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SINAPIC ACID-AN ALLELOPATHIC AGENT IN THE WEED, *CROZOPHORA ROTTLERI*

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All plant parts of *Crozophora rottleri* bear inhibitor (s). Leaf leachate contains more or stronger inhibitors as compared to stem and root leachate. Leaf leachate causes greater inhibition on mustard than on wheat and rice. In case of mustard, only one day soaked leaf leachate at 1:2.5 dilution causes 97.3% inhibition in shoot length and 95.4% inhibition in root length. In case of rice and wheat, however, longer soaking (4-day) is required for a similar degree of inhibition. Inhibitory activity increases with the day of soaking. Degree of inhibition is directly proportional to the concentration of the extract. After acid hydrolysis both organic and aqueous phase show inhibitory activity. TLC of ether extract revealed a band which was identified to be sinapic acid. Aqueous extract yielded a white crystal. MS, IR and UV suggest that this is a 16-C aliphatic acid with a mol. wt. 362.

Molisch (1937) first coined the term allelopathy to describe the action (inhibitory or stimulatory) of a species of higher plant on another. Allelopathy has received increasing attention of explaining vegetation pattern in plant communities and as an important aspect of weed crop interaction. These interactions among plants often lead to a superiority of one species at the detriment of another under natural conditions. Our study deals with the presence of natural chemical retardants or inhibitors in weed that infest uncared lands and cultivated fields and the allelopathic influence of such weeds on seed germination and seedling growth of crops. The present paper is concerned with the allelopathic principle associated with the leachate of different organs of a seasonal weed, *Crozophora rottleri*, Euphorbiaceae. It appears in the month of February, the flowering stage is in June to July and it completely disappears in late August. It grows to a maximum height of 70-80 cm and forms dense bushes. It occurs along the road side, rail-way lines, in pond areas and is also observed at the edge of cultivated land. Rice, wheat and mustard are the crops on which its allelopathic action has been detected in this work.

MATERIALS AND METHODS

Vigorously growing individuals of *Crozophora rottleri* were collected from the site of Kanchrapara, Halisahar, Barrackpore, Dunkuni, Baranagar (West Bengal, India) in June/July 1995. The root, stem and leaf from an individual plant were detached. Aqueous leachate was prepared by soaking 100 gm each of fresh plant part in 200 ml distilled water for 1, 2 and 4 days. It was filtered through filter paper (Whatman no. 1) and the filtrate made upto 250 ml with water; this constituted the standard or stock solution (1:2.5) from which dilutions (1:5 and 1:10) were made.

Effect of leachate (of different parts) of *Crozophora* on germination and growth were detected by bioassay with three replicates for each set containing 50 seeds. For bioassay, rice, mustard and wheat seeds were used. Seeds were sterilized with 0.1% mercuric chloride solution, washed with distilled water and placed on a filter paper in Petri dish. A proper control was maintained by treating with equal volume (10 ml) of distilled water. After 3 days shoot and root length in the control and treated set were measured.

Chemical analysis:

Extraction procedure

1. A crude aqueous extract was prepared by soaking 100 g of fresh leaf material in 500 ml of distilled water for 48 hours. The extract was decanted, filtered through cheese-cloth and the solids discarded. the filtrate was centrifuged and the supernatant decanted.

2. The aqueous extract (fraction 1) was re-extracted with 100 ml hexane to remove lipids (fraction 2).

3. The resulting aqueous solution (fraction 3) was again extracted with diethyl ether to collect aglycones (fraction 4).

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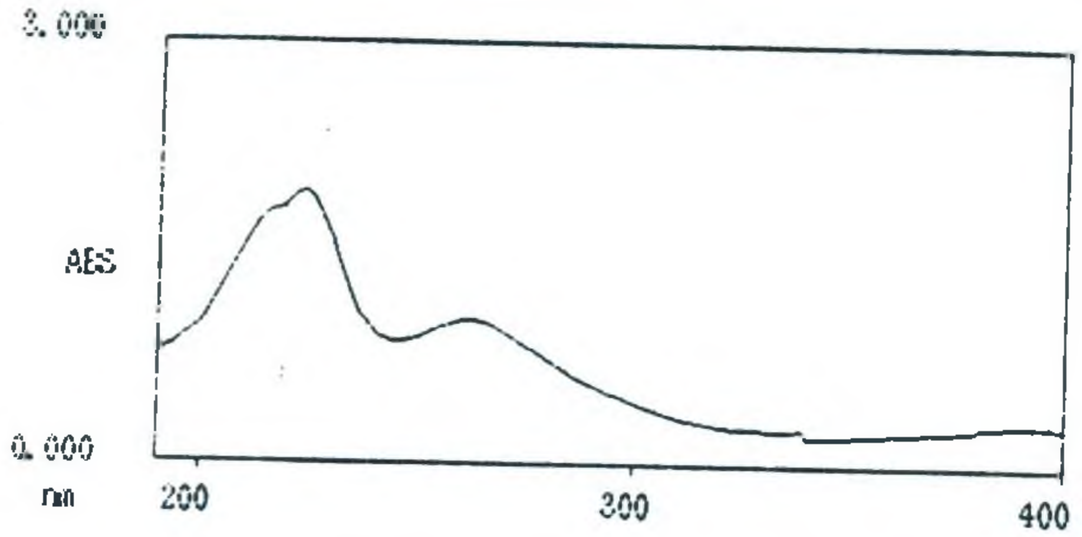


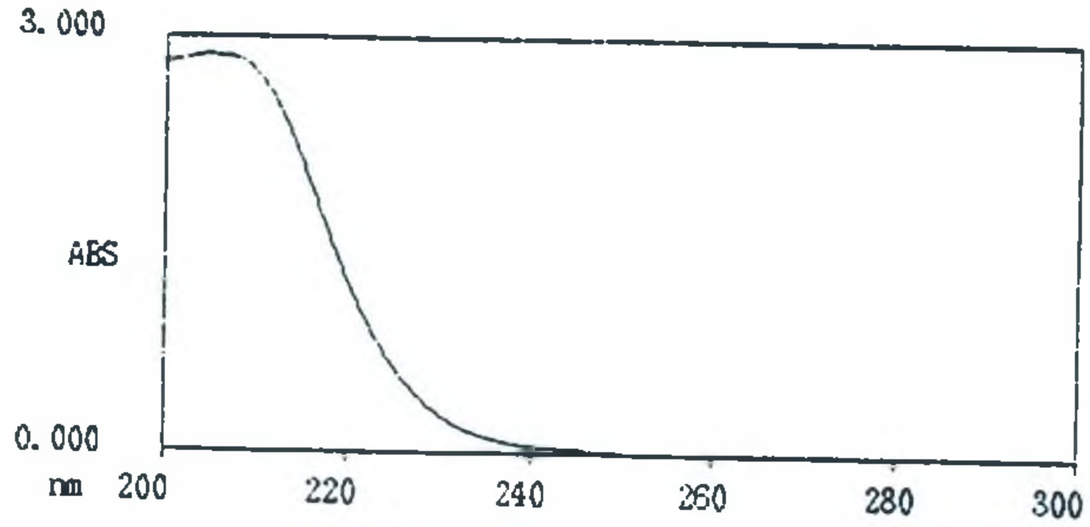
No.	PEAK nm	PEAK ABS	VALLEY nm	VALLEY ABS
1	339.5	0.259	341.0	0.202
2	262.5	1.092	334.5	0.257
3	223.5	1.938	246.0	0.880

Figure 1. Graph shwoing UV analysis of sample showing its benzene ring nature

Table 1: Effects of 2-day soaked leachate of different parts of Crozophora at 1:2.5, 1:5, 1:10 dilution on germination and seedling growth of rice. Mean of 3 replications each with 50 seedlings recorded in mm ('-' indicates inhibition and '+' indicates stimulation).

Test organ	Tested solution	Shoot length	Root length	Percentage inhibition (–)/ stimulation (+) in SL*	Percentage inhibition (–)/ stimulation (+) in RL*
Loaf leachate	control	20.7	41.3	-	-
	1:2.5	14.8	13.7	-28.5	-66.8
	1:5	16.5	27.4	-20.2	-33.6
	1:10	19.4	30.6	-6.2	-25.9
Stem leachate	control	9.7	25.7	-	-
	1:2.5	8.4	18.0	-13.4	-29.9
	1:5	9.2	19.0	-5.1	-26.0
	1:10	10.1	21.4	+4.1	-16.7
Root leachate	control	9.0	24.8	-	-
	1:2.5	8.0	16.6	-11.1	-33.0
	1:5	8.7	22.2	-3.3	-10.4
	1:10	9.3	24.1	+3.3	-2.8

SL*=Mean shoot length in mm and RL*=Mean root length in mm



No.	PEAK nm	PEAK ABS	VALLEY nm	VALLEY ABS
1	204.6	2.872	295.0	–0.139

Figure 2. Showing absortion spectrum with absorption peak at 204 nm.

Table 2: Effects of 2-day soaked leaf leachate of *Crozophora* (at 1:2.5, 1:5, 1:10 dilution) on germination and seedling growth of rice, wheat and mustard. Mean of 3 replications each with 50 seedlings recorded in mm ('-' indicates inhibition and '+' indicates stimulation).

Tested seed	Tested solution	Shoot length	Root length	Percentage inhibition (–)/ stimulation (+) in SL*	Percentage inhibition (–)/ stimulation (+) in RL*
Rice	control	21.0	44.5	-	-
	1:2.5	10.4	0.7	-50.4	-98.4
	1:5	11.7	10.9	-44.2	-75.5
	1:10	18.4	23.7	-12.3	-46.7
Wheat	control	13.3	29.5	-	-
	1:2.5	1.8	3.5	-86.4	-88.1
	1:5	9.7	18.3	-27.0	-37.9
	1:10	17.2	40.9	+29.3	+38.6
Mustard	control	20.3	56.8	-	-
	1:2.5	0.0	0.0	-100	-100
	1:5	0.7	0.9	-96.5	-98.4
	1:10	6.1	9.3	-69.9	-83.6

SL*=Mean shoot length in mm and RL*=Mean root length in mm

4. The remaining aqueous fraction (fraction 5) was subjected to acid hydrolysis.

a. Acid hydrolysis was carried out in a water bath at 40°C for 30 minutes in IN HCI to release the potential allelochemical moieties from water soluble conjugates.

5. The hydrolysate was extracted with diethyl ether. The organic (ether) phase is fraction 6 and the aqueous phase is fraction 7.

The ether phase (fraction 6) was concentrated and spotted on TLC (silica gel G) plate using solvent-chloroform: acetic acid: 90 10 (Harborne, 1984) along with the eight standard phenolics (syringic acid, caffeic acid, ferulic acid, 3-4 dihydroxy benzoic acid, vanillic acid, 4-hydroxy benzoic acid, coumaric acid, sinapic acid).

On concentration of aqueous phase (fraction 7), fine whitish crystals were found. These were repurified by running in the solvent system-methanol: ammonia 99: 1. These crystals were then subjected to UV spectrophotometer, IR and MS analysis.

RESULTS

1. Bioassay: Two sets of measurements were performed, the treated set with test solution and the control with distilled water. After 3 days, shoot and root length in the control and treated set were measured. Table 1 shows the effect of leachate of different plant parts of *Crozophora* on germination and seedling growth of rice var.IET 1444. Leaf leachate shows more inhibitory activity than stem and root leachate. Table 2 shows the effect of two day soaked leaf leachate of *Crozophora* on germination and seedling growth of rice, wheat, mustard. Leaf leachate causes greater inhibition on mustard than on wheat and rice. In case of rice and wheat, leaf leachate at 1:10 dilution shows some stimulatory effects on shoot length. Inhibitory activity increases with the day of soaking. Dilution of the extract reveals lesser inhibition.

Table 3 shows the inhibitory activity of fraction 6 (ether phase) and fraction 7 (aqueous phase). Aqueous phase causes 93.6% inhibition in shoot length and 100% inhibition in root length whereas ether phase causes 51% inhibition in shoot length and 90% inhibition in root length.

2. Chemical analysis of acid hydrolysed ether and aqueous phase:

Concentrated ether extract was spotted on the TLC (silica gel G) run in the solvent system-chloroform: acetic acid: 90:10 along with standard phenolics. after run the plate was observed under UV light (365 nM). A number of bands were observed one of which coincides with the standard sinapic acid, On UV spectrophotometer analysis this band showed a peak

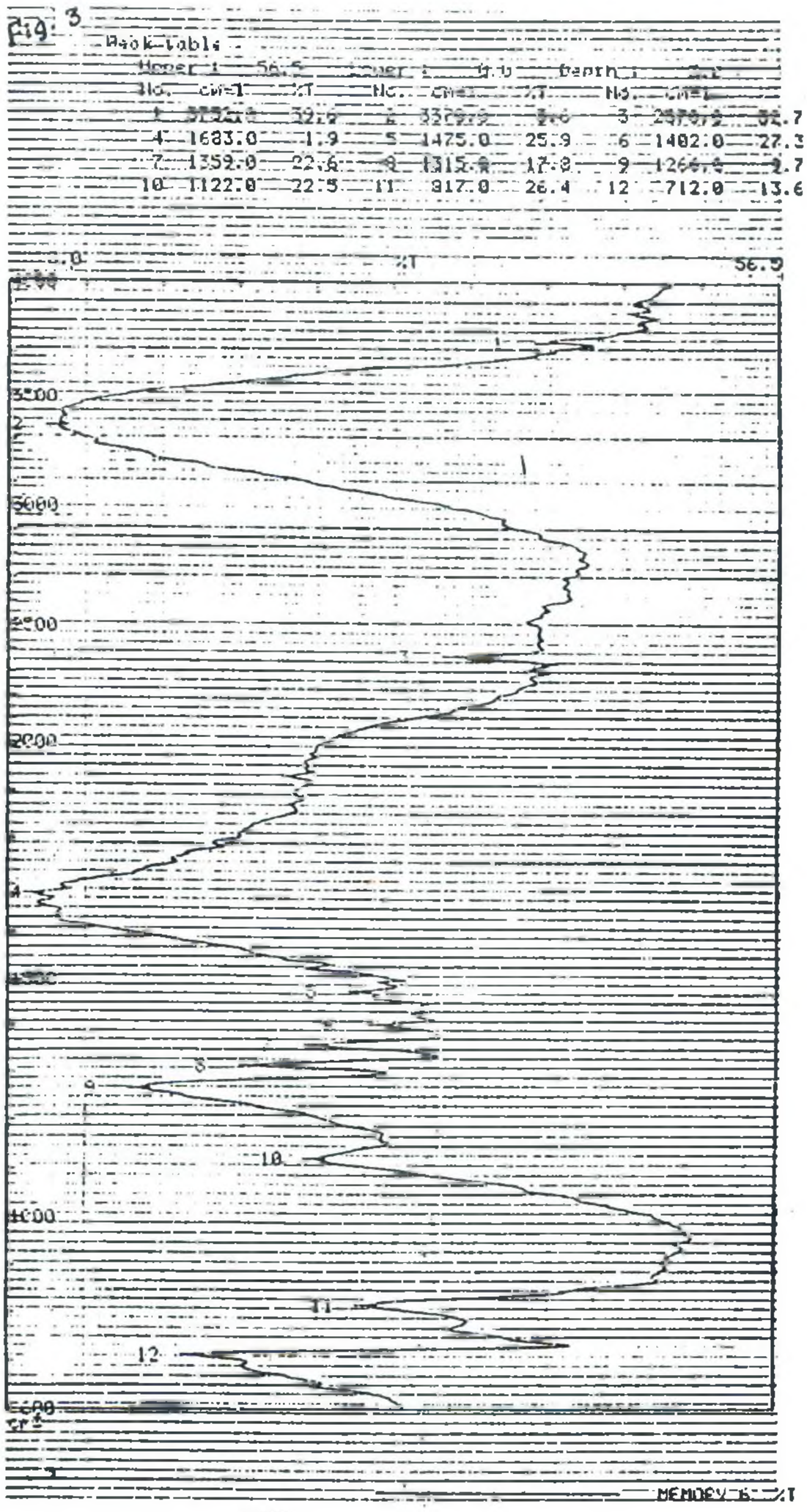


Figure 3. Showing IR and MS analysis of crystals.

Table 3: Effects of ether phase (fraction 6) and aqueous phase (fraction 7) of acid hydrolysed *Crozophara* leaves on germination and seedling growth of rice. Mean of 3 replications each with 50 seedlings recorded in mm.

No. of observation	Tested solution	Control		Treatment		Percentage inhibition	
		SL	RL	SL	RL	in SL*	in RL*
1	Aqueous phase	39.0	57.2	0	0	-100	-100
2		14.4	34.0	2.7	0	-80.8	-100
3		15.1	37.3	0	0	-100	-100
1	Ether phase	19.8	43.4	8.8	3.0	-55.1	-93.0
2		34.9	49.5	17.9	4.2	-48.4	-91.3
3		14.8	40.0	7.4	5.6	-49.5	-85.9

SL*= Mean shoot length in mm and RL*=Mean root length in mm

at 262 nm that further suggests that it is a compound which contains benzene ring (Fig. 1).

Crystals obtained from the aqueous phase show methyl red positive staining. They had an absorption peak at 204 nm (Fig.2). These crystals were also subjected to IR and MS analysis (Fig.3). MS results indicate a molecular ion at 362 and certain fragments (215, 307, 347).

DISCUSSION

The extent of phytotoxicity depends on the plant parts from which the leachate is prepared. Leaf leachate

of *Crozophora* shows more inhibitory effect on mustard than on wheat and rice. Degree of inhibition was in increasing order with increasing concentration of the extract. But in case of wheat both one day and two day-soaked leaf leachates at 1:10 dilution showed some stimulatory activity. Therefore, in wheat field presence of some *Crozophora* plants may give some good results. Leaf leachate contains a number of phenolic compounds one of which is sinapic acid. Again, a substance of mol. wt. 362 and comprising of 16-C atoms acts as an inhibiting allelopathic agent. Its absorption peak (204 nm) suggests that this is not a phenolic compound (which should have a peak beyound 240 nm). This substance requires further study for complete characterization.

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