

PRELIMINARY OBSERVATIONS ON AUTO- AND RECIPROCAL TOXICITIES IN *ACHYRANTHES ASPERA* L. AND *PERISTROPHE BICALYCVULATA* (RETZ.) NEES

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ABSTRACT

Germination and seedling growth of *Achyranthes aspera* L. and *Peristrophe bicalyculata* (Retz.) Nees under influence of each other's aqueous shoot and root extracts or against their own extracts, expressed as single value of inhibition index across the whole range of extract concentrations indicated more Autotoxic sensitivity in *P. bicalyculata* than *A. aspera*. *P. bicalyculata* was more susceptible to *A. aspera*, which was reciprocally less susceptible to *P. bicalyculata*. Shoot extract of both species was more toxic than root extract. Furthermore, shoot extract of one species was more toxic to root growth of other species (overall inhibition: 56.2 – 60.6%) than to the shoot growth (overall inhibition: 22.0 – 30.6%). In both cases root extracts didn't show any deleterious effects on shoot growth of other species, however, root extracts inhibited root growth more or less equally in either case (overall inhibition: 8.7 – 12.8%).

Key Words: *Achyranthes aspera* L., *Peristrophe bicalyculata* (Retz.) Nees, Aqueous shoot & root Extracts
bioassay, auto- and reciprocal phytotoxicity

INTRODUCTION

Achyranthes aspera L. is an erect perennial herb (often woody below and somewhat suffrutescent) and *Peristrophe bicalyculata* (Retz.) Nees is a tall annual arid land species (Townsend, 1974; Jafri, 1966). These invasive species often form almost pure populations of variable sizes along roadside or in derelict areas in Karachi. Phytosociological studies of 13 *A. aspera* dominated and 11 *P. bicalyculata* dominated stands of vegetation in Karachi indicated that out of 61 species harboring these stands, 20 were exclusive to *P. bicalyculata* stands, 15 to *A. aspera* stands and 38 species were common among these stands i.e., compositional similarity on the basis of common species between *A. aspera* and *P. bicalyculata* stands was around 62.3%, which remained more or less same (61.8%) if evaluated on the basis of importance values of the constituent species. These stands were characterized with relatively low diversity (mean number of species per stand: 9.54 ± 1.02 in case of *A. aspera* and 12.45 ± 0.54 in case of *P. bicalyculata*) and geometric pattern of relative abundance among the species. The overall representation of *A. aspera* and *P. bicalyculata* in each other's stands was extremely poor (Khan, 1980).

Allelopathy has attracted the attention of ecologists in interpreting community structure and distribution pattern of several plant populations (Lodhi, 1975; Einhellig, 1995; Hegazy, 1999; El-Khatib, 1998, 2000; Erwin & Wetzel, 2000; El-Khatib *et al.* 2004; Chen *et al.*, 2005; Khan and Shaukat, 2006a & b). This phenomenon is characterized with reduction in emergence or growth of some target species in the community by an allelopathic species. Khan and Shaukat (2006a) have reported allelopathic nature of *A. aspera* against some species found growing sympatric with, but subordinate to *Achyranthes* and some cultivated species as well. The degree of auto- and reciprocal toxicities in *A. aspera* and *P. bicalyculata* are evaluated in this paper to check whether these species are mutually phytotoxic.

MATERIALS AND METHODS

The vigorously growing plants of *A. aspera* and *P. bicalyculata* were collected from 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.

Reciprocal phytotoxicity of aqueous extracts of *A. aspera* and *P. bicalyculata*:

Aqueous extracts of shoot and root of *A. aspera* and *P. bicalyculata* 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 each other. 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 or root extract was added. Controls received glass-distilled water. 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. Seeds of *A. aspera* showed no dormancy. The seeds of *P. bicalyculata* were after-ripened in dry storage for three months to remove dormancy (Khan *et al.*, 1984).

Auto-toxicity

The response of *A. aspera* and *P. bicalyculata* was also tested against root and shoot extracts of their own for any possible auto-toxicity. Seeds of *P. bicalyculata*, before putting to experiment, were after-ripened to remove dormancy. Germination counts were made daily and root and shoot lengths were recorded at 96 h of growth.

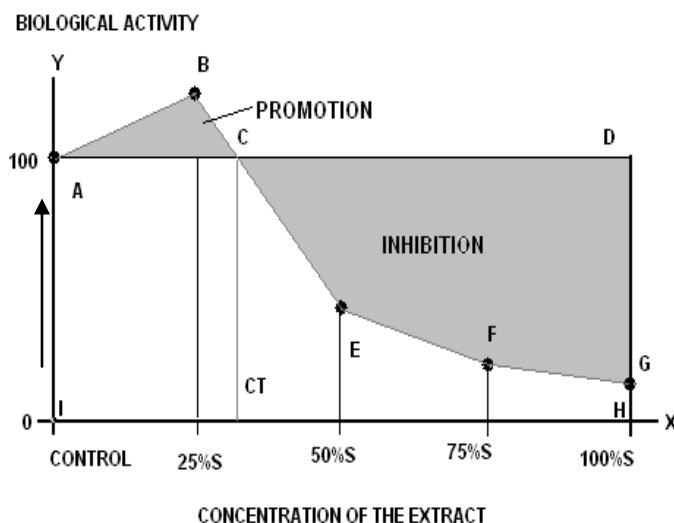


Fig. 1. Diagrammatic representation of typical biological response to the extract concentration.

While assessing the growth data, instead of assessing the effect of individual concentration of extract on test species, the overall effect that is response across the whole range of extract concentration was considered. An *et al.* (2005) has calculated overall biological activity across the whole range of concentrations, represented by single value – inhibition index defined as the percentage of the inhibition area to the total area expressed in percentage i.e., Inhibition index = [Inhibition area / Total area] 100.

If C is the extract concentration and CT is the threshold concentration of the extract causing inhibition in the test species (Fig. 1) and $f(C)$ is the mathematical function describing the non-linear dose-response relationship with promotion at lower and inhibition at relatively higher concentration of the extract, inhibition and promotion indices may mathematically be described as:

$$\text{Inhibition index (\%)} = [\text{Area CDGFE} \div \text{Area ADHI}] 100 = \left[\int_{CT}^{100} [100 - f(c)] dC \div \int_0^{100} 100 dC \right] 100$$

$$\text{Promotion index (\%)} = [\text{Area ABC} \div \text{Area ABCDHI}] 100 = \left[\frac{\left[\int_0^{CT} (f(c) - 100) dC \right] - (100 C_T)}{\int_0^{100} f(c) dC + \int_{CT}^{100} [100 - f(c)] dC} \right] 100$$

In the present investigation, for the sake of convenience, easiness and accuracy, the areas were determined by actual count of the area graphically and the indices for overall inhibition [(Area CDGFE / Area ADHI) 100] and promotion [(Area ABC / Area ABCDHI) 100] were calculated for all the curves presented in Fig. 2-5 for their objective comparison.

RESULTS

Auto-toxicity in *A. aspera* and *P. bicalyculata*:

The final germination percentage of *A. aspera* was not significantly affected by its shoot or root extracts. The rate of germination was, however, significantly declined in both extracts (Fig. 2). 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 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.

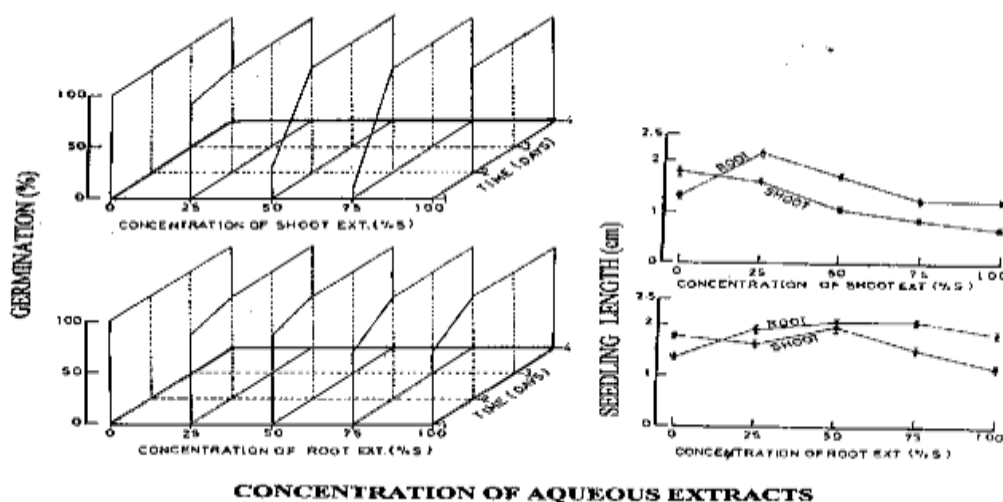


Fig.2. Auto-toxic effects in *A. aspera*. Figure shows the germination response of *A. aspera* seeds and seedling growth of this species to its own aqueous shoot and root extracts.

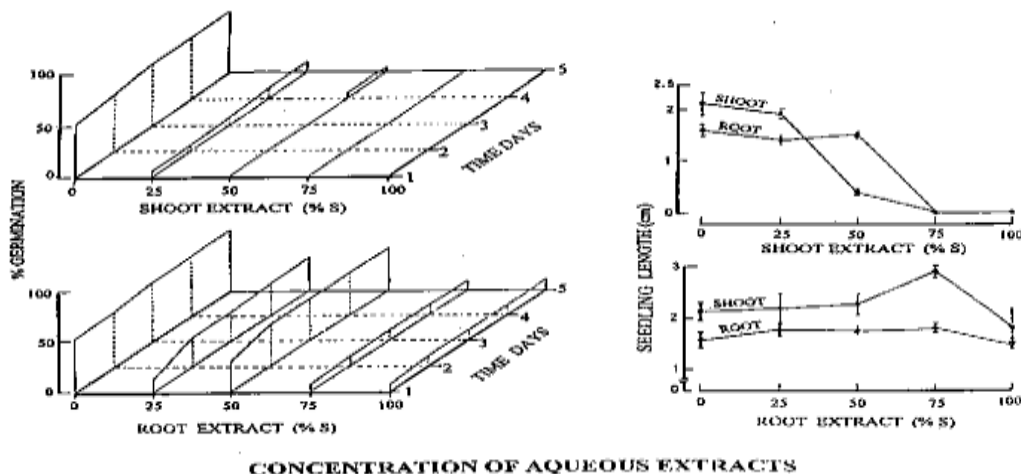


Fig.3. Auto-toxic effects in *P. bicalyculata* – response of germination and seedling growth of species to its own shoot and root extracts.

The germination of *P. bicalyculata* was significantly inhibited at 25% S of its root or shoot extract (Fig. 3). The shoot extract was more toxic. The inhibitory effects increased with the concentration of the extracts. No germination occurred at 75(S) of the shoot extract. Shoot extract inhibited seedling growth more drastically than the root extract. Shoot elongation was significantly ($P < 0.001$) suppressed at 50 (%S) and radicle elongation at 75 (%S) of the shoot extract. Shoot elongation in root extract didn't differ from the control at any concentration except at 75 (%S) where significant promotion took place. Root elongation was significantly promoted at all concentrations of the root extract.

Comparing the overall auto-toxicities in the two species (Table 1), there were greater auto-toxic effects in *P. bicalyculata* than in *A. aspera*. In both cases, shoot contained more toxicity than root.

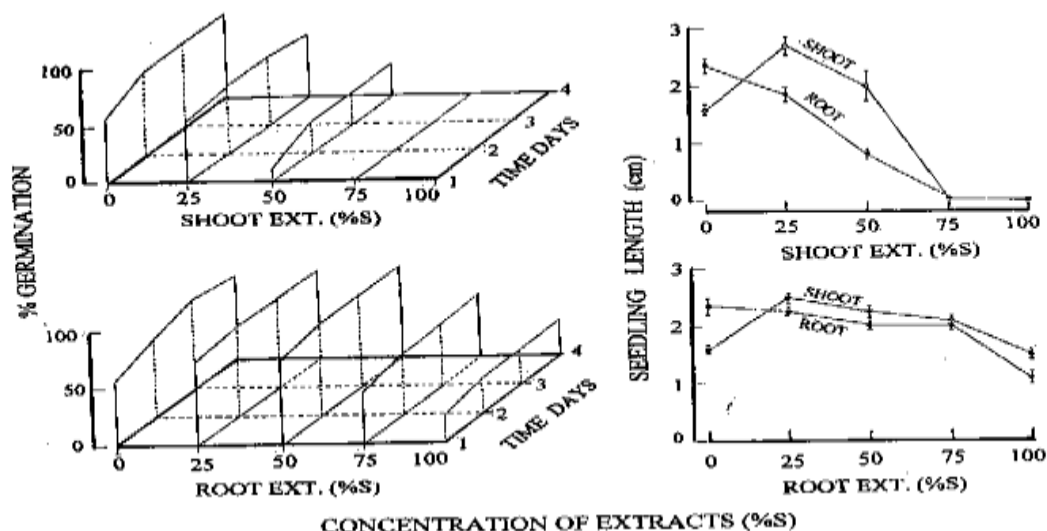


Fig.4. Effects of aqueous shoot and root extracts of *A. aspera* on germination and seedling growth of *P. bicalyculata*.

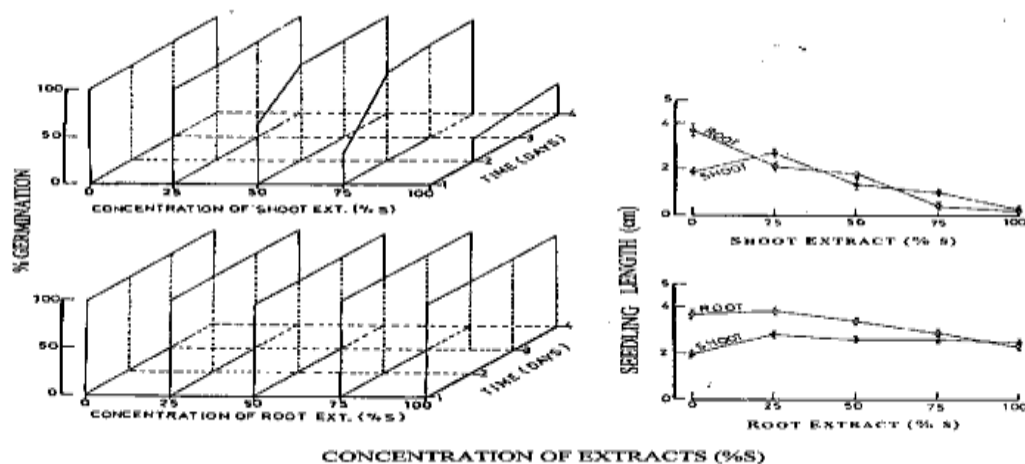


Fig.5. Effects of aqueous shoot and root extracts of *P. bicalyculata* on germination and seedling growth of *A. aspera*.

Reciprocal Phyto-toxicity between *A. aspera* and *P. bicalyculata*:

P. bicalyculata was quite susceptible to aqueous extracts of *A. aspera*. Its seeds couldn't germinate in 75 and 100 (%S) concentration of *A. aspera* shoot extract. *Achyranthes* root extract was, however, substantially lesser inhibitory to *Peristrophe* seed germination (Fig. 4).

Aqueous extract of *P. bicalyculata*, on the other hand were found to be less toxic to *A. aspera* seed germination. Full strength shoot extract of *Peristrophe*, however, not only delayed germination (Fig. 5) but also inhibited the final germination of *A. aspera* seeds significantly. Root extract had practically no effect on seed germination of *Achyranthes*.

Seedling performance of the two species under influence of each other's shoot and root extracts, in terms of overall inhibition or promotion is presented in Table 1. Shoot extract of both species was more toxic than root extract. Shoot extract of one species was more toxic to the root growth of other species (overall inhibition: 56.2 – 60.6%) than to the shoot growth (overall inhibition: 22.0 – 30.6%). In both cases root extracts didn't show any effect on shoot growth of either species, however inhibited root growth more or less equally (overall inhibition: 8.7 – 12.8 %). *A. aspera* was relatively more toxic to *P. bicalyculata*.

DISCUSSION

The examination of the influence of aqueous extracts of root and shoot of *A. aspera* and *P. bicalyculata* tested against themselves indicated greater autotoxic effects in *P. bicalyculata* both at germination and seedling growth

stage. The auto toxic effects in *A. aspera*, on the contrary, were mild. In both species shoot extract was more toxic than root extract.

Table1. Auto- and reciprocal toxicities in *A. aspera* and *P. bicalyculata* in terms of overall inhibition or promotion index of the seedling growth.

Species / Species Treatment	Extract Tested	Inhibition Index		Promotion Index	
		Shoot	Root	Shoot	Root
AUTOTOXICITY					
A. aspera	Shoot	33.86	Zero	Zero	22.89
	Root	9.56	Zero	0.89	30.14
P. bicalyculata	Shoot	61.14	39.78	Zero	Zero
	Root	0.46	Zero	9.88	10.40
RECIPROCAL TOXICITY					
A. aspera on P. bicalyculata	Shoot	30.36	60.55	15.34	Zero
	Root	Zero	12.49	22.49	Zero
P. bicalyculata on A. aspera	Shoot	22.02	56.22	8.03	Zero
	Root	Zero	8.70	25.10	1.08

A. aspera appeared to be more phytotoxic to *P. bicalyculata* at germination stage. The overall reciprocal toxicity of one species on other's seedling growth was, however, more or less of equal magnitude – shoot extract of the two species being inhibitorier than root extract. Aqueous extracts of many species are reported to be phytotoxic in nature (Rice, 1974, Shaukat *et al.*, 1983; Erwin & Wetzel, 2000; Prati & Bossdorf, 2004; Adetayo, *et al.*, 2005; Khan and Shaukat, 2006a & b). Several plants produce a number of bioactive allelochemicals such as phenolics, terpenoids, non-protein aminoacids, saponins, flavonoids, etc. as secondary metabolic products and release them into the environment where they exert toxic effects on specific target species. The presence of phenolics and saponins in *A. aspera* shoots has been shown to be phytotoxic to a number of associated species such as *Chloris barbata*, *Cenchrus pennisetiformis* and *C. setigerus* (Khan and Shaukat, 2006a). Shoot extract of *P. bicalyculata* has also been reported to be phytotoxic against *Triticum aestivum* and *Chloris barbata* (Khan, 1980). Strong phytotoxicity between *A. aspera* and *P. bicalyculata* against each other may likely be one of the reasons for low representation of one species in other's stands of vegetation.

P. bicalyculata, in present investigation, was found to possess more autotoxic sensitivity than *A. aspera*. Autotoxicity has been reported in a number of plants - *Typha latifolia* (Mc Naughton, 1968)), *Phytolaca americana* (Edwards *et al.*, 1988), *Cirsium vulgare* (deJong and Klinkhamer, 1985), *Pennisetum glaucum* (Saxena *et al.*, 1996), *Juncus effuses* (Erwin and Wetzel, 2000), *Lolium rigidum* (Canals *et al.*, 2005) etc. Alfalfa's autotoxicity is a well-documented fact (Webster *et al.*, 1967, Miller, 1983, Hedge and Miller, 1992; Nelson, *et al.*, 1997) due to a number of phytotoxins which affects its own germination and seedling performance. The re-establishment of *Medicago sativa*, is often unsuccessful due to this allelopathic suicide created by the allelopathic effects of the crop on its seedlings. Perry *et al.* (2005) have reported auto-inhibitor (\pm - catechin) in *Centurea maculosa* roots exudates.

In competitive species, Autotoxicity is considered to play a potential role in spatial and temporal dispersal of seed germination and seedling establishment (Edwards *et al.*, 1988). For species whose germination is inhibited by autotoxins, this strategy would avoid intraspecific competition between adults and seedlings. In other words the ecological implication of autotoxicity is the fact that seeds are more dispersed through space and time.

It is clear that in *P. bicalyculata* the autotoxic allelochemical (s) exist in leaves and stem. They are water-soluble and inhibit germination of its seeds. *P. bicalyculata* is reported to contain coumarin in leaves (Duke, 1977), which is highly inhibitory (Itoh, 1976; Hedge and Miller, 1992) and could be the key allelochemic in *P. bicalyculata* autotoxicity. Further research is needed to elucidate ecological implication of autotoxicity in the life history *P. bicalyculata* – a short-lived ruderal species.

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(Accepted for publication November 2006)