extract suppressed germination in order: *C. barbata* < *T. aestivum* < *C. setigerus*, *P. bicalyculata* < *C. pennisetiformis*. Shoot extract was inhibitorier than root extract. Reduction in seedling growth was species specific. In general, inhibition was a function of the extract concentration. The suppression of seedling growth in test species was in the order: *C. barbata* < *C. pennisetiformis* < *C. setigerus* < *P. bicalyculata* i.e., *C. barbata* being more resistant and *P. bicalyculata* most susceptible to *Achyranthes* toxicity - *C. pennisetiformis* and *C. setigerus* being moderately susceptible. Decaying *A. aspera* root and shoot were pernicious to germination and growth of *T. aestivum*. Artificial rain-drip arrested lettuce seedling growth at higher concentration only. Root and shoot extracts were autotoxic to *A. aspera* at higher concentrations only. Phytotoxic principles of *A. aspera* 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 extract indicated at least two Phenolic inhibitors in the shoot and three in the root. Radicle growth bioassay of *Brassica campestris* indicated at least two saponins/glycosides in shoot and two in the root. The toxic effects of the extracts facilitated explanation to the observed field behaviour of the test species in terms of their sociological peculiarities in *A. aspera* dominated stands.

Key Words: *Achyranthes aspera*, Medicinal Weed, Phytotoxicity, Biochemical Assays

INTRODUCTION

Allelopathy has attracted the attention of ecologists in interpreting community structure of several plant species (Einhellig, 1995; Hegazy, 1999; El-Khatib, 1998, 2000; El-Khatib *et al.*, 2004). 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.

Achyranthes aspera L. is one of the tropical weedy species, referred to as a medicinal weed by Pankaj Oudhia (www.Botanical.com/) wide spread in Indo-Pak subcontinent – not only infesting crop fields but also colonizing derelict areas to form more or less pure stands of vegetation. Khan *et al.* (1987) have studied the sociological characteristics of this ruderal species. Almost pure stands dominated by this species in the suburbs of Karachi were found to have low diversity (mean number of species per stand = 9.54 ± 1.02) and characterized with geometric relative abundance pattern of species. The conspicuousness (IVI) of *A. aspera* in its stands related negatively with species diversity, equitability and species richness. On the basis of X^2 analysis, this species with a mean density of 104 plants.m⁻² exhibited negative association with a number of species of more or less of similar ecological requirements viz. *Cenchrus biflorus* (occurring in one stands out of 13 with density around 5 plants.m⁻² *C. pennisetiformis* (occurring in 8 stands out of 13 with density of 5.2 individuals. m⁻², *C. setigerus* (occurring in two stands out of 13 with density around 1.4 plants.m⁻², and *Leucas urticifolia* (occurring in 3 stands out of 13 with density around 3 plants.m⁻², and positive association with none except a marginally insignificant positive association with *Chloris barbata* which occurred in 8 stands out of 13 with density around 10 plants m⁻². In *A. aspera* sites pattern was non-random and contagious and the large patches of *A. aspera* alternated with the small patches of its associates. As per our studies the arcsine transformed percentage density proportion of *A. aspera* in its stands is also related negatively ($r = -0.9984$) with that of rest of the associates (cumulatively) indicating a sort of negative interaction among *Achyranthes* and its associates in the field.

The above given data led us to hypothesize that *A. aspera* may have allelopathic activity which may play a part in its sociological peculiarities. The present investigations focused on using its aqueous extracts to study the hypothesis that water-soluble materials of *A. aspera* may be released from the plant tissue and exert phytotoxic effects on the neighboring species. Such information should further elucidate ecology of this ecologically and agriculturally significant species.

MATERIALS AND METHODS

The vigorously growing plants of *A. aspera* 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 toxicity were also collected from the same locality.

Phytotoxicity of aqueous extracts of *A. aspera* against a cultivated species and some of its field associates:

Aqueous extracts of shoot and root material of *A. aspera* 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 root and shoot extracts was tested against a cultivated species namely *Triticum aestivum* cv Z76 and four field associates of *A. aspera* (Khan *et al.*, 1987) viz. *Chloris barbata*, *Cenchrus setigerus*, *C. pennisetiformis* and *Peristrophe bicalyculata*.

Twenty surface sterilized (2% sodium hypochlorite for 5 min.) seeds were placed on Whatman No. 1 filter paper in 9 cm diameter sterile petriplates and 5 ml of either shoot or root extract were added to each. Controls received glass-distilled water. The seeds of *P. bicalyculata* had undergone dry storage of six months – this species needed at least three months dry storage of seeds to recover dormancy (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. In case of *T. aestivum* the number of seminal roots were also recorded. Seedlings were oven-dried at 80 °C for dry matter measurement.

Phytotoxicity of decaying *A. aspera*:

The shoot and root of *A. aspera* 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 *T. aestivum* were sown in each pot. Each treatment and control was replicated three times. The pots were kept at 25 ± 2 °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 °C for dry weight measurement.

Auto-toxicity:

The response of *A. aspera* was tested against the root and shoot extracts of its own for any possible auto-toxicity.

Artificial rain-drip and leaching of phytotoxins:

The technique employed for this experiment was essentially the same that described by Naqvi and Muller (1975). Air-dried leaves of *A. aspera* 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:

Fifty surface sterilized naked seeds of *A. aspera* or those enclosed within bracts were kept on moist Whatman No. 1 filter paper and incubated at 26 ± 1 °C for 24 h. The seeds were removed now and 20 seeds of *Lactuca sativa* v. Grand Rapids were sown on the same filter paper. Plates were kept at 26 ± 1 °C under light intensity of 4000 Lux at the top of plates for 14 h day length. Controls received distilled water only. The toxicity of the exudates from bracts alone was also tested by soaking 0.5 g of air-dried bracts in 15 ml of distilled water for 24 h at room temperature. The filtrate was tested against *L. sativa* v. Grand Rapids.

Activity of soil and soil-micro-organisms against phytotoxins:

To check the activity of soil or soil microbes against the *A. aspera* 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 °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 °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 °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 or shoot of *A. aspera* was crushed in 200 ml distilled water, homogenate centrifuged and adjusted to 3 with 0.5N H₂SO₄. The homogenate was then extracted three times with peroxidase free ether and pooled ether fraction was evaporated to dryness over CaCl₂ 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 upper 3 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 °C, the length of the coleoptile segments was measured.

Extraction and biochemical activity of saponins:

Saponins from the aqueous extracts of *A. aspera* 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 H₂S 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 H₂S 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 H₂S gas was passed through this solution, warmed a filter. The filtrate contained acidic saponins. The two samples of saponins were pooled and evaporated to dryness over CaCl₂ 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 campestris* 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 °C. After 48 h of incubation, germination counts and radicle-length measurements were made.

RESULTS

Phytotoxicity of aqueous extracts of *A. aspera* against a cultivated species and some of its wild associates:

Shoot extract of *A. aspera* reduced the germination percentage of wheat particularly at stock concentration. The root extract was comparatively lesser effective (**Fig. 1**). The rate of wheat germination was, however, substantially impeded by root as well as the shoot extract. Shoot extract significantly stimulated the root and shoot elongation of wheat at 25 (%S) but inhibited the root growth at 50, 75, and 100 (%S). Shoot growth remained more or less unaffected. In comparison to shoot extract, root extract was more toxic to shoot and root growth of wheat, the both of them reduced regularly with the increase of extract concentration. Root extract also inhibited the development of root hairs progressively with increase of extract concentration – in higher concentration the root tip was burned completely. In root as well as shoot extract, the root became coiled and directed upwards. The seminal roots slightly increased in number but the dry matter yield of shoot and root progressively declined in shoot extract. Their dry matter remained more or less indifferent to the root extract.

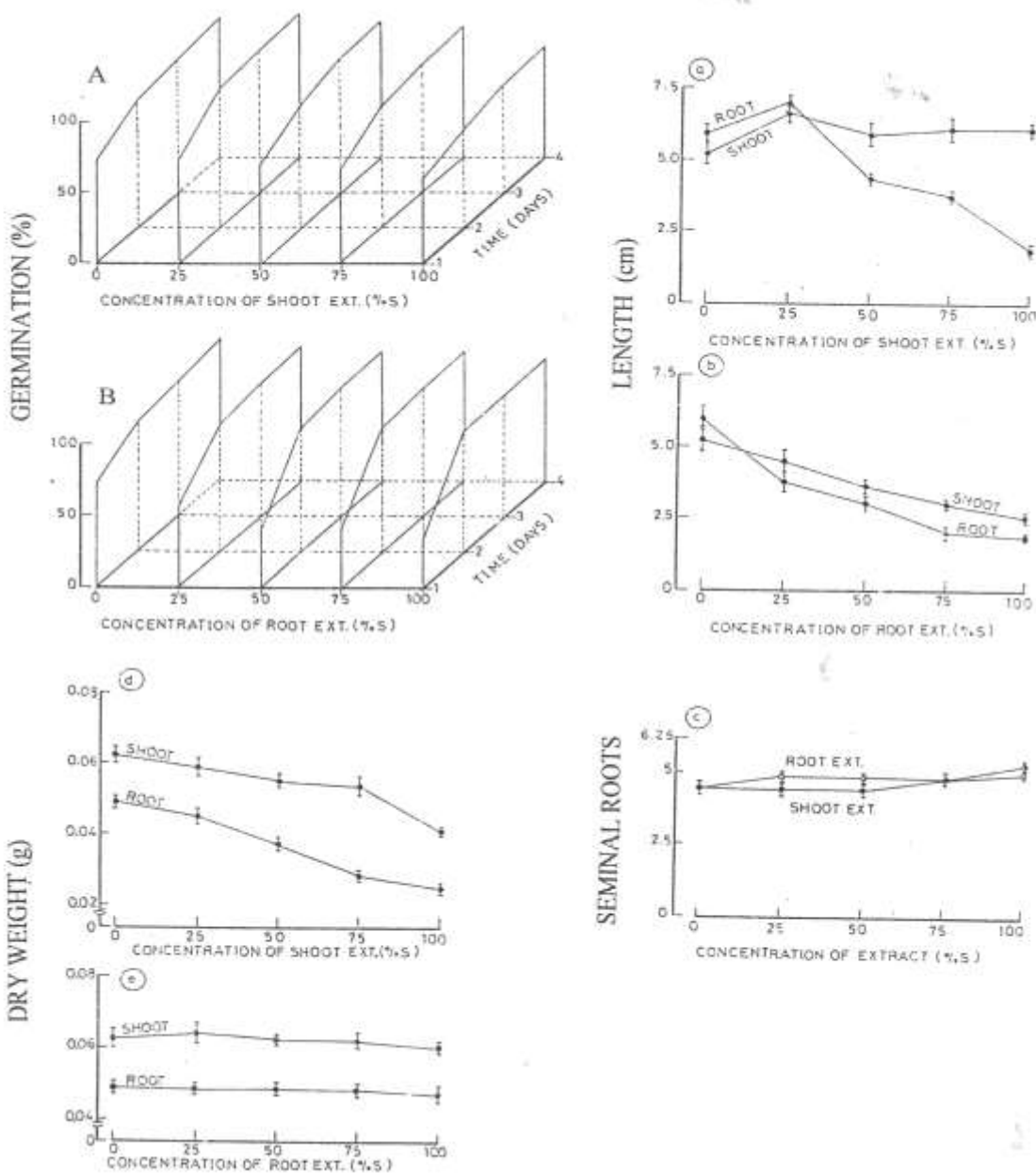


Fig.1. Effects of aqueous shoot and root extracts of *Achyranthes aspera* on germination (A & B) and seedling growth (a to e) of *Triticum aestivum* cv. Z76.

Amongst the wild associates, no significant effect on final germination percentage of *C. barbata* was observed in either extracts- rate of germination of, course, declined substantially in 50-100 (%S) concentration of shoot extract. The final germination percentages of *C. setigerus* and *C. pennisetiformis* were drastically suppressed by shoot extract and to somewhat lesser degree by the root extract (**Fig. 2**). *C. pennisetiformis* was more susceptible than *C. setigerus*. Amongst all, *P. bicalyculata* was the most susceptible species, which couldn't germinate in 75 and 100 (%S) concentration of shoot extract. Root extract was slightly lesser inhibitory to it.

Root elongation of *C. barbata* was significantly retarded ($p < 0.05$) at only 100 (%S) of root extract of *A. aspera*, otherwise root as well as shoot growth of this species remained almost unaffected at any concentration of the extracts (**Fig. 3**). The shoot elongation of *C. setigerus* and *C. pennisetiformis* was significantly ($p < 0.05$) stimulated at lower but inhibited at higher concentrations of the shoot extract. The tips of some 22% of the roots

of *C. pennisetiformis* became black and desiccated at stock concentration of shoot extract. Shoot growth of this species substantially stimulated at lower concentration of root extract but declined thereafter. Root growth of both of these grasses declined progressively. Shoot extract was more detrimental to seedling growth of *C. pennisetiformis* than root extract. Shoot growth of *P. bicalyculata*, which was initially stimulated ($p < 0.01$) declined sharply with the increase of shoot extract concentration. Both shoot and root growths decreased sharply to reach zero at 75 and 100 (%S) of shoot extract. Root growth declined more in shoot extract than in the root extract.

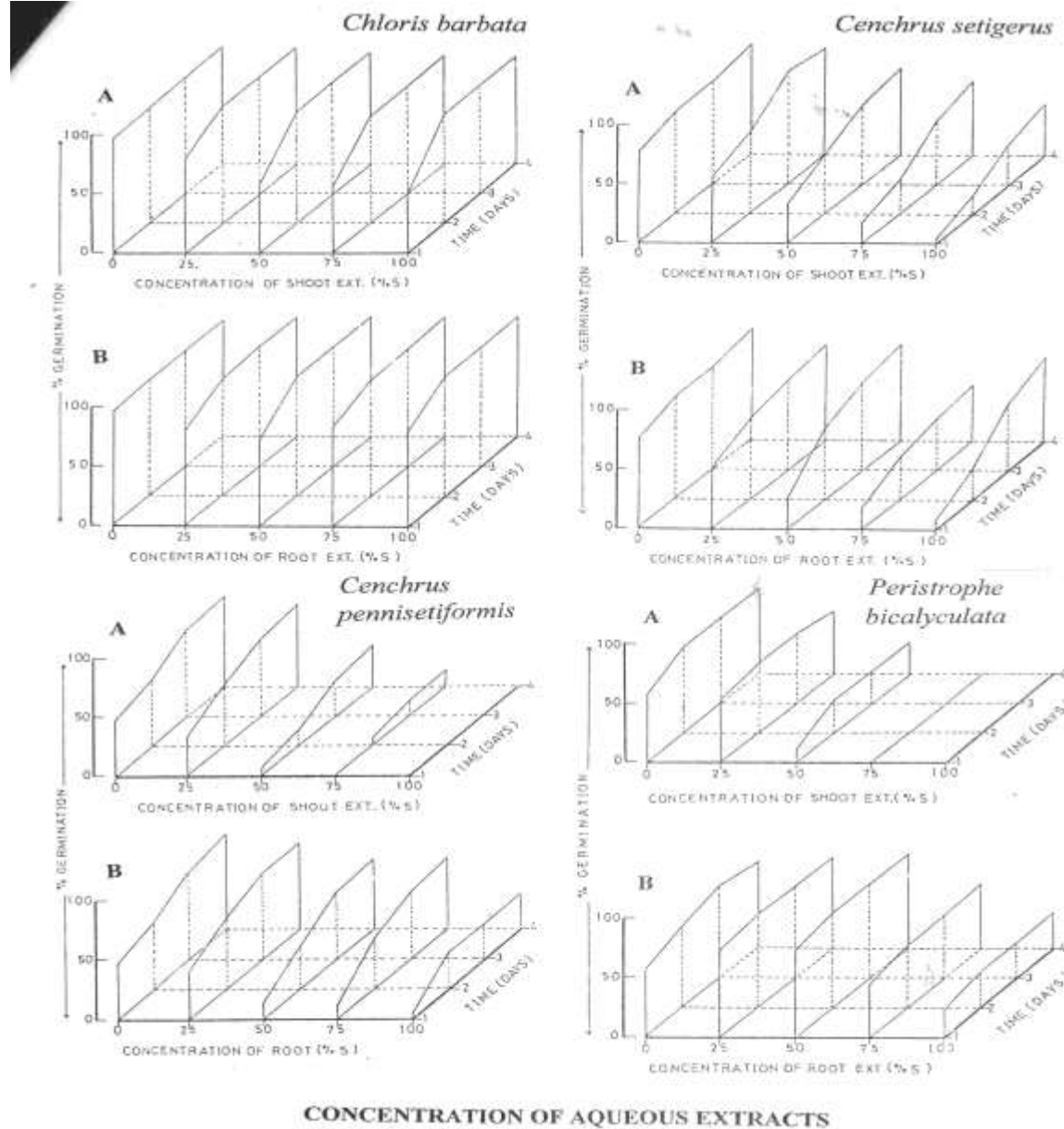


Fig.2. Effects of aqueous shoot (A) and root (B) extract of *Achyranthes aspera* on germination of some of its major wild associates in its populations.

Phytotoxicity of decaying *A.aspera*:

The germination percentage of *Triticum aestivum* was significantly ($p < 0.01$) reduced in soils incorporated with 5, 10, and 20g of decaying *A. aspera* shoots and roots (Fig. 4). Decaying roots had slightly greater influence on germination than that of decaying shoots. The root elongation was significantly ($p, 0.001$) stimulated in soil incorporated with 5g shoots or roots but declined in soils with 20g shoot significantly ($p < 0.05$). The root and shoot lengths were significantly ($p < 0.001$ and $p < 0.01$, respectively) larger at each

treatment of root material incorporation in soil. Dry weight of seedlings shoot was significantly reduced in soils incorporated with *A. aspera* shoots ($p < 0.001$) and roots ($p < 0.01$) at each level of treatment. Dry weight of roots of wheat seedlings was significantly low ($p < 0.05$) in soil with 20g root material of *Achyranthes* only.

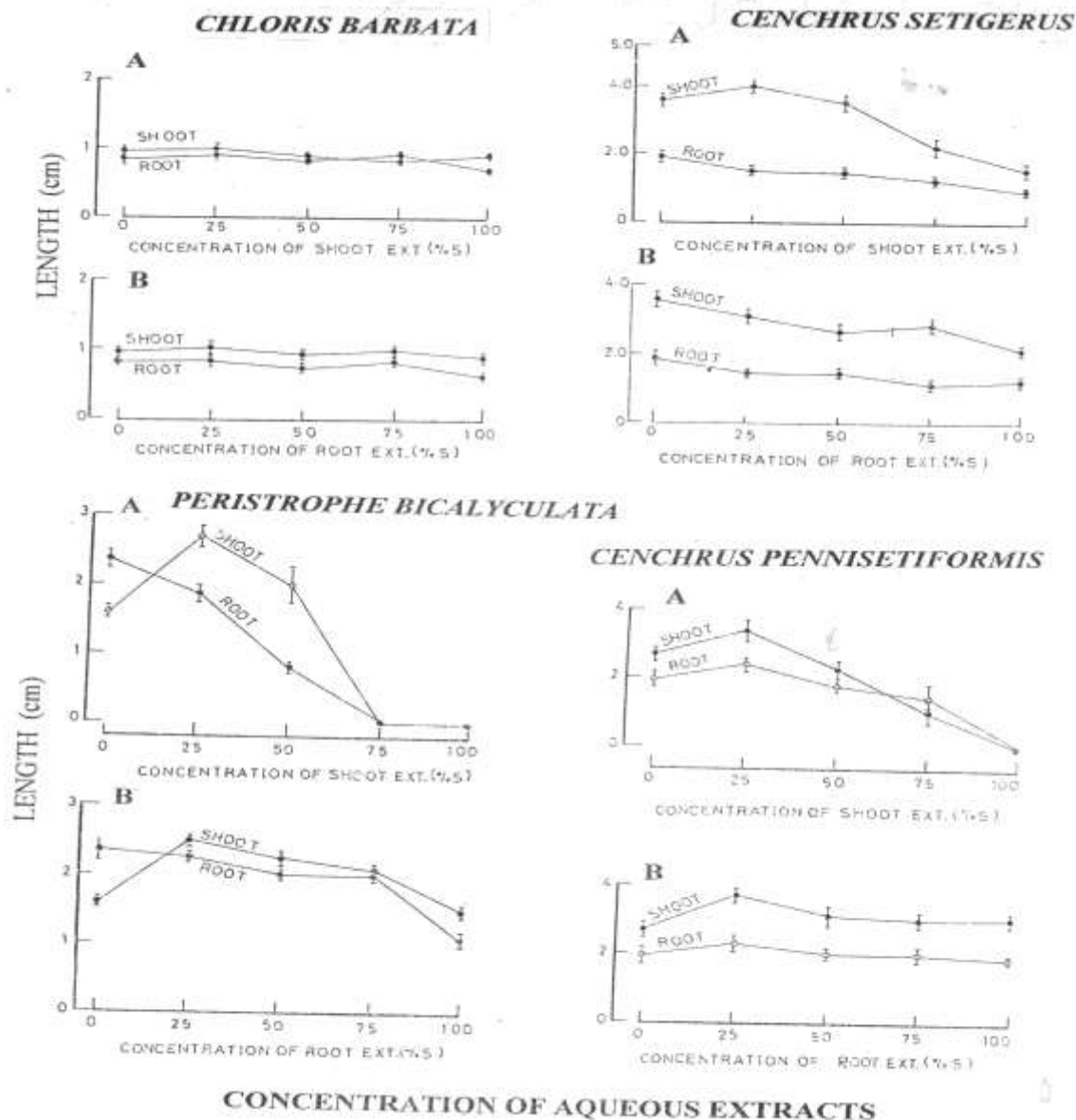


Fig.3. Effects of aqueous shoot (A) and root (B) extract of *Achyranthes aspera* on seedling growth of some of its wild associates in its populations.

Autotoxicity:

The final germination percentage of *A. aspera* was not significantly affected by any of its shoot or root extracts. The rate of germination was, however, significantly declined in both extracts (Fig. 5). Shoot extract was more delaying in sense that the seeds didn't germinate up to 48 h of incubation in 100 (%S) concentration of shoot extract. Root elongation exhibited significant stimulation at 25 and 50 (%S) of shoot extract ($p < 0.001$ and $p < 0.05$, respectively) and didn't decline below controls in higher concentrations. Shoot length, on the other hand, declined regularly in shoot extract. Shoot elongation retarded significantly only at 75 and 100 (%S) concentrations of root extract. Root elongation remained significantly stimulated ($p < 0.001$) at all concentrations of root extract tested.

Artificial rain-drip and leaching of phytotoxins:

The original 1X or 4X concentration of the leachate collected from *A. aspera* did not significantly alter the seed germinability of *L. sativa*. However, 1X concentration of leachate significantly ($p < 0.05$) stimulated the shoot elongation and 4X concentration significantly inhibited both root ($p < 0.05$) and shoot ($p < 0.01$) growth of lettuce seedlings.

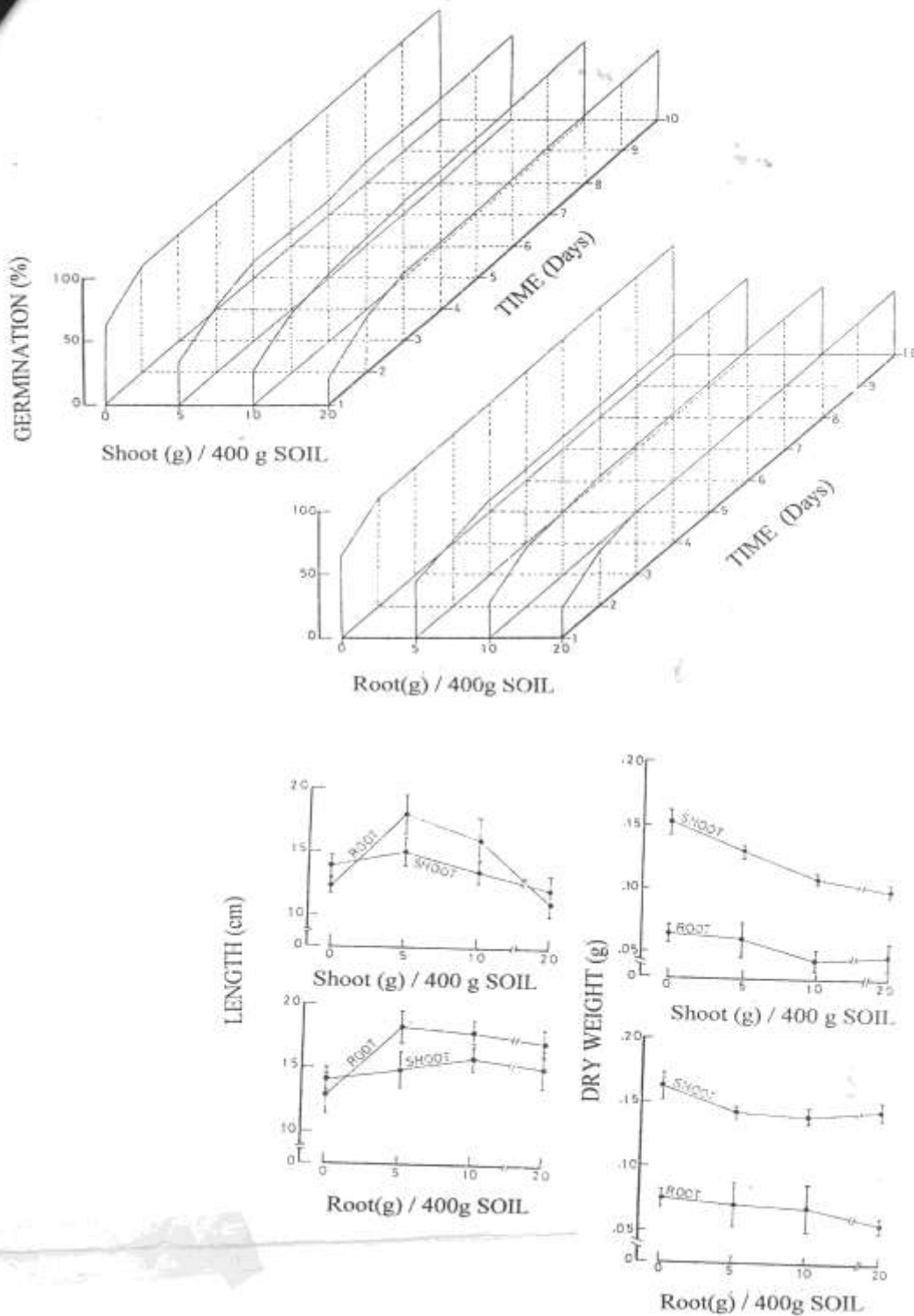


Fig.4. Effects of decaying shoot and root materials of *A. aspera* on germination and seedling growth of *Triticum aestivum* cv. Z76.

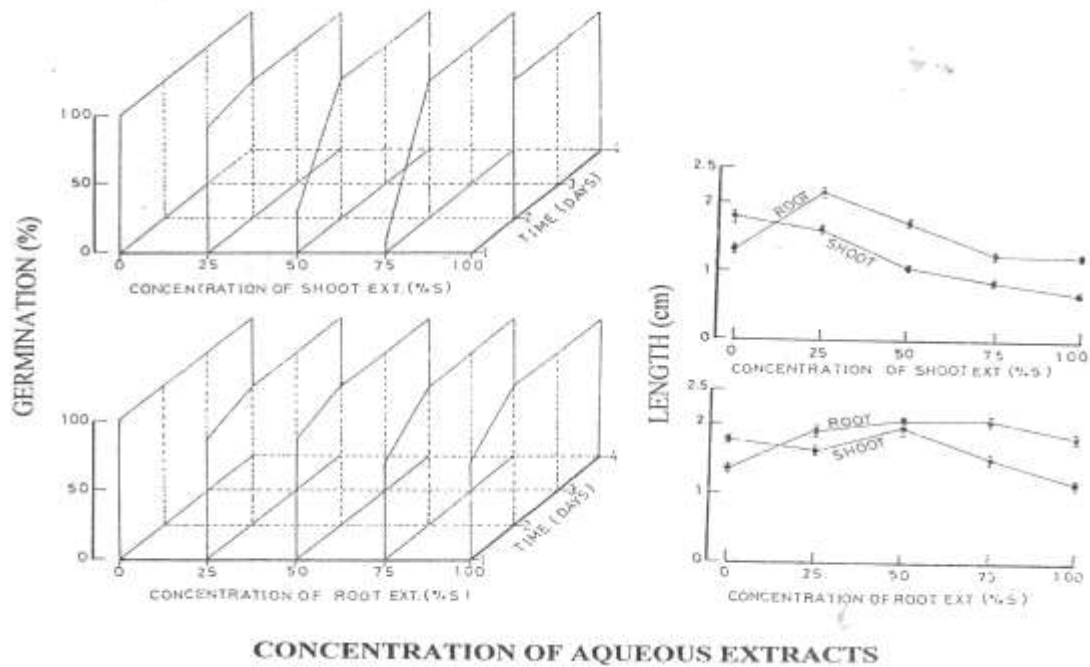


Fig.5. Auto-toxic effects in *A. aspera* Figure shows the germination response of *A. aspera* seeds and seedling growth of this species to its own aqueous shoot and root extracts.

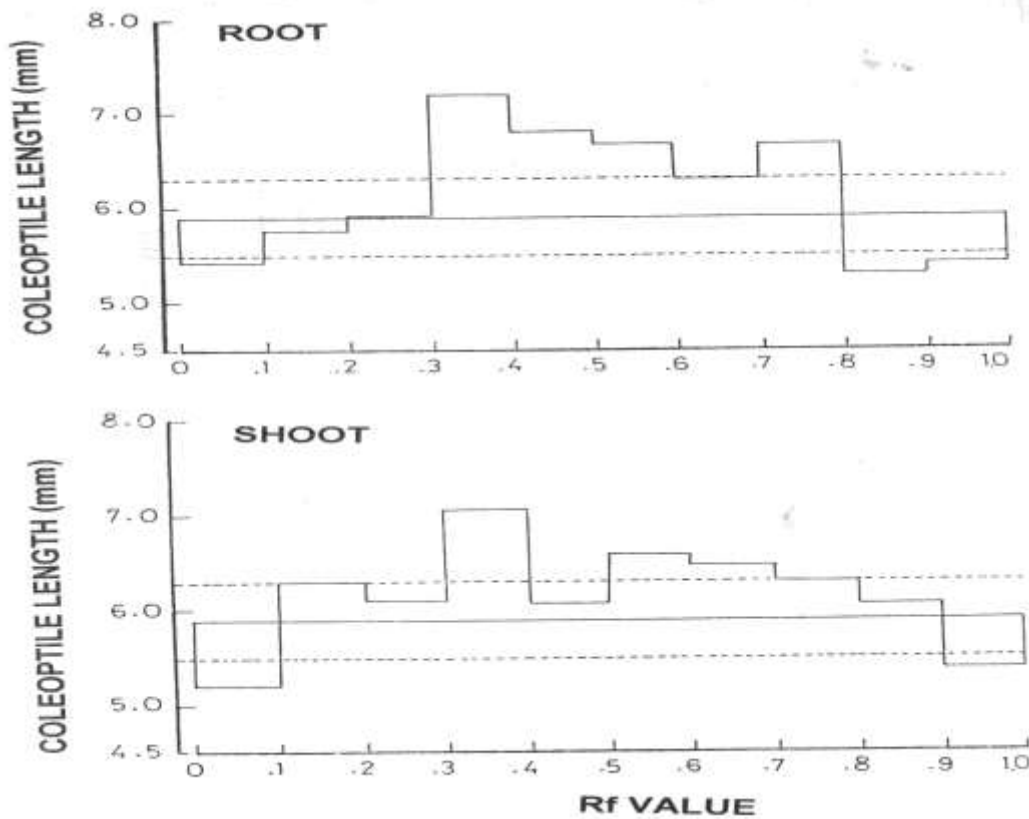


Fig.6. Histograms of ether fraction of extracts of *A. aspera*, chromatographed on Whatman #1 filter paper and developed in solvent, isopropanol:Ammonia:water (10:1:1 v/v/v). Dotted line represents 95% confidence interval.

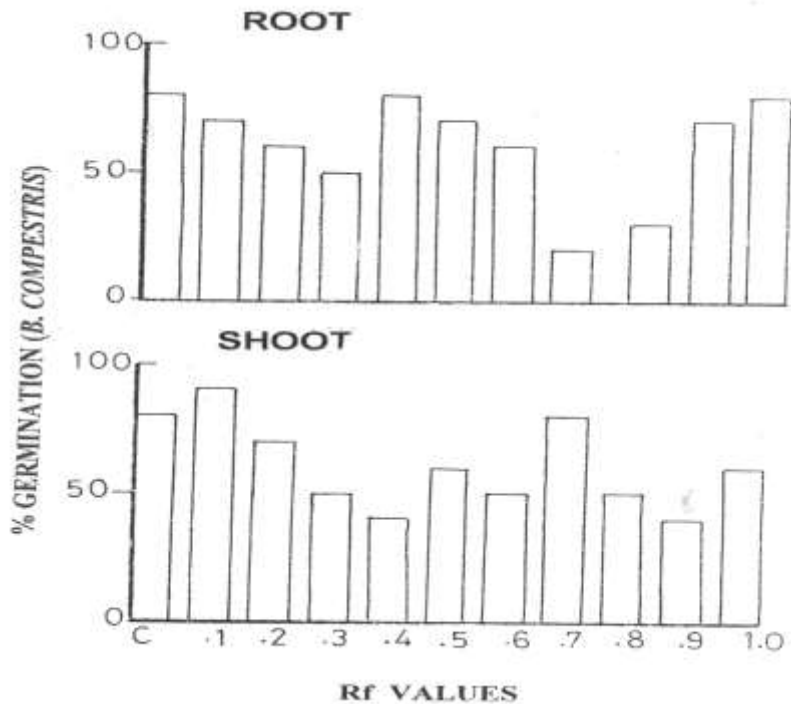


Fig.7. Germination behaviour of *Brassica compestris* at Rf values of chromatogram of crude saponins / glycosides extracted from *A. aspera* root and shoot by precipitation method with lead acetate and run on Whatman #1 filter paper in solvent chloroform:acetone (4:1 v/v).

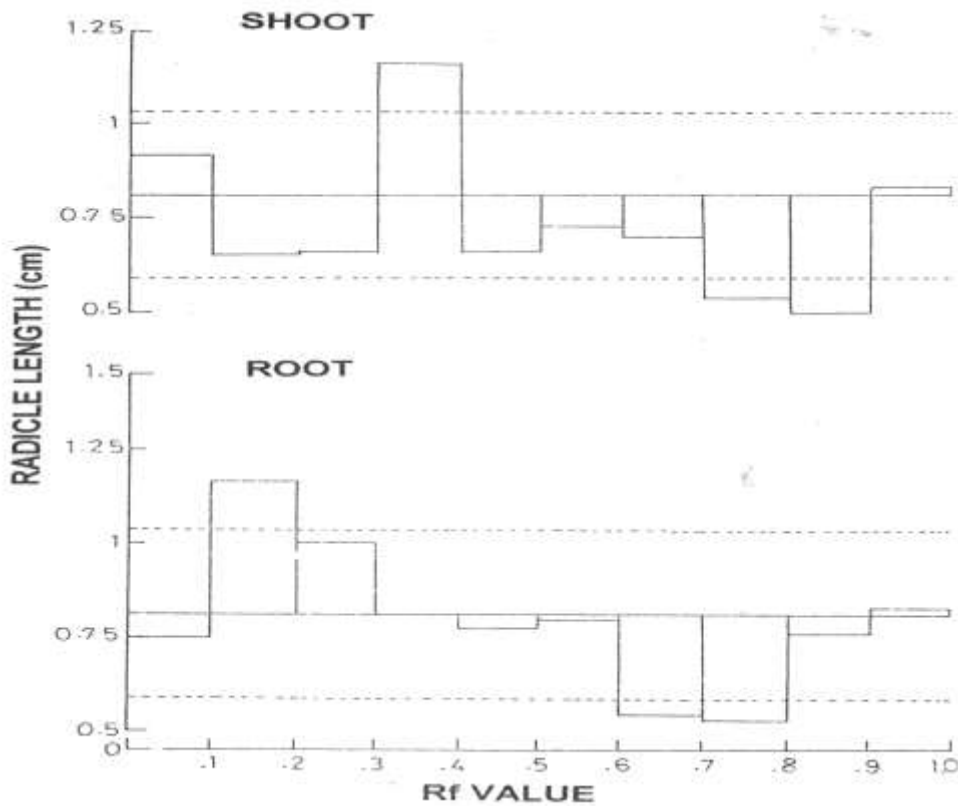


Fig.8. Histograms of radicle length of *Brassica compestris* at various Rf values of chromatogram of crude saponins extracted from *A. aspera* shoot and root by precipitation method with lead acetate and run on Whatman # 1 filter paper in solvent, chloroform:acetone (4:1 v/v). Dotted line represents 95% confidence interval.

Exudation of phytotoxins from germinating seeds:

The shoot as well as root growth of lettuce seedlings was inhibited significantly ($p < 0.001$) by the exudates of bracts which encloses seeds, germinating seeds and germinating seeds enclosed within bracts of *A. aspera* (Table 2).

Activity of soil and soil microorganisms against phytotoxins:

The germination of lettuce seeds remained practically unaltered in sterilized and unsterilized aqueous extract of roots of *A. aspera* with or without sterilized or unsterilized soil but it was significantly lower than that in the controls (Table 3). The sterilized as well as unsterilized aqueous extract of shoot in the presence or absence of sterilized soil inhibited germination completely. However, substantial loss of inhibitory activity of unsterilized and sterilized shoot extract occurred in presence of unsterilized soil, yet the germination percentage in this treatment was significantly low than that in the controls. Compared to the controls, shoot and root growth of lettuce seedlings were inhibited significantly in all the treatments of sterilized or unsterilized root extract supplemented with or without sterilized or unsterilized soil. The seedling growth in sterilized root extract supplemented with sterilized soil was not significantly different from that of the unsterilized root extract supplemented with unsterilized soil. Both root and shoot growth of lettuce increased significantly when sterilized root extract was supplemented with unsterilized soil. The activity of the root extract was not altered significantly by sterilization as both the root and shoot growth of lettuce were equally suppressed by unsterilized or sterilized root extract when the germination substrate was extract + filter paper only (no soil). The shoot growth of lettuce in sterilized soil supplemented with sterilized root extract was significantly higher than in sterilized soil on paper media. However, root growth didn't show such a response. The shoot extract appeared to be more toxic to lettuce growth. Significant increase in growth of root and shoot of lettuce seedlings occurred when the unsterilized or sterilized shoot extract was supplemented with unsterilized soil. The results indicated that both soil and its micro flora provided but slight protection to lettuce growth against toxicity of root and shoot extracts of *A. aspera* and the phytotoxins present in the extracts were thermostable or thermoconvertible to other phytotoxins.

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

Leachate Concentration	Germination (%)	Shoot Length (cm)	Root Length (cm)
Control	96.6 ± 3.33	1.80 ± 0.03	5.61 ± 0.08
1 x	100 ± 0 ns	1.96 ± 0.01 **	5.32 ± 0.10 ns
4 x	96.6 ± 6.66 ns	1.62 ± 0.02 **	5.21 ± 0.08 *

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

Table 2. Effects of exudates of bracts, seeds and seeds + bracts of *A. aspera* on early seedling growth of *Lactuca sativa* var. Grand Rapids.

Source of Exudate	Treatment	Shoot Length (cm)	Radicle Length (cm)
Bracts	Control	1.75 ± 0.033	5.53 ± 0.079
	Test	1.39 ± 0.046 ***	2.24 ± 0.089 ***
Seeds	Control	1.75 ± 0.033	5.53 ± 0.079
	Test	1.32 ± 0.050 ***	2.47 ± 0.135 ***
Seeds + Bracts	Control	1.75 ± 0.033	5.53 ± 0.079
	Test	1.02 ± 0.055 ***	3.67 ± 0.122 ***

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

Partial characterization of phytotoxins:

i. Wheat Coleoptile Bioassay: The bioassay of ether fraction of the aqueous extracts indicated significant growth inhibition at Rf values 0-0.1 and 0.9-1.0. Bioassay also revealed growth promotion at Rf values 0.3-0.4, 0.4-0.5, 0.5-0.6 and 0.6-0.7 in the shoot extract and at Rf values 0.3-0.4, 0.4-0.5, 0.6-0.7 and 0.7-0.8 in the root extract (**Fig. 6**).

ii. Germination and radicle growth bioassay of *Brassica campestris*: The crude saponins extracted by precipitation method using lead acetate were tested for their biochemical activity against *B. campestris*. On the chromatogram of the root extract of *A. aspera* the germination of *Brassica* was reduced significantly at Rf values 0.2-0.3, 0.6-0.7 and 0.7-0.8 by a quantum of 31.5, 75, and 62.5 %, respectively (**Fig. 7**). In the shoot extract the germination was inhibited at Rf values 0.2-0.3, 0.3-0.4, 0.5-0.6, 0.7-0.8 and 0.8-0.9 by 37.5, 50, 37.5 and 50%, respectively.

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

Treatment	Root Extract			Shoot Extract		
	Germination (%)	Shoot Length (cm)	Root Length (cm)	Germination (%)	Shoot Length (cm)	Root Length (cm)
US + H2O + Filter paper (No Extract)	96.66 ± 1.66 a	2.40 ± 0.056 a	5.01 ± 0.16 a	96.66 ± 1.66 a	2.40 ± 0.056 a	5.01 ± 0.16 a
SS + H2O + Filter paper (No Extract)	95.00 ± 2.86 a	2.52 ± 0.06 a	5.12 ± 0.11 a	95.00 ± 2.86 a	2.52 ± 0.06 a	5.12 ± 0.11 a
US Ext. + Filter paper	76.66 ± 3.33 b	0.21 ± 0.014 b	0.32 ± 0.13 b	0.00 b	0.00 b	0.00 b
S Ext. + Filter paper	76.66 ± 6.66 b	0.22 ± 0.062 b	0.36 ± 0.13 b	0.00 b	0.00 b	0.00 b
US + US Ext. + Filter paper	81.66 ± 9.28 ab	0.35 ± 0.021 c	0.37 ± 0.017 b	35.00 ± 10.41 c	0.99 ± 0.013 c	0.66 ± 0.081 c
US + S Ext. + Filter paper	83.33 ± 3.33 ab	0.64 ± 0.015 d	0.71 ± 0.033 c	33.33 ± 3.33 c	0.82 ± 0.15 d	0.99 ± 0.057 d
S S+ US Ext. + Filter paper	86.66 ± 6.66 ab	0.26 ± 0.015 b	0.27 ± 0.014 d	0.00 b	0.00 b	0.00 b
SS + S Ext. + Filter paper	86.66 ± 6.66 ab	0.36 ± 0.021 c e	0.39 ± 0.043 b	0.00 b	0.00 b	0.00 b

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.

Radicle growth bioassay of *Brassica* indicated inhibition of radicle growth at Rf values 0.7-0.8 and 0.8-0.9 in the shoot extract and at Rf values 0.6-0.7 and 0.7-0.8 in root extract (**Fig. 8**). The promotion of radicle growth was observed at Rf values 0.3-0.4 in the shoot extract and at 0.1 and 0.1-0.2 in the root extract.

DISCUSSION

The examination of the influence of the aqueous extracts of root and shoot of *A. aspera* on germination of some associated herbs in field and a cultivated species disclosed that the shoot extract was more inhibitory to the process of germination in comparison to the root extract. The suppression of germination in shoot extract was in the order: *C. barbata* < *T. aestivum* < *C. setigerus* < *C. pennisetiformis* < *P. bicalyculata*. The root extract, although less suppressive, exhibited following order of germination inhibition: *C. barbata* < *T. aestivum* < *C. setigerus* < *P. bicalyculata* < *C. pennisetiformis*. The aqueous extracts of many species are known to inhibit seed germination (Le Tourneau *et al.*, 1956; Quarterman, 1973; Datta and Sinha-Roy, 1975; Naqvi and Muller, 1975; Mubarak and Hussain, 1978, Burhan and Shaukat, 1999; 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). 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 O₂ (Mirchaim *et al.*, 1975).

The shoot extract of *A. aspera* was more phytotoxic to seedling development than was the root extract. However, *Triticum* was comparatively more susceptible to root extract. Both root and shoot extract of *Achyranthes* at low concentration even stimulated the seedling growth in some instances. Such a non-linear response (hormesis) in allelopathic dose-response data is fairly common (An *et al.*, 2005). The root growth of various species was retarded by the shoot extract in the order: *C. barbata* < *T. aestivum* < *C. setigerus* < *C. pennisetiformis* < *P. bicalyculata* where as shoot growth in the order: *C. barbata* < *C. pennisetiformis* < *C. setigerus* < *T. aestivum* < *P. bicalyculata*. The root growth under the influence of root extract exhibited the same order as under the influence of the shoot extract. The root extract inhibited the shoot growth in order: *P. bicalyculata* < *C. barbata* < *C. pennisetiformis* < *C. setigerus* < *T. aestivum*.

The effects of the aqueous extracts on germination and early seedling growth of the test species 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.

The decaying shoots and the roots of *A. aspera* were found to be pernicious to both germination as well as seedling growth of wheat – shoot material being inhibitorier. The reports on the toxicity of decaying materials of plants are contradictory. Le Tourneau and Haggnes (1957) found no evidence of phytotoxicity even after incorporation of 20g material of various sparges 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.*, *Triticum aestivum*. In such experiments the texture of soil used is of considerable importance as the phytooxins 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 inhibitory nature of the artificial rain drip indicated that phytotoxins present in *A. aspera* are highly water soluble and under natural conditions it is very likely that they 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. Leaching of phytotoxins from germinating seeds and bracts in our experiment is indicative to this effect. The 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 *Achyranthes* 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. Antibacterial activity of stem and leaf extracts of *Achyranthes* is reported by Ikram and Haq (1980). Dhar *et al.* (1968) have reported the plant extract to be antifungal against *Aspergillus niger* and *Candida albicans*. 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 at least two phenolic inhibitors in the shoot and three phenolic inhibitors in the root of *A. aspera*. Radicle growth bioassay of *B. compestris* indicated at least two saponins in shoot and two in the root. Saponins are glycosides of both triterpenes and sterols and have been detected in over 70 families of plants (Basu and Rastogi, 1967). Khastgir and Sengupta (1958) isolated a saponin from the root of *A. aspera* and Sarkar and Rastogi (1960) obtained four spots of saponins from this plant. Recently, Michl *et al.* (2000) have reported two new bisdesmosidic triterpenoid saponins from *A. aspera* besides three already known saponins from its methanolic extract. 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 O₂. 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. They 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).

Dragondorff reagent indicated absence of alkaloid(s) in *A. aspera* aqueous root and shoot extracts. Ikram and Islam (1963) also reported no alkaloids in the leaves and stems of *A. aspera*. However, Zafar (1994) has reported *A. aspera* to contain a medicinally active water alkaloid, Achyranthine. Betaine is another alkaloid present in *A. aspera* (Kapoor and Singh, 1966. Ind. J. Chem.4: 461. www.himalayahealthcare.com).

From the foregoing discussion *A. aspera* appears to be a fairly phytotoxic plant and effects of its aqueous extracts on germination, growth and development of its field associates indicate to its allelopathic potential. It appears that *A. aspera* is a significant factor in structuring pattern in its populations and negative association between *A. aspera* with its associates (the test species) under field conditions as observed by Khan *et al.* (1987) can be explained by its allelopathic effects on these species.

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