Allelopathic Potential of Adhatoda vasica NEES

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Adhatoda visica NEES의 알레로페티 효과

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ABSTRACT

Adhatoda vasica Nees is a shrubby component of tropical and subtropical vegetation in Pakistan. It harbours relatively few unhealthy associated species in and around its thickets. Aqueous extracts, rain leachates, litter from shoots and soil underneath it invariably reduced germination, early growth, biomass, moisture and chlorophyll contents of Pennisetum americanum, Setaria italica, Zea mays, Brassica campestris and Triticum vulgare in different laboratory experiments. Chromatographic analysis revealed the presence of caffeic, ferulic, vanillic, p-coumaric, p-OH-benzoic, and tannic acids in aqueous extracts. The phytotoxicity was related to the test species used, part assayed and parameter measured. It is suggested that the preclusion of the associated species and the dominance of A. vasica is primarily due to allelopathy. Negative grazing also provides better chances for its establishment.

INTRODUCTION

Allelopathy is an important ecological factor governing the vegetation dynamics, pattern and sociability of the species in nature (Chou, 1980; Kil and Yim, 1983; Rice 1984). It is a negative factor in the formation of associations and communities. Low species diversity due to exclusion of the susceptible species from the common habitat is also attributed to allelopathy.

Hussain et al. (1979) observed unhealthy association of other plants with Datura innoxia. Hussain et al. (1982) attributed inhibition followed by the elimination of associated species by Cenchrus ciliaris and Bothriochloa pertusa due to allelopathy. Muller (1970) reported spacing in the vicinity of Artemesia californica owing to allelopathy. Lodhi (1975) reported bare understory below Celtis laevigata. Similarly, Melia harbours relatively low and unhealthy understory due to phytotoxicity (Hussain et al., 1985). There are strong evidences concerning the exclusion of

associated species resulting in the dominance of allelopathic species (Rice, 1984).

Adhatoda vasica is an important understory shrubby plant in the degraded dry thorny tropical and subtropical vegetation in Pakistan. Deforestation and overgrazing has degraded the original forests into an open Adhatoda scrub. It has the tendency to dominate as understory plant and excludes the associated species. Negative grazing might also help in its dominance. Few unhealthy species can grow within and adjacent to Adhatoda thickets. Keeping in mind the role of allelopathy and the tendency of Adhatoda to form almost pure thickets the present investigation was, therefore, carried out to find its allelopathic potential and to identify the phytotoxins responsible for this phenomenon.

MATERIALS AND METHODS

Nature plants of Adhatoda vasica were collected from Attock Nizam Pur area. They were separated into stems and leaves and stored at room temperature (25–30°C). Glassware, thoroughly washed with tap water, were sterilized at 170°C for 4 hours before use. General preparation of the experiments is given by Hussain et al. (1979, 1985, 1984).

Aqueous extract biassay. Five gram of dried and crushed stems or leaves was separately soaked in 100ml distilled water at 25°C for 24 hours, filtered and designated as cold water extracts.

Similary, 5 gram of plant parts was separately boiled in 100ml distilled water for five minutes, filtered, cooled to room temperature and designated as hot water extracts. These various extracts were used against Setaria italica, Pennisetum americanum, Triticum vulgare, Zea mays and Brassica campestris following Hussian and Gadoon (1981), Hussain et al. (1984). There were five replicates, each with 10 seeds.

Germination, radicle and plumule growth were recorded sowing after 48 h. Twenty uniform seedlings were selected from each treatment for the determination of fresh and dry weight. Moisture contents were calculated on oven dry basis. Seedlings were dried at 65°C for 72 hrs.

In another experiment pots were filled with 200 gram of thoroughly sterilized soil and seeds of the aforesaid test species were sown. There were 10 seeds in each pot with 5 replicates. The pots were provided with 60 ml hot water extracts or distilled water for making test or control, repectively.

They were incubated at 25°C for 7 days. After recording the germination the plants were thinned to 4 uniform healthy seedlings per pot and transferred to 16h photoperiod for fortnight. Height, fresh and dry weight and chlorophyll contents were determined at the end. Chlorophyll contents were spectrophotometrically determined using spectronic-20 following Harborne (1973).

Litter-bed bioassay. One gram of dried and crushed leaves was evenly spread in a Petri dish and topped with a single sheet of filter paper. Ten seeds were sown on it with 5 replications. Ten ml of distilled water was added to each dish. Control was similar to the test

except that litter was replaced with filter papers. The dishes were incubated as above mentioned and same paramters measured.

Aqueous culture experiment. Three week old, uniform and healthy seedlings of the test species, raised in organically rich soil, were carefully rooted out and their roots thoroughly washed with water. They were individually transferred to a sterilized glass vial containing 30 ml of test or control solution. Test solution was prepared by mixing equal volumes of 5 hot water extract with Hoagland nutrient solution. Control was a mixture of equal volumes of distilled water and Hoagland solution. There are 10 replicates. The vials were plugged with cotton and covered with brown paper to avoid light penetration. The seedlings were kept under 16 h light period for 15 days. Mortality was recorded on 2nd, 4th, 6th and 8th day. Moisture contents were determined on oven dry basis.

Mulching experiment. Glass pots were filled with 200 gram of thoroughly washed and sterilized coarse river sand. One gram of powdered shoots was incorporated into the top layers for making test. Control had the same amount of fine pieces of filter papers. Each pot was provided with 60 ml Hoagland nutrient solution. Seeds of the same test species were used. The pots were incubated for one week at 25°C. The seedlings were thinned to 4 uniform and healthy seedling per pot and transferred to 16 h photoperiod at room temperature.

Height, fresh and dry weight and chlorophyll contents were determined after another 14 days. There were 5 replicates with 10 seeds in each pot.

Natural rain leachate bioassay. Fifteen gram of crushed stems and leaves was spread over the filter papers in large funnel. They were exposed to slow drizzling rain in such a way that rain dripped through plant material into a collecting flask lying beneath the funnel. A similiar arrangement without any plant material was made for collecting simple rain water for making control. The entire rain-leachate collecting assembly was kept 1 ml above the soil after it had rained for almost half an hour. The rain leachates and simple rain water were then used against the same aforesaid test species as before. Same parameters were determined at the end of bioassay.

Soil residual toxicity. Soil was collected from interior of *Adhatoda* thickets and from a bare area in the same locality for making test and control respectively. It was air dried, meshed through 2 mm sieve and used in the following experiments.

Soil bed bioassay. Ten gm of test or control soil was uniformly spread in a Petri dish and topped with a single sheet of filter paper and seeds placed on it. Every dish was moistened with 10 ml distilled water and incubated following Hussian and Gadoon (1981).

Soil extract bioassay. Twenty gm of test or control soil was separately shaken in 100 ml distilled water for 4 hours and filtered. These soil extracts were then used against the same test species following Hussain and Gadoon (1981). There were replicates, each with 10 seeds.

Germination, radicle and plumule growth, number of seminal roots, fresh, dry weight and moisture contents of the seedlings were determined in each experiment.

Identification of phytotoxins. Ten percent aqueous extracts from shoots were concentrated to 1/3 of to original volume and acidified to pH 2.5. It was extracted three times with ether. The three etherial fractions were mixed and evaporated to dryness. The residue was dissolved in 2 ml ethanol and spotted on Whatmans No. 1 chromatographic paper. The chromatograms were developed in 6 AA (6:94, acetic acid: water) and BAW (63:10:27, n-buthanol: acetic acid: water). The dried chromatograms were inspected under short (256 nm) and long (366 nm)UV lights and sprayed with diazotized p-nitroaniline, diazotized sulfanilic acid and ferric chloride-potassium ferricyanide reagents. Standard compounds were simultaneously run in the same way for comparsion.

RESULTS

Cold water extract. The germination of Setaria italica and Brassica campestris respectively decreased to 11 and 74 in leaf extract and to 63 and 72 in stem extracts. The remaining species were unaffected. The plumule and radicle growth of all the test species, except B. campestris in leaf extract and Triticum aestivum in stem extracts, were severely reduced in the test condition. The seminal roots of T. aestivum and Zea mays decreased in both the extracts. The moisture contents of S. italica, Pennisetum americanum and T. aestivum in leaf extracts and of Z. mays and T. aestivum in stem extracts declined(Table 1).

Hot water extract bioassay. The germination of P. americanum and B. campestris in leaf extract and of all the test species, except T. aestivum in stem extracts, was severely retarded in the test. The plumule and radicle growth of all the test species, except plumule growth of B. campestris, was highly reduced by both the extracts.

The seminal roots of T. aestivum and Z. mays decreased in the test condition. The moisture contents of P. americanum and T. aestivum in leaf and stem extracts and that of S. italica in stem extract largely declined (Table 1).

Pot experiment. The germination of all the test species, except Z. mays and T. aestivum, was severely inhibited by the added extracts (Table 2). The fresh weight of S. italica, P. americanum and B. campestris shoots and those of S. italica and T. aestivum roots declined severely under the test condition. The dry weight of S. italica roots and B. campestris shoots largely decreased while the remaining species were unaffected. The moisture contents of S. italica and B. campestris shoots and of the roots of latter species severely decreased. The total and horophyll "a" and "b" reduced in all the test species.

'er bed bioassay. Excepting T. vulgare, the germination of all the test species significantly reduced when they grew upon the litter beds. The germination ranged from 38% to 80% among the species but one species. The plumule and radicle growth of all the test species were significantly retarded in the growth medium containing litter. It ranged from 4 (Zea) to 35" (Pennisetum) among the species. While the raticle growth varied form zero (Zea) to 57% (Brassica) among the species. T. vulgare and Z. mays faild to produce seminal roots in the test

Table 1. Effect of aqueous extracts and litter form Adhatoda vasica on the germination and early growth of test species. All values are expressed as percentage of control

	Co	Hot		_	
Test species	Leaves Stems Lea		Leaves	Leaves Stems	
	Germination				
Setaria italica	11.20	63.41	100.00	58.20	52.2
Pennisetum americanum	100.00	100.00	78.00	80.00	80.0
Triticum vulgare	113.14	102.02	113.14	200.00	200.0
Zea mays	106.07	106.08	109.09	38.80	38.8
Brassica campestris	74.00	72.00	40.00	68.57	68.58
	Plumule Grown	h			
Setaria italica	44.47	41.83	13.86	42.4	21.217
Pennisetum americanum	4.57	37.01	57.14	42.85	35.45
Triticum vulgare	18.07	260.84	23.4	40.96	6,404
Zea mays	32.82	61.06	23.66	29.00	4.35
Brassica campestris	156.50	65.39	320.24	470.04	33.25
	Radicle Growtl				
Setaria italica	4.14	7.96	13.8	87.38	2.71
Pennisetum americanum	84.08	17.56	44.36	43.26	50.58
Triticum vulgare	12.34	160.49	28.39	40.5	2.06
Zea mays	47.52	5.44	69.4	62.5	0
Brassica campestris	94.08	17.56	44.36	43.26	57.36
	No. of Semina	l Roots			
Triticum vulgare	1.8	2.5	5.9	2.9	-
Zea mays	1.5	3.1	2.1	2.4	_
	Moisture Conte	1118			
Setaria italica	60	100	643.88	60	54.67
Pennisetum americanum	43.38	106.52	59.03	79.02	86.93
Triticum vulgare	69.62	86,89	63.62	66.28	43.09
Zea mays	160.62	87.94	272.702	90.99	45.46
Brassica campestris	288.92	253.08	182.45	442.96	160.35

Table 2. The results of aqueous culture experiment. All values are expressed in percentage

Test species	Germi-		Dry	N4 :	Chlorophyll		
	nation		Moisture -	Total	a	ь	
Sciaria italica shoot	58.89	74.73	121.69	58.99	83.009	47.56	83.84
Root		59.59	82.72	959.43			
Pennistum americanum							
shoot	82.95	78.67	260.08	120.8	71.63	41.99	74.89
Root		172.73	106.78	179.97			
Triticum vulgareshoot	94.57	95.26	100	94.57	79.39	46.83	54.09
Root		80.99	128.702	188.93			
Zea mays shoot	131.54	121.48	97.85	104.35	67.34	34.94	33.95
Root		245.58	97.408	134.37			
Brassica campestris							
shoots	32.94	25.82	84.74	32.94	87.98	83.52	85.55
Roots		137.57	117.94	52.26			

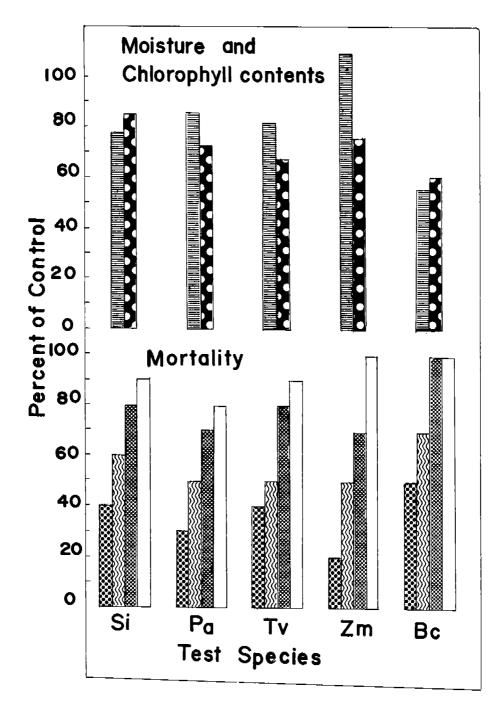


Fig. 1. Effect of aqueous extracts on the moisture contents (≥), chlorphyll contents (≥), mortality on 2nd (≥), 4th (≥), 6th (≥) and 8th (□) of Scraria italica (SI), Penniserum americanum (Pa), Triticum vulgare (TV), Zea mays (Zm) and Brassica campestris (Bc) seedlings. All values are significantly different from control. Each bar is a mean of 10 seedlings.

conditions. The moisture contents of all the test species, except B. campestris, distinctly decreased due to litter (Table 1).

Aqueous culture experiment. All the test species exhibited 20–50% mortality on the 2nd day of treatment which increased to 80% (*Pennistum*), 90% (*Triticum*) and to 100% (*Zea* and *Brassica*) on the 8th day (Fig. 1).

The shoot moisture and chlorophyll contents of all the test species, except moisture contents of Z. mays, very declined under test condition in nutrient solutions containing extracts.

Mulching experiment. The germination of all the species except Z. mays decreased in the test condition. The weight of all the species, the fresh and dry weight of T. vulgare and Z. mays roots and shoots severely decreased in the growth medium containing mulch (Table 4). However, there was no remarkable difference in the moisture contents of the shoots, except

a slight reduction in Z. mays and P. americanum. The total chlorophyll and chlorophyll "a" and

Table 3. Effect of natural rain leachateand effected soil on the germination and growth of test species. All values are expressed as % of control

	Natura	Natural rain			
	Leachat	Bioassay	Extract		
	leaves	Stem	•	Bioassay	
•	Gernination				
Seraria italica	85.00	95.00	97.43	104.75	
Pennisetum americanum	84.73	84.10	75.51	102:32	
Triticum vulgare	97.9	100.00	95.91	223.3	
Zea mays	84.6	4.6	62.5	133.3	
Brassica campestris	90.00	90.00	67.74	94.59	
	Plumule Growth				
Setaria italica	32.21	89.27	54.48	5.78	
Pennisetum americanum	38.12	74.82	53.14	8.2	
Triticum vulgare	5.88	9.80	109.02	3.()4	
Zea mays	15.97	12.91	33.99	3.2	
Brassica campestris	51.85	118.51	3.02	2.5	
	Radicle Growth				
Setaria italiça	15.63	73.85	84.19	63.22	
Pennisetum americanum	29.28	48.33	83.56	92.48	
Triticum vulgare	26.70	62.78	2 5.79	44.66	
Zea mays	80	90	30.18	124.07	
Brassica campestris	75.60	48.78	76.93	63.82	
	No. of Seminal Ro	ots			
Triticum vulgare	100	79.4	95.91	116.30	
Zea mays	-	-	_	-	
	Moisture Contents				
Setaria italica	96.63	106.61	128.44	106.3	
Pennisetum americanum	70.33	256,87	129.05	154.41	
Triticum vulgare	71.49	97.71	62.73	79.51	
Zea mays	96,01	79.26	95.35	139.69	
Brassica campestris	74.63	6.09	85.53	164.97	

Table 4. Effect of Adhotoda mulch on the germination and early growth of test species

					Dry weight	Moisut- re	chloro-	Chlorophyll	
Test species		Germi- nation	Height	Fresh weight				phyll	chloro- phyll
Setaria italica		85,82	89,39	99.80	99.98	99.94	phyll 82.98	55,98	<u>b</u> 16.45
	shoot Root			99.82	99.92	99.99			
Pennistum americano	nn								
	shoot	37.5	75.39	99.77	99.99	99.98	85.85	27.98	83.72
	Root	-	-	99,94	99,99	99.95			
Triticum vulgare	shoot	66,76	57 .67	99.73	98.7	101.14	89.27	37.69	53.39
	Root	_	_	24.03	5.93	111.6			
,	shoot	74.52	87.84	99.15	99.14	100.2	77	88.59	24.64
	Root	_	-	50.05	57.23	87.54			
Brassica campestris	al	87.9 2	66.46	99.82	99,99	100.33	67 F3	31.67	137.95
•	shoot	01.72	66.46	77.8 <u>4</u>	<i>9</i> 9.99	100.32	67.52	31.07	137.93
	Root	-	-	99.78	99.85	100.48			

[&]quot;b" of all the species declined in the test condition (Table 2).

Natural rain leachate bioassay. The germination of S. italica, P. americanum and Z. mays in leaf and stem leachates and that of S. italica in leaf leachate severely decreased suggesting the presence of inhibitors (Table 3) with in the rain. The leachates severely decreased the growth of seminal roots of Z. mays. The plumule and radicle growth of all the test species, except Z. mays, severely decreased in test condition (Table 3). The moisture contents of Z. mays, T. vulgare and B. campestris in stem leachates remarkably decreased in the test condition.

Soil residual toxicity. There was no effect on the germination of any of the species tried. The germination of P. americanum, Z. mays and B. campestris severely reduced during their growth on the soil beds. The plumule growth of all the test species except that of T. vulgare was heavily reduced by the affected soil. The Seminal roots of Z. mays and the radicle growth of all the species except P. americanum and Z. mays in soil extract was severely retarded by the Adhatoda soil. The moisture contents of T. vulgare in both the experiments and that of B. campestris in soil bed decreased suggesting inability of the species to absorb moisture from the growth medium (Table 3). The reduced germination and growth of susceptible species in Adhatoda soil suggest the presence and negative effect of phytotoxins in it.

Identification of phytotoxins. Some of the identified phytotoxins from the aqueus extract of *A. vasica* shoots were *p*-coumaric, *p*-hydroxybenzoic, vanillic, ferulic, caffeic and tannic acids. All of them are proven allelopathic substances and, therefore, they were not assayed for their phytotoxicity (Hussain *et al.*, 1987; Naqvi, 1976; Rice, 1984).

DISCUSSION

The release and subsequent accumulation of stimulatory or inhibitory water soluble substances from plants are a natural process that affects the physicochemical properties of soil. The plants deposite litter to apparantly improve fertility and physical nature of the soil. However, litter from some plants is capable of suppressing the growth of associated species due to phytotoxicity as observed in the present study.

The soil beneath Adhatoda thickets is rich in organic matter but harbours few unhealthy species. The aqueous extracts from shoots not only retard the germination and early growth but also reduce the biomass and moisture contents of the susceptible species. This suppression was due to some inhibitors released in the extracts. Muller (1970) reported aqueous extract of Salvia to be allelopathic. Hussain et al. (1985) observed aqueous extracts from Melia to be phytotoxic. Datura innoxia (1979), Eragrostis poaeoides (Hussain et al. 1984) and Trianthema portulacastrum (Hussain et al., 1987) were similarly phytotoxic. Many other shrubby plants exhibit allelopathy (Rice, 1984) our findings agree with them. The test species were inhibited more when they grew directly upon the Adhatoda vasica litter suggesting the release and effectivity of phytotoxins. Litter from Dichanthium annulatum (Dirvi and Hussain, 1979), Melia (Hussain et al., 1985) and Salvia (Muller, 1970) likewise suppressed the growth of test species and the present findings agree with them. The released toxins create and undesirability of the immediate habitat owing to their effectivity in the soil which is and important factor to manifest allelopathy. The test species exhibited retarded germination and growth in nutrient rich substrate containing shoot litter or aqueous extracts of A. vasica suggesting the failure of nutrients to climinate the phytotoxicity. Similar findings have been reported by other workers (Dirvi and Hussain 1979; Hussain et al., 1985, 1987; Lodhi, 1975)and they also support our view. Rain helps transport the water leachable toxins into the soil. This view was strengthened by the strong inhibitory nature of natural rain leachates from A. vasica shoots. The test species growing in the rain leachates were significantly retarded in germination and growth due to phytotoxins. Distilled water and simple rain water had no effect on the germination and early growth of test species.

Moist soils are capable of extracting the toxins from wet litter in amounts sufficient to retard the growth and germination of susceptible species as demonstrated by this study. The inhibitory effects of *A. vasica*, soil suggest that it acquired toxicity owing to *A. vasica* which contain caffeic, ferulic, vanillic, *p*-coumaric, *p*-hydroxybenzoic and tannic acids and all of them are proven allelopathic agents (Naqvi, 1976; Rice, 1984) and have been isolated from affected

soils (Lodhi, 1975). We, therefore, trusting upon them did not make further assay the phytotoxicity of these toxins. The phytotoxins also decreased the moisture and chlorophyll contents. The reduction in moisture contents was due to some physiological disorders in plant functions (Hussain et al., 1979; Hussain et al., 1985; Hussain et al., 1987). The test species had low chlorophyll contents that will directly hamper in food production and offer slow food starvation of the affected species. Rice (1984) and Hussain et al. (1987) reported reduced chlorophyll and our findings agree with them. The laboratory findings agree with the situation expected in nature. A. vasica will set free toxins with rain, dew and moisture into the soil to intoxicate it. The observed poor and unhealthy association of few species with A. vasica and its ultimate dominance seems to be due to allelopathy. The negative grazing of A. vasica gives it a further edge over the palatable species to increases the allelopathic stress. Allelopathy might further deplete the soil fertility by retarding the nitrogen cycle and root nodulation in nature. Further study is in progress to envisage its allelopathy against some of its most important natural associates, to isolate and identify the toxins from the affected soil.

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적 요

파키스탄에 나는 Adhatoda vasica는 열대, 아열대성 관목으로 덤불을 이루고 있는데 그 주위에는 식물이 비교적 드물고 잘 자라지 못한다. 그래서 이 식물의 수용추출액, 빗물세탈액, 낙엽 낙지 및 임상토양을 써서 5종 실험 식물의 종자발아, 유식물 생장, 생체량, 수분과 엽록소 함량을 조사해 본 결과 상당히 억세됨을 알았다. 크로마토그래피 분석으로 caffere acid등 5종의 화학물질을 확인했으며 이들은 A. vasica식물이 우점하며 그 주위 식물에 비치는 영향 즉 알레로패티작용에 일차적으로 관계가 있다고 본다.

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