ALLELOPATHIC EFFECTS OF DATURA INNOXIA MILL.

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Abstract

Relatively bare areas are frequently present under and around *Datura innoxia* Mill thickets, although several herbaceous species may grow well under other adjacent plants causing a shade equal to that the causing a shade equal to the causing and growth of test species by root exudates, aqueous extracts from various parts, leachates and substances volatilizing from its shoot. Soil collected under and around *Datura* was inhibitory against the test species. The phytotoxicity depended upon the part assayed, its age, test species used and the physiological process involved. The presence of relatively bare areas under and around *D. innoxia* was due primarily to allelopathy.

Introduction

Datura innoxia Mill widely distributed in warm climates including Pakistan, prefers loamy soil under semi-shade conditions. Relatively bare areas frequently occur under and around it. Several herbaceous species have been found profusely growing under other plants, causing an equal shade to that of Datura in the same area. The elimination of herbaceous species under and around Datura might be due to release of some biochemical inhibitors. The vorks of Qadir & Abbasi (1971), and Mubarak & Hussain (1978) have indicated the possibility of seed germination inhibitors responsible for delayed and irregular germination in Datura seeds.

Evidences concerning the role and importance of allelopathy have been widely reported (Muller, 1965, 1966, 1967, 1969; Naqvi & Muller, 1975; Akhtar et al., 1978; Dirvi & Hussain, 1979; Hussain et al., 1979). The suppression of growth and presence of bare areas due to allelopathy by Salvia and other shrubs has been confirmed (Muller, 1965, Muller & Muller, 1964; Muller et al., 1964, 1968). The absence of herbaceous

plants under *Celtis* (Lodhi & Rice, 1971; Lodhi, 1975), *Adenostoma* (McPherson & Muller, 1969; McPherson *et al.*, 1971), *Eucalyptus* (delMoral & Muller, 1970) was due to allelopathy.

Keeping in view the poor representation of vegetation under and around *D. innoxia* thickets and from aforementioned evidences, we hypothesized that *Datura* exhibits allelopathy, responsible for the reduction of associated species. The present studies were therefore undertaken to prove this hypothesis.

Materials and Methods

Datura plants, collected from Peshawar University Campus, were dried at room temperature (25-30 °C) in shade. Aqueous extracts were stored at 5-10 °C and used within a week. Petri dishes and other glass ware were sterilized at 170 °C for 4 h (Meynell & Meynell, 1970). The results were statistically analysed using "Z & t test" (Cox, 1967), Nomenclature followed is that of Stewart (1972).

Results

1. Field Experiment.

Datura was grown in mixed cultures with Capsicum annuum, Lycopersicon esculentum, Pennisetum americanum and Setaria italica in loamy field plots in alternate rows. Mono cultures of Datura or test species were made by extending the rows of mixed cultures on either side which served as control.

There were 4 rows of each species in each combination. Each row was considered as a replicate. Moisture deficiency was avoided. Five plants were randomly selected in each row from each treatment after 8 weeks. Plants were harvested and dried at 60 °C for 72 h.

Datura reduced height and dry weights of all the test species in mixed cultures. Datura gained both in height and dry weight in mixed cultures with Capsicum and Lycopersicon but was inhibited by Pennisetum and Setaria (Table 1). The reduction in dry weights of the test species was more than 50%.

2. Pot Experiment.

Equal volume of litter free loamy soil was taken in 20x22 cm pots, lined with polyethylene sheets. *Datura* was grown in combination with the same 4 aforementioned test species having either root mixed or root separated treatments. The roots of the interfering species were separated by polyethylene partitions in the pots. Each combination had 5 replicates, each with 5 plants of either *Datura* or test species in each half of the pot.

Competition for moisture and nutrient was avoided by providing equal amount of water and Hoagland's solution weekly. The pots received uniform environmental condi-

Table 1. Height and dry weight of above ground parts of Datura innoxia and interacting species in the field and pot experiments.

					Z	ERACTIN	G SPECIE	Š		
Species	Sowing		Capsic	nın	Lycope	rsicon	Pennise	nm	Setaria	ria
	Conditions		Height D	Dry	Height	Dry	leight Dry Height Dry	Dry		Dry
			(CIII)	(g)	(cm)	(g)		(g)		weigint (g)
I. Field Experiment	T T T T T T T T T T T T T T T T T T T			*		1) 	1 P 5 F 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	: : : : : : : : : : : : : : : : : : :	
Test Species	Alone	Mean	13.00	1.75	8.85	0.75	39.50	4.43	56.95	1.53
		±SE	2.35	0.21	1.72	0.05	6.25	1.22	7.59	0.03
	Mixed	Mean	10.25*	0.72*	8.94	0.54*	28.61*	2.00*	43.25*	0.65*
		HS H	2.58	0.01	1.19	0.03	4.97	16.0	6.37	0.01
	Alone	Mean	27.25	8.53	18.85	4.95	11.40	3.12	16.60	3.03
		±SE	4.33	2.16	4.91	1.05	2.99	1.10	2.98	1.05
Datura innoxia.	Mixed	Mean	31.16*	11.08*	21.32*	5.74	11.65	1.31*	12.35*	1.80*
		∓SE	5.23	2.76	4.32	1.12	2.18	60.0	3.10	0.05
II. Pot Experiment										
Test Species	Root									
	Separated	Mean	6.15	0.11	7.14	0.18	19.48	0.15	24.36	0.19
		±SE	1.52	0.01		0.01	3.34	0.02	4.62	0.03
	Root Mixed	Mean	3.77*	0.04*	7.88	0.12*	22.06	0.13*	22.32	0.16*
		±SE	1.22	0.01	2.01	0.01	5.15	0.01	4.91	0.05
Datura innoxia	Root									
	Separated	Mean	7.92	0.18	7.82	2.23	7.60	0.22	5.98	0.17
		±SE	1.56	0.02	1.10	10.0	2.01	0.01	1.00	0.03
	Root Mixed	Mean	8.30	0.27*	7.23	0.24*	6.50*	0.14*	06.9	0.19*
		±SE	1.39	0.03	1.19	0.03	1.95	90.0	0.95	0.03

Each value is mean of 5 replicates, each with 5 plants.

*Significant at P = 0.05

tions and were regularly weeded by hand. Shoot length and dry weight of tops were determined after 8 weeks.

The dry weights of all the test species decreased in the condition of root mixing while heights remained unaffected. *Datura* gained in dry weights in mixed cultures with all the test species except in *Pennisetum* and *Datura* combination (Table 1).

Field and pot experiments confirm the results obtained in each case. Datura retarded growth of all the test species in presence of nutrients. Most probably some phytotoxins have been released from Datura roots into the soil responsible for inhibiting the growth. Moreover, substances volatilizing from Datura might have played a role in growth inhibition, as revealed by the subsequent experiments.

3. Relative toxicity of Datura parts.

Aqueous extracts were prepared by soaking separately 5 g mature or young leaves, peeled or unpeeled stems, bark, flowers, capsule wall, seeds and roots in 100 ml double distilled water for 12 h at $25\,^{\circ}\mathrm{C}$.

The extracts were used against *Brassica campestris, Lactuca sativa* and *Setaria italica* in standarad filter paper bioassay following Mubarak & Hussain (1978). Each species in each treatment had 10 replicates, each with 10 seeds. Germination and radicle growth recorded after 48 h incubation at 26 °C showed that *Brassica*, could not germinate in any of the treatments except that of seed extract (Table 2). The germination of most of the species was significantly inhibited. Mature leaves were more toxic than young leaves which closely approached it. while seed extract was least toxic. All parts inhibited the germination but response was species related.

The radicle growth of all the test species was significantly inhibited by all the extracts. Mature leaves had more toxicity than other parts, followed by stem. Flower, capsule wall and roots were third in order of toxicity followed by young leaves. The toxicity depended upon the part assayed and test species used. *Brassica* was the most sensitive followed by *Lactuca*, *Setaria* and *Pennisetum*, respectively.

4. Artificial Leaching Experiment.

P. americanum and S. italica, sown in 10x10 cm pots having litter free loamy soil, were kept under Datura plants. Control pots were kept under a similar shade away from Datura. Tests were made by spraying equal amount of double distilled water over Datura shoots which dripped down to the pots. Control pots were watered directly. There were 4 replicates, each with 15 seeds.

The experiment was conducted under field conditions in June, 1977. Germination was recorded after 10 days and plants thinned to 4 per pot. Plants were harvested after 3 weeks and dry weight determined.

Table 2. Relative toxicity of Datura innoxia parts against germination (G) and radicle growth (R) of the test species.

Extracts		1 T T B B T B B F F F B B B B B B B B B B	1	Test S	Test Speceies			1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	Brassica campestris	a is	Lactuca sativa	p.	Pennisetum americanum	т пт	Setaria italica	z i
	9	x	Ö	R	Ö	R	Ö	R
Mature Leaves.	* *	*	* * 	* *	16.66**	**29"0	15.30**	1.12**
Young Leaves.	* *	*	8.53**	1.17**	98.86	72.25**	97.95	24.34**
Unpeeled stem.	*	*	**/6.01	10.53**	101.11	38.48**	64.28**	14.94**
Peeled stem.	* *	*	40.24**	3.98**	95.55	51.29**	83.67**	10.41**
Bark.	*	*	25.25**	2.60**	102.22	35.00**	62.24**	9.51**
Flower.	*	*	*	*	88.88	**96.61	*67.68	14.83**
Capsule wall	* *	*	*	*	95.55	14.70**	56.12**	5.91**
Seed.	**16.09	20.20	68.29**	30.67**	102.22	90.70	97.95	88.16*
Root.	**	* *	* *	*	17.77**	1.96**	87.75*	21.57**
								1

Each value is the mean of 10 replicates, each with 10 seeds, expressed as % of their control.

^{*}Significant at P = 0.05 **Significant at P = 0.01

Table 3. Effect of artificial leaching from *Datura innoxia* on germination and radicle growth of test species.

Test Species	Control	Test	% of control
	Germination (%)		
Pennisetum americanum	75.00	58.33	77.77**
Setaria italica	40.00	36.66	91.65
	Dry Weight ± SE (mg	g)	
Pennisetum americanum	167.95±10.87	109.80±7.56	65.37**
Setaria italica	73.80± 8.96	34.25±4.25	46.23**

Germination is a mean of 4 replicates, each with 15 seeds while dry weights are mean of 4 replicates, each with 4 plants.

The germination of *Pennisetum* and dry weights of both the test species was significantly inhibited by leachates form *Datura* (Table 3). The inhibition could not be due to shade factor since control received a similar treatment.

5. Toxicity through Volatilization.

Volatile inhibitors have been found inhibitorry against the germination and growth of plants (Muller, 1964; McPherson et al, 1971; Muller et al, 1964). Following bioassays were run to assay the nature of volatiles from Datura:

a) Intact shoot bioassay in the field.

The bioassay was carried out in the field using 30x20 cm twice folded Whatman No. 1 filter paper as seed beds in 40x20 cm plastic bags. Thirty seeds of the test species were placed on these moist seed beds and topped with a single sheet of filter paper. Tests were made by introducing a Datura or Cestrum nocturnum shoot with 5 or 6 leaves into the each bag, to compare the effects of CO₂ on germination and radicle growth. The direct contact of shoots with the seed beds was avoided. Controls were without any shoot. All the bags were loosly closed at their open ends and wrapped with brown paper to avoid light penetration. P. americanum, Trifolium resupinatum and S. italica, used as test species, had 3 replicates. The bioassay was run in June, 1977, under natural conditions. Germination and radicle growth was recorded after 48 h. The germination of Pennisetum and Setaria was significantly inhibited in Datura atmosphere where as in Cestrum there was no affect (Table 4).

^{**}Significant at P' = 0.01

Table 4. Effect of volatile from intact shoot of *Datura innoxia* on germination and radicle growth of the test species grown under natural conditions.

Test Species	Control	Cestrum Atmosphere	% of Control	Datura Atmosphere	% of Control
	Germinati	ion (%)			
Pennisetum americanum	93.30	86.60	92.81	70.00	75.02**
Trifolium resupinatum	93.33	90.00	96.46	90.00	96.44
Setaria italica	95.00	93.33	98.24	73.33	77.18*
	Radicle G	rowth (mm)			
Pennisetum americanum	32.80	25.70	78.35*	4.50	13.71**
±SE	6.29	3.75		1.05	
Trifolium resupinatum	15.23	13.78	90.47	6.08	39.92**
±SE	4.35	2.98		1.22	
Setaria italica	28.10	14.69	52.27*	6.06	21.56**
±SE	4.91	2.77		1.67	

Each value is the mean of 30 seeds in triplicate.

The radicle growth of all the test species was significantly reduced (p-0.01) in Datura atmosphere. The growth was not more than 40% of control in any of the species (Table 4). Cestrum retarded growth of Pennisetum and Setaria. The comparison of Cestrum and Datura shoot atmospheres indicates that CO_2 alone cannot inhibit the growth to such an extent as observed in Datura. In the case of Datura volatile toxins inhibited the growth and CO_2 might have accelerated the toxic mechanism.

b) Detached shoot bioassay in Laboratory.

Twenty g fresh mature leaves were placed in 15x6 cm containers. Seeds of Brassica campestris, S. italica, Lactuca sativa and P. americanum placed on twice folded Whatman No. 1 filter paper, were placed in these containers simulating the atmosphere in the proximity of Datura. Control were made similarly by replacing Datura leaves with moist filter paper. Containers with or without leaves were sealed. There were 5 replicates, each with 20 seeds. Germination and radicle growth was recorded after 48 h incubation at 26 °C.

The radicle growth of all test species was significantly inhibited while germination

^{*}Significant at P = 0.05

^{**}Significant at P = 0.01

Table 5. Effect of volatile from detached shoot of *Datura innoxia* on germination and radicle growth of test species, grown under laboratory conditions.

Test Species	Control	Test	% of Control
00 D D D D D D D D D D D D D D D D D D	Germination (%)	는 마마 마마 마마 마마 마마 에 마마 마마 마마 마마 마마 마마 마마 마	(C) 안 된 및 (A) 10 분석 및 선물 (C) 및 (C)
Brassica campestris	92.00	90.00	97.82
Setaria italica	95.00	93.00	97.89
Lactuca sativa	80.00	83.00	103.75
Pennisetum americanum	96.00	83.00	86.45*
	Radicle Growth ±SE	(mm)	
Brassica campestris	9.21±2.35	2.68±0.53	29.09**
Setaria italica	15.18±3.10	5.30±1.01	34.91**
Lactuca sativa	6.76±1.92	4.84±1.00	71.59**
Pennisetum americanum	10.01±2.16	4.38±0.98	43.75**

Each value is the mean of 5 replicates, each with 20 seeds.

was unaffected. Brassica was the most sensitive species followed by Setaria (Table 5). The inhibition was due to some substances volatilizing from Datura leaves in the containers.

c) Absorption of Volatile Toxins on the filter papers.

To minimize the possible suspected effects of CO₂, evolved during the respiration by *Datura* shoot, another experiment was performed. Twice folded moist filter papers were subjected to volatiles from *Datura* leaves for 7 days in the field. These filter papers were then used as the seed beds for *B. campestris* and *S. italica* in standard filter paper bioassay. Germination and radicle growth of 10 replicates, each with 10 seeds, incubated at 26 °C for 48 h was recorded.

Germination was unaffected but there was significant reduction in growth (Tabel 6). This inhibition could be due to volatile toxins absorbed on moist filter papers. The possibility of growth inhibition by CO_2 was almost ruled out. In earlier two bioassays the toxins played a primary role while CO_2 might be a secondary factor in inhibiting growth and germination.

The results of these bioassays clearly revealed the presence of volatile inhibitors in *Datura*. These toxins were absorbed on moist filter papers thereby inhibiting the growth

^{*}Significant at P = 0.05 **Significant at P = 0.01

Table 6. Effect of *Datura innoxia* shoot volatile, absorbed on the filter papers, on germination and radicle growth of the test species.

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Test Species	Control	Test	% of Control
			10 EMP - O GT B 10 T- O T- OT T- III II I
	Germination (%)		
Brassica campestris	95.00	93.00	97.89
Setaria italica	94.00	92.00	97.87
	Radicle Growth±SÉ	(mm)	
Brassica campestris	10.11±2.11	4.84±1.05	47.87**
Setaria italica	13.25±1.92	8.38±1.21	63.24**

Each value is a mean of 10 replicates, each with 10 seeds.

and germination, A somewhat similar phenomenon is expected in nature. These toxins, after volatilization, would accumulate on moist soil particles, thus rendering it toxic for the growth of susceptible species.

6. Soil Residual toxicity.

Whether the water soluble toxins leaching from living or dead plant parts reaching the soil remain effective or not was analysed by collecting soil underneath *Datura* thickets from upto 5 cm depth, referred to as "test soil". Control soil was collected similarly in the vicinity of *Datura* without any vegetational cover. The soils were dried at room temperature and sieved through 2 mm mesh. Control soil served as the true control in the following bioassays:-

a) Soil-bed bioassay.

Ten g of control or test soil, uniformly spread in separate Petri dishes with equal amount of double distilled water, was topped with a single sheet of filter paper. Seeds of L. sativa, B. campestris and S. italica were placed on these filter papers. Simultaneously, a 2nd distilled water control was used to compare the nutrient status of soils. Dishes were sealed with parafilm. Each species had 10 replicates, each with 10 seeds. Germination and radicle growth was recorded after 48 h incubation at $26\,^{\circ}\mathrm{C}$.

Radicle growth of all the test species was significantly inhibited in both the test soils. Soil taken from underneath the canopy was more inhibitory than root zone soil. *Lactuca* and *Brassica* were the most affected species (Table 7).

^{*}Significant at P = 0.05 **Significant at P = 0.01

Table 7. Effect of *Datura innoxia* soil beds on germination and growth of the test species.

Test Species	Distilled	Control		Datur	ra soils	
•	Water	Soil	Unde	er Canopy	Roo	t Zone
			Test	% of Control	Test	% of Control
	Germ	ination (%)				
Lactuca sativa	84.00	80.00	88.00	110.00	85.00	106.25
Brassica campestris	91.00	89.00	80.00	89.88*	76.00	85.39*
Setaria italica	96.00	93.00	93.00	100.00	92.00	98.92
	Radio	ele Growth (mm).			
Lactuca sativa	8.34	10.88	5.93	54.50**	5.16	87.01**
$\pm SE$	1.23	1.98	1.11		1.15	
Brassica campestris	10.18	9.46	6.07	63.74**	6.36	67.23**
±SE	2.05	1.72	1.02		1.29	
Setaria italica	21.51	18.95	15.14	79.89**	15.44	81.47**
±SE	3.55	2.39	2.06		3.45	

Each value is a mean of 10 replicates, each with 10 seeds.

*Significant at P = 0.05 **Significant at P = 0.01

b) Soil Extract bioassay.

Twenty g test or control soil was thoroughly shaked in 100 ml double distilled water for 12 h and filtered. These extracts along with a distilled water control were used against *L. sativa* and *B. campestris* in standard filter paper bioassay. There were 10 replicates, each with 10 seeds. The dishes were incubated at 26 °C for 48 h. Germination of *Lactuca* and radicle growth of both the test species was significantly inhibited by *Datura* soil extract (Table 8).

The observed reduction in germination and radicle growth was due to toxins released by *Datura* plants into potentially good soil. The improved growth in control soil over distilled water was an evidence of nutrients in the soil. These toxins were added to soil in the form of leachates, volatile, root exudates or by decaying dead plant parts rendering it toxic for the growth of other species.

Discussion

The reduced growth and germination of test species under *Datura* thickets was apparently not due, primarily, to physical factors. Growth of species was significantly

Test Species	Distilled Water	Control Soil Extract	<i>Datura</i> Soil Extract	% of Control
	Germination (%)			
Lactuca sativa	83.00	91.00	75.00	82.41**
Brassica campestris	85.00	92.00	84.00	91.30
	Radicle Growth	±SE (mm)		
Lactuca sativa	9.52±3.16	11.56±2.69	6.77±2.01	58.86**
Brassica campestris	13.48±3.65	15.79±2.87	6.50±1.95	41.16**

Table 8. Effect of Datura innoxia soil extract on germination and growth of test species.

Each value is a mean of 10 replicates, each with 10 seeds.

inhibited in the presence of nutrients. The presence of relatively bare areas under and around *D. innoxia* was not due to competition. In our field and laboratory experiments all the suspected competitive factors were eliminated and the only possible mechanism which could otherwise interfere was allelopathy. The observed inhibition might have been due to toxins relased by *Datura*.

Laboratory bioassays and extraction of water soluble toxins under controlled coditions were more or less simulating the process in the natural conditions. The toxins were transported from living or dead decaying parts as rain or fog drips, water leachates and root exudates to soil. The different parts assayed invariably contained phytotoxins. The mature leaves were more inhibitory than other plant parts. These findings are in agreement with McPherson & Muller (1969) and delMoral & Muller (1970) who obtained similar results for *Adenostoma* and *Eucalyptus*.

The volatile toxins provided an additional allelopathic mechanism in *Datura*. The inhibition of growth in field and laboratory biossays provided concrete evidences on the presence of toxins in *Datura* volatiles. The growth retardation of test species in these bioassays cannot be solely attributed to high CO₂ concentration. More inhibition of growth of test species in *Datura* than *Cestrum* atmosphere is undoubtly due to toxic principles in *Datura*. Moreover, when the shoot was used indirectly, for absorbing volatiles, if any, there was still significant growth inhibition. Muller & Muller, (1964), Muller et al (1964), Muller et al (1964), Muller et al (1967), Muller et al (1969) and Friedman et al (1977) have proved the presence of volatile growth inhibitors in *Salvia*, *Adenostoma* and *Artemesia*, responsible for spacing the vegetation. Our results regarding the allelopathic effects of *Datura* are in agreement with these findings. Similar results were obtained by delMoral & Muller (1970) for *Eucalyptus*. The toxins, released by *Datura*, accumulated in suffi-

^{**}Significant at P = 0.01

cient quantity in the top layers of soil, thereby rendering it toxic for the growth of test species.

The artificial leaching experiment suggested a possible mechanism for the transport of phytotoxins to soil and this is what we expect in nature. The soil was not nutrient deficient, since the seedlings in the control soil exhibited a better growth than those grown in the distilled water. Soil-plant phytotoxicity due to *Celtis* and its possible role in vegetation patterning has been reported (Lodhi & Rice, 1971; 1975). Guenzi & McCalla (1966) and Wang *et al* (1967) isolated phytotoxins from soils. The same could be true for the soil under and around *Datura* plants.

The present study suggested an allelopathic mechanism in *Datura*, responsible for the reduction and exclusion of herbaceous plants in its vicinity. The mechanism is active through release of toxins from living or dead plant parts by rain, fog, dew or through volatilization. The moist soil acts as an absorption and accumulation medium. Other factors of the environment might play a secondary role. It therefore, appears that allelopathy is an important ecological factor determining the composition and structure of plant communities.

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