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ALLELOPATHIC EFFECTS OF PAKISTANI WEEDS *XANTHIUM STRUMARIUM* L.

Bushra Inam, Farrukh Hussain* and Farhat Bano

Pakistan Museum of Natural History, Islamabad

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Xanthium strumarium L. is both a weed and waste land species in Pakistan. Aqueous extracts from different parts, litter incorporated into growth medium and rain leachates severely reduced either germination, early growth, fresh or dry mass of *Lactuca sativa, Brassica campestris, Pennisetum americanum* and *Zea mays* in various experiments. Chromatographic study revealed the presence of caffeic, *p*-coumaric, *p*-OH-benzoic, chlorogenic and ellagic acids in rain leachate and shoot extracts. The toxicity depended upon the test species and the part assayed. The findings suggest strong allelopathy by *X. strumarium* L. which is subjected to modification by other factors of the environment.

Key words: Xanthium strumarium, weed, allelopathy, growth inhibitors, crop.

INTRODUCTION

X. strumarium L. is a common weed of cultivation and wasteland from plains up to 1500 m. in Pakistan. Hussain *et al.* [5, 7] and Hussain and Rashid [11] reported its distribution in different crops. Weeds compete for the physical resources of the habitat and exhibit allelopathy against associated or sequentially growing species. Allelopathic effects involve the reduction of growth and yield of susceptible species by rendering soil toxic and delaying the nitrogen cycle. Many Pakistani weeds have been reported to exhibit allelopathy. Hussain [2] reported allelopathic effects of *Euphorbia granulata.* Later on Hussain *et. al.* [9] demonstrated phytotoxic effects of *E. helioscopia. Cenchrus ciliaris* [3] retards the growth of crops in mixed culture in the field and laboratory. *Eragrostis poaeioides* [8] reduces germination and growth of many species. *Stachys parviflora* [10] also exhibits allelopathy. Hussain *et al.* [6] observed the allelopathic effects of *Setaria italica.* Dirvi and Hussain [1] reported allelopathy by *Dichanthium annulatum* against cultivated species. Naqvi and Muller [13] demonstrated phytotoxicity of *Lolium multiflorum.* Putnam and Duke [14], Tukey [16] and Rice [15] documented allelopathic effects of many weeds. The importance of allelopathy in agroeco-systems cannot be under estimated. No reference is, however, available on the phytotoxicity of *X. strumarium.* The present investigation was, therefore, conducted to investigate its allelopathic behaviour and to identify the toxins involved in allelopathy.

*Department of Botany, University of Peshawar, Peshawar.

MATERIAL AND METHODS

Mature plants of *X. strumarium* were collected, separated into leaves, stems, roots, fruits and inflorescences, and dried in shade at room temperature (25-30°). Sterilization of glassware, extraction of water soluble substances in distilled water, general bioassay techniques and statistical analysis of the data have been given by Dirvi and Hussain [1] and Hussain *et al.* [8,10]. Germination and early growth of test species were recorded at 25° after 72 hrs. There were always 10 replicates, each with 10 seeds.

1. *Aqueous extract bioassay.* Five g dried and crushed leaves, stems, fruits, inflorescences and roots were separately soaked in 100 ml distilled water for 12 hours at 25-30° and filtered. These extracts were used against *Lactuca sativa, Brassica campestris, Pennisetum americanum* and *Zea mays* in standard bioassay following Dirvi and Hussain and Hussain *et al.*.

2. *Litter-bed bioassay.* One g dried and crushed shoots or roots were separately placed in a petri dish and topped with a single sheet of Whatman No. 1 filter paper. Control was similar to test except that litter was replaced with fine pieces of filter papers. Five ml distilled water was added to each dish. Seeds of the aforementioned test species were used and incubated as above following Hussain *et al.*

3. *Litter mulching experiment.* Five g crushed litter was evenly incorporated into 6x5 cm pots containing an equal amount of sterilized coarse river sand to prepare the test. Control pots were similarly prepared by replacing litter with fine pieces of filter paper. Ten seeds of the same

test species, except *B. campestris*, were sown in the test and control pots. There were 5 replicates. An equal volume of distilled water was added to every pot and the pots were incubated at 25°. Germination was recorded after 5 days and plants thinned to 4 per pot. The pots were then transferred to a daily 16 hour light period and allowed a further growth of 15 days. Fresh and dry mass was recorded at the end. Further details were given by Dirvi and Hussain [1].

4. *Natural rain-leachate bioassay.* Fifteen g shoots were spread in large funnels over a single sheet of filter paper. A collecting flask was kept below the funnel. The entire assembly was then subjected to rain during March, 1986, on a 1-meter high branch of *X. strumarium*. Simple rain water was simultaneously collected. The slow drizzle lasted for 8 hr. providing sufficient leachate and rain water. A portion of the leachate was concentrated to half of its original volume at 50°. Seeds of the aforesaid test species were used against both the concentrated leachate and the rain water control. Part of the rain leachate was saved for chromatographic analysis.

5. *Soil residual toxicity.* Soil with or without *X. strumarium* thickets was collected, air dried and sieved through 2-mm mesh. These soils were used against the same test species following our standard soil extract and soil bed bioassay techniques [4,8,10].

6. *Identification of toxins.* Ten percent aqueous shoot extract or rain leachate was concentrated to 1/3 of its original volume and acidified to pH 2.5. It was extracted 3 times with ether by reflux shaking followed by the separation of upper ether and lower aqueous fractions [17]. The three ether fractions were mixed and concentrated in a rotavapour. The residue was dissolved in 2 ml ethanol and used for spotting Whatman No. 1 chromatographic paper at a distance of 2.5 cm from the base. The chromatograms were developed in 6 % AA(6:94, acetic acid: water) and BAW (63:10:27, *n*-butanol: acetic acid: water) in ascending direction. The dried chromatograms were inspected under short (254 nm) and long (360 nm) UV light, sprayed with diazotized *p*-nitro aniline, diazotized sulphanillic acid, and ferric chloride-potassium ferricyanide reagents. The colours and Rf were determined and compared with standard reference compounds which were simultaneously developed and treated in the same way.

RESULTS

1. *Aqueous extract bioassay.* the germination of *Lactuca* was decreased in extracts of leaves and inflorescences, and radicle growth of all the test species was significantly reduced by aqueous extracts from different parts (Table 1). Leaves and inflorescences were generally more inhibitory. The toxicity was species and part specific.

2. *Litter bed bioassay.* The germination of *Lactuca* and *Brassica* was reduced in shoot litter, and radicle growth of all the test species was adversely retarded by both shoot and root litter (Table 1). The growth varied from 2.95 % by shoots (*Brassica*) to 86.87 % by roots (*Pennisetum*) among the species.

3. *Litter mulching experiment.* Litter incorporated into the nutrient rich growth medium significantly decreased germination of *Lactuca* and *Pennisetum*, and fresh and dry mass of all the test species (Table 1). The dry weight varied from 15.25 % (*Lactuca*) to 60.23 % (*Zea*).

4. *Natural rain leachate bioassay.* Non-concentrated

Table 1. Effect of aqueous extract and litter of *X. strumarium* on the germination and growth of test species. (All values are expressed as % of control).

Test species	Aqueous extracts					Litter bed bioassay		Litter mulching experiment	
	Leaves	Inflore-scences	Fruits	Stems	Roots	Shoots	Roots		
Germination									
Lactuca sativa	70.00**	80.00*	100.00	100 00	100.00	80.00	100.00	60 00*	
Brassica campestris	100.00	100.00	100.00	100.00	100.00	70 00*	100.00	N.T.	
Pennisetum americanum	100.00	100.00	100.00	100.00	100.00	100.00	100 00	80.00*	
Zea mays	100.00	100.00	100.00	100.00	100.00	100.00	100.00	94.00	
Radicle Growth							Fresh mass	Dry mass	
L. sativa	9.28**	8.78**	57.53**	61.64**	81.66*	53.02*	83.09*	79.52*	15.25**
B. campestris	52.30**	48.73**	74.07**	66.42**	68.35**	2.95**	39.58**	N.T.	N.T.
P. americanum	21.46**	26.07**	65.48**	65.48**	43.20**	44.78**	86.87*	71.42*	46.23*
Z. mays	58.11**	80.03*	63.08*	77.89*	84.23*	49.85**	80.53*	81.84*	60.23**

N.T. = Not Tested, * = Significant at P = 0.05, ** = Significcant at P = 0.01.

rain leachate neither reduced germination nor the radicle growth of any test species except for the radicle growth of *Pennisetum* (Table 2). In one case there was stimulated radicle growth. The concentrated leachates reduced germination of *Lactuca* and *Pennisetum* and radicle growth of all the test species (Table 2). The inhibition varied from 78.11 % (*Lactuca*) to 88.98 % (*Brassica*) among the species.

5. *Soil residual toxicity.* Soil collected from beneath *Xanthium* did not inhibit germination and early growth of any test species due to leaching of toxins by rains. Rains had washed off the soil to almost non-toxic level and, therefore it was not inhibitory.

6. *Identification of phytotoxins.* Caffeic, *p*-coumaric, p-hydroxybenzoic, chlorogenic, and ellagic acids were identified as the possible inhibitors in shoot extracts and rain leachates (Table 3). All of them are easily water extractable and proven allelopathic substances and therefore, they were not assayed for their phytotoxicity.

Table 2. Effect of natural rain leachate of *X. strumarium* on the germination and early growth of test species. (All values are expressed as % of control).

Test Species	Germination		Radicle growth	
	Non-concentrated	Concentrated	Non-concentrated	Concentrated
L. sativa	94.00	76.00	95.00	88.11**
B. campestris	100.00	92.00	110.00	88.98*
P. americanum	100.00	82.00*	85.66*	84.20*
Z. mays	96.00	94.00	91.25	86.15*

* = Significant at P = 0.05

DISCUSSION

Weeds suppress the growth in pre and post-emergence phases of life due to allelopathy. Inhibition also retards the soil fertility by delaying the nitrogen cycle [15]. *Xanthium* is an annual weed which regularly sheds litter after completing the life cycle. The accumulated litter might set free water soluble toxins during soaking by natural or irrigation water. It was observed that aqueous extracts from different parts adversely reduced the germination and growth of test species to varying extents suggesting the presence and leachability of some water soluble toxins. Aqueous extracts from other weeds [1-3, 6,8] similarly retard the germination and growth of cultivated species. Similarly seeds growing in contact with wet litter exhibited retarded germination and growth. Extraction of toxins in the laboratory simulate the natural release of toxins. This is also indicated by the fact that natural rain leachates were inhibitory like the aqueous extracts. Rain is one of the important toxin transporting agency. The litter from *Xanthium* incorporated into nutrient rich growth medium reduced both germination and biomass of all the test species. This was primarily due to the release of phytotoxins from the added litter. Likewise, litter from *Dichanthium* [1] and *Eragrostis* [8] exhibit allelopathy. Test species had poor growth in the substrate containing

Table 3. Identification of some phytotoxins from *X. strumarium* shoots.

Compound	Rf on		UV		Colours with		
	6AA	BAW	Short (254 nm)	Long (360 nm)	FFC	DSA	DPA
1. Known caffeic acid	.47	.91	Light blue	Bright blue	Blue	Yellow brown	Pale yellow
Suspected caffeic acid	.46	.90	Light blue	Bright blue	Blue	Yellowish-brown	Light yellow
2. Known *p*-coumaric acid	.70	.97	Absorbed	Absorbed	Blue	Red	Off white
Suspected *p*-coumaric acid	.69	.98	Absorbed	Absorbed	Blue	Red	Off white
3. Known *p*-hydroxy-benzoic acid	.34	.80	Light blue	Bright blue	Blue	Orange red	Off white
Suspected p-hydroxy-benzoic acid	.34	.81	Light blue	Bright blue	Blue	Yellowish red	Off white
4. Known chlorogenic acid	.61	.68	Light blue	Bright blue	Blue	Light brown	Light brown
Suspected chlorogenic acid	.61	.69	Light blue	Bright blue	Blue	Brownish	Light brown
5. Known ellagic acid	.02	.34	Absorbed	Absorbed	Blue	None	None
Suspected ellagic acid	.02	.35	Absorbed	Absorbed	Blue	None	None

6 % AA = 6:94, acetic acid:water BAW = 63:10:27, *n*-butanol: acetic acid: water; FFC = Potassium ferricyanide-ferric chloride, DSA = diazotized sulphanillic acid; DPA = diazotized *p*-nitroaniline.

Euphorbia litter [2,9]. These observations strengthen these authors' view regarding the release of phytotoxins from *Xanthium*. The accumulation and effectivity of toxins in the soil is an important factor. Soil collected from underneath *Xanthium* had almost no toxicity. This was most probably due to the lack of accumulation of phytotoxins owing to soil leaching. The soil was collected during July – August and it is the time when frequent rains might have washed the added toxins leaving behind a toxin-leached soil. Muller [12] attributed the failure of *Fransiera* to exhibit allelopathy in field due to lack of accumulation of allelopathic substances. Similarly Hussain *et al.* [10] demonstrated that soil underneath *Stachys* did not express allelopathy due to rain leaching in spite of its strong allelopathic behaviour. This might be true in the present study. A similar observation was recorded when the test species remained unaffected by non-concentrated rain leachates but were severely retarded in their growth by the concentrated rain leachates. This suggests that the amount of phytotoxins and its accumulation are ecologically important in allelopathic processes. Rice [15] recognized that low concentrations of allelopathic substances may be stimulatory or non-toxic but higher concentration of the same substance are quite inhibitory. We, therefore suggest that intoxicated soil becomes favourable due to leaching by rains. The identification of caffeic, *p*-coumaric, *p*-hydroxy benzoic, chlorogenic, ferulic and ellagic acids in shoot extracts and rain confirms the allelopathic nature of *X-anthium*. These are all water soluble and proven allelopathic agents [15] and, thus, we did not assay their phytotoxicity against the present test species. The findings reveal that phytotoxins from the deposited litter will release into the soil and make it unfavourable for susceptible species, provided other environmental factors favour allelopathy. It is, therefore, suggested that litter from this weed should not be allowed to decay in the fields due to allelopathy. Since phytotoxicity depends upon the sensitivity of the species, more susceptable species might be eliminated from the common habitat. Allelopathy by *X. strumarium* may indirectly affect the crop species by suppressing the nitrogen cycle [15], but we did not test this possibility. However, allelopathic effects always depend upon a number of related environmental factors that can modify its influences.

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