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BIOCHEMICAL INHIBITION EXHIBITED BY *DATURA INNOXIA* M SEEDS

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Abstract

Germination studies of *Datura innoxia* seeds showed that the possible factors responsible for the dormancy were hard testa and biochemical inhibitors in the seeds. Laboratory bioassays were run to analyse the possibility of seed residual toxicity. The leachate, artificial rain-drip, water extracts from whole seed, seed coat and cotyledon proved to be inhibitory against the germination and early growth of its own seeds as well as of other seeds tested. The toxicity of seed parts extracts was species related. Whole seed and seed coat extracts were more toxic than the cotyledon extract. Presence of inhibitors was one of the possible causes of delayed, irregular and reduced germination in *Datura innoxia* seeds.

Introduction

Datura innoxia M. of the family Solanaceae is world wide in distribution, specially in warm temperate climates. The genus is annual or biennial preferably growing in loamy soils (Mubarak, 1976). All parts of the plant are poisonous and the seed contains hyoscine and hyoscyamine (Mukerji, 1953). Evenari (1949) and Went (1957) observed germination inhibiting substances in seeds of many plants. Such seeds remain dormant and germinate only after they receive a specific amount of moisture. Siddiqi & Scott (1975) investigated that the seed extract of *Abrus precatorius* were toxic to the germination of seeds used in their bioassays. Koller (1957) found in *Atriplex dimorphostegia* that the seed coat provides chemical inhibitors for growth. Qadir & Abbasi (1971) classified *Datura* seeds as "exclusivly inhibitory". Mubarak (1976) also pointed out the possible occurrence of inhibitory substances in these seeds. An investigation was therefore conducted to find out the possible locus of inhibitory substance in *Datura innoxia* seeds and its effect on the germination and subsequent growth of the plants.

Materials and Methods

Sterilized Petri dishes of 80 mm size were used. The dishes were always sealed with "parafilm M" during the incubation to retain proper moisture. *Brassica campestris, Setaria italica* and *Datura innoxia* were used as the standard test species. There were ten replications each with 10 seeds. The tests were conducted by using the extract while in

control for each species distilled water was used. Seeds of *Datura innoxia* were used after the removal of testa and incubated for up to 7 days. The germination and radicle growth was recorded at the end of the experiment. The experiments were repeated atleast twice with similar results.

I. Residual toxicity of the seed exudates.

Datura Seeds after surface sterilization with alcohol and rinsing with water, were kept on three layers of Whatman No. 1 filter paper, moistened with distilled water. They were incubated at 26°C. After 7 days *Datura* seeds were removed and the seeds of the test species were sown on the same filter papers and incubated at 26°C for 48 h.

II. Artificial rain-drip bioassay.

Twenty g of *Datura* seeds were kept on 6 layers of Whatman filter paper in a sieve. Two hundred ml distilled water was dripped by 2 burettes and this water was passed 5 times from the seeds. The rain drip, thus collected, was used in the bioassay against the test species. They were incubated at 26°C for 48 h. except *Datura.*

III. Whole seed extract bioassay.

Five and 10 g of crushed Whole seeds of *Datura* were separately soaked in 100 ml of distilled water for 12 h. and the filterates used in bioassays. Tests as well as controls were incubated at 26°C for 48 h.

IV. Relative toxicity of Datura seed parts.

Seed coat of *Datura* was separated from cotyledon including embryo. These and the whole seeds of *Datura* after grinding separately were soaked, 5g seed material in 100 ml distilled water. After 12 h the filterates were used in bioassay. The dishes were incubated at 26°C for 72 h except *Datura.*

All the data was subjected to statistical analysis using "Z and t tests" following Cox(1967).

Results

I. Residual toxicity of Datura seed exudates.

The radicle growth of all the test species was significantly inhibited ($P < 0.01$) indicating the presence of gremination and growth inhibitors in *Datura* seeds (Table 1). The most affected species was *Datura* itself followed by *Setaria.* The germination (Table 5) of all the test species except *Brassica* was significantly retarded ($p < 0.01$).

II. Artificial Rain-drip bioassay.

The radicle growth of *Brassica* was significantly inhibited at 0.01 level while that of *Setaria* and *Datura* was almost unaffected (Table 2.) The germination of *Setaria* was significantly reduced ($p < 0.01$) (Table 5).

TABLE 1. Effect of *Datura* seed exudates on the radicle growth of test species.

Test Species		Radicle Growth (mm) Mean ± SE		% of Control
Brassica campestris	Control	9.24	0.64	
	Test	6.67**	0.59	72.1
Setaria italica.	Control	12.28	0.93	
	Test	6.34**	0.65	50.0
Datura innoxia.	Control	22.50	2.12	
	Test	6.70**	0.53	29.7

** = Significant at 0.01 level of probability.

III. Whole seed extract bioassay.

The radicle growth of all the three test species was significantly inhibited ($p < 0.01$) at both the concentrations (Table 3). The increasing extract concentration was associated with decreasing radicle growth. The germination of *Brassica* and *Setaria* was inhibited at 0.01 level (Table 5). The effect of increasing concentration was, however, not directly proportional to the inhibition. The effect on growth and germination was species specific.

TABLE 2. Effect of artificial rain-drip of *Datura* seeds on the radicle growth of test species.

Test Species		Radicle Growth (mm) Mean ± SE		% of Control
Brassica campestris	Control	28.62	1.17	
	Test	15.92**	0.31	55.60
Setaria italica	Control	29.48	0.81	
	Test	29.00	1.91	100.0
Datura innoxia	Control	22.50	2.12	
	Test	21.22	1.74	94.30

** = Significant at 0.01 level of probability.

TABLE 3. Effect of Whole seed extract of *Datura innoxia* seeds in two different concentrations on the radicle growth of the test species.

Test species	Control Radicle growth (mm) Mean ± SE		Test x 5 Radicle growth (mm) Mean ± SE		% of Control	Control Radicle growth (mm) Mean ± SE		Test x 10 Radicle growth (mm) Mean ± SE		% of Control
Brassica campestris.	16.03	0.44	4.06**	0.98	25.32	16.32	0.67	7.52**	0.62	34.0
Setaria italica.	24.14	0.98	15.64**	1.93	64.70	22.02	0.68	13.45**	1.80	61.00
Datura innoxia.	6.33	0.47	4.08**	0.26	64.46	22.50	2.12	4.93**	0.76	22.10

X 5 and X 10 present 5 and 10 g concentrations, respectively.

** = Significant at 0.01 level of probability.

TABLE 4. Relative toxicity of *Datura* seed parts against the radicle growth of the test species.

Test Species	Test Conditions. Radicle growth (mm)											
	Control		Whole Seed Extract			Cotyledon Extract			Seed Coat Extract			
	Radicle growth (mm) Mean ± SE		Mean ± SE		% of Control	Mean ± SE		% of Control	Mean ± SE		% of Control	
Brassica campestris.	16.03	0.44	4.06**	0.36	25.32	9.27**	0.74	57.82	6.08**	0.48	37.92	
Setaria italica.	15.84	0.40	7.11**	0.55	44.82	9.68**	0.23	61.11	8.79**	0.49	55.49	
Datura innoxia.	6.33	0.47	4.08	0.26	64.46	3.48**	0.30	55.00	4.36**	0.33	69.00	

** = Significant at 0.01 level of probability.

TABLE 5. Effect of aqueous seed extract of *Datura innoxia* on germination of test species in different bioassays expressed as % of control.

Test species	Residual Toxicity of Seed Exudates.	Artificial Raindrip Bioassay.	Whole Seed Extract Bioassay.		Realative Toxicity of Seed parts.		
			X 5	X 10	Whole Seed Extract	Coty-ledon Extract	Seed-coat Extract
Brassica campestris.	94.6	96.0	67.0**	98.0	67.0**	76.0*	76.0**
Setaria italica.	67.8**	83.0**	81.0**	95.0	97.0	96.0**	88.0**
Datura innoxia.	72.0**	100.0	106.0	100.0	106.0	103.0	94.0

X 5 and X 10 present 5 and 10 g cencentration, respectively.

* = Significant at 0.05 level of probability.

** = Significant at 0.01 level of probability.

IV. Relative toxicity of Datura seed parts.

The radicle growth of all the 3 test species was inhibited ($p < 0.01$) in the whole seed, seed coat and cotyledon extracts (Table 4). The maximum growth inhibition was observed in whole seed extract followed by seed coat extract. The radicle growth of *Datura* was inhibited more by cotyledon extract and less by whole seed extract. Generally the whole seed and seed coat extracts were more toxic than the cotyledon extract. The germination of *Brassica* and *Setaria* was reduced significantly ($p < 0.01$) in the seed coat extract (Table 5).

Discussion

Germination studies on *Datura innoxia* (Mubarak, 1976) revealed that either it was due to the structural limitations of the hard testa or the presence of inhibitory substances in seeds that the germination was slow and irregular. In prelimnary bioassays with *Datura* seeds inhibitors were allowed to leach on the filter paper seed beds. It was observed that radicle growth of *Brassica, Setaria* and *Datura* was significantly inhibited. Artificial rain-drip bioassay was conducted to correlate the germination of *Datura* seeds with natural conditions. In nature the seedlings of this specie emerge in March. They grow, flower in summer and then die after shedding the seeds in late autumn. After this, winter rain starts which continues till March. So it is most probable that during the winter rains the inhibitors are washed off from the seeds, thus allowing the germination. Hard testa was an additional factor in inhibiting the germination of *Datura* seeds.

Experiments with *Datura* seeds also confirm the results of some earlier findings that certain substances inhibit the germination and early growth of other species. The whole seed and seed coat extracts had almost an equal inhibition potentiality. The inhibition was more pronounced in these two extracts than the cotyledon extract. It would appear that in the early stages of germination of *Datura* seeds the seed coat simply provides inhibitors for itself and for other species. The presence of phenolic inhibitors in the integument of apple seeds has been reported (Come, 1969). These can be easily oxidized in moist seed coat forming a sort of film around the embryo, thus, preventing the oxygen supply to the embryo. The oxidation of phenolic compounds of apple seeds is more under such conditions, specially if the temperature is raised. The embryo thus receives very little oxygen because more is fixed by phenolics and less is dissolved.

In *Datura* seeds, the irregular germination observed at 20°C, suddenly decreased at 30°C and absolutely negative results were obtained at 40°C (Mubarak, 1976). With the removal of testa, 92.5 % germination was observed after 15 days at 20°C. The unidentified inhibitors in the seed coat of *Datura* acted in a similar manner as the phenolic in the integuments of apple seeds. The inhibitors, if allowed to accumulate in physiologically significant quantity in the soil, can inhibit the germination and early growth of other species growing in the vicinity of *Datura*. It is, therefore, concluded that the germination of *Datura innoxia* seeds was self-inhibited by the phytotoxins present in the seed coat and cotyledon of the seeds. The inhibition of germination and radical growth was, however, species related.

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