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ACKNOWLEDGEMENTS. We thank Dr S. J. Ankegowda, Scientist (Plant Physiology), Cardamom Research Centre, Appangala for help in statistical analysis and Indian Council of Agricultural Research, New Delhi for financial assistance.

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Chemical ecology of certain tropical fruit pulp II: non-zoochoric

In the first part[1] we showed the presence of stimulators and inhibitors of germination in the pulp of *Acacia erioloba*, a fruit dependent on zoochoric agents for dispersal. Recently Uma Shaanker *et al.*[2] surmised that natural selection favoured the production of laxative substances in the fruit pulp consumed by birds and other animals. According to these authors, natural selection should act differently on zoochoric and non-zoochoric fruit. The latter, for example, would require no pulp with laxative or nutritive properties.

It is therefore of interest to compare the properties of a number of zoochoric and non-zoochoric fruit pulp. Following the study of stimulators and inhibitors in *A. erioloba*, a zoochoric fruit, we report here our findings on *Sterculia foetida* which is *not* dispersed by animals. The thick wooden rind bursts and the seeds are liberated (Figure 1 *a*,*b*). Here the seeds are not embedded in the pulp. Also, because of the thick wooden case, water, necessary for germination, cannot easily reach the seeds. Nonetheless we find such substances as described below.

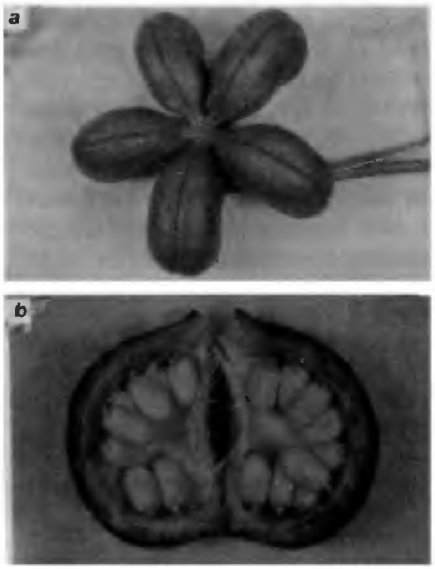

Figure 1. *a*, Bunch of *Sterculia foetida* fruit; *b*, Two halves of a single fruit with seeds.

Around Calcutta, the flowering stage of *Sterculia* is from April to May. During October, *Sterculia* fruit is green in colour. In January, the colour turns red, and seeds are dispersed in February after bursting of the pod.

Green pods of *Sterculia* were collected from the tree and cut open with a knife and water extract of the pulp was prepared. Pulp (50 g) was macerated and then 100 ml distilled water was added to it. It was then filtered through cheese cloth. 25 ml of distilled water was added to the residue and refiltered. The resultant solution was then centrifuged. The supernatant constituted the standard or stock solution of 1 : 2.5 dilution, which was further diluted for certain experiments, as detailed below.

Isolation and detection of the putative active compounds in the fruit pulp extract were attempted with the help of paper chromatography. Whatman no. 3 paper was used to develop the chromatogram.

For phenolic compounds, the solvent was *n*-butanol : acetic acid : water :: 4 : 1 : 2.2. Silver nitrate reagent was used for staining phenolics[3].

Effects of the pulp extract of *S. foetida* on germination and growth were detected by bioassay with three replications for each set containing 40 seeds. For bioassay, rice seeds (var. IET 1444 collected from Chinsura Rice Research Institute, Chinsura, West Bengal) were used, which 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 (20 ml) of distilled water (instead of aqueous pulp extract). After 4 days, the length of shoot and root in the control and treated set was measured. Natural variation between the two sets of control was 0.3% in the shoot length and 1.6% in the root length respectively.

Bioassay was attempted after preparative chromatography (using solvent system *n*-butanol : acetic acid : water :: 4 : 1 : 2.2) with 0.5 ml pulp extract. One vertical section of the chromatogram was stained with $AgNO_3$ reagent[3], the remaining larger unstained portion was divided into 7 unequal sections depending on the location of spots. Height of the chromatogram was 16 cm. Sections 1 and 2 are 2.3 cm; the remaining 5 sections are 2 cm. Twenty five rice seeds were placed on each section and the corresponding control (same sized pieces cut out of a paper after

Table 1. Effect of *Sterculia foetida* pulp extract on germination and seedling growth of rice

Tested solution	Shoot length				Root length				%inhibition	
	M	SD	SE	*t* value*	M	SD	SE	*t* value	in SL	in RL
1 : 2.5	0	–	–	–	0	–	–	–	100	100
1 : 5	2.9	1.65	0.30	11.6	0.9	1.95	0.35	22.1	59.7	95.7
1 : 10	4.2	2.12	0.35	7.3	1.8	2.74	0.45	20.3	41.6	91.9

The observed difference in SL and RL between the control and tested solution is considered statistically significant in all levels, because our calculated *t* value (**df* = 40 and $p = 0.2, 0.1, 0.05, 0.02, 0.01, 0.001$) is much higher than the table value.
M, SD, SE, SL and RL indicate mean, standard deviation, standard error, shoot length and root length respectively. Mean of 40 seeds recorded in mm.

Strips stained with $AgNO_3$	Corresponding strips for bioassay		
	Shoot length	Section number	Root length
	+ 24.41%	7	+ 32.62%
	+ 13.05%	6	+ 38.49%
	+ 18.57%	5	+ 39.93%
	- 25.21%	4	- 29.77%
	+ 17.53%	3	+ 17.67%
	+ 33.67%	2	+ 24.79%
	+ 46.76%	1	+ 28.41%

Figure 2. Inhibitory and stimulatory influences of different sections of the chromatogram developed with 0.5 ml of *Sterculia foetida* pulp extract. Solvent– butanol : acetic acid : water :: 4 : 1 : 2.2. Width of the sections 1 and 2 is 2.3 cm and the remaining sections is 2 cm. (Mean of 25 seeds recorded in mm.)

blank run in identical conditions). Three such experiments were performed. Shoot and root length were measured after 4 days.

For bioassay, two sets of measurements were performed, the treated set with aqueous pulp extract of *S. foetida* and the control with distilled water. Length of shoot and root in the two sets was measured after 4 days. Table 1 shows inhibition in shoot and root length in a treated set.

Paper chromatography showed three spots (one brown, one violet and one black or slate colour) which were visible after staining with $AgNO_3$ reagent.

A. erioloba reveals 6 phenolic spots[1] but the upper three are missing in *S. foetida*.

Bioassay on different sections of preparative chromatogram shows inhibition of both shoot and root length only at section 4 (Figure 2). Stimulation of both shoot and root length was observed in sections 1–3 and 5–7. Three phenolic spots were observed in sections 2–4.

Thus, two stimulators and one inhibitor were revealed. The inhibitory effect on the shoot length is more pronounced than on the root length.

Thus the situation is very close to that of *A. erioloba*, a zoochoric fruit[1]. This is not what should be theoretically expected. But in the case of *S. foetida* the pulp of the fruit was not fully mature. Perhaps in view of the thick wooden rind, water is not easily accessible to the seeds. Thus we have to address an interesting question – whether the stimulators and inhibitors have evolved without any zoochoric selection pressure or, in other words, whether these are metabolic by-products of the basic physiology and biochemistry of the plants? Generation of a large set of data on the chemical aspects of zoochoric and non-zoochoric fruits including the presence of laxatives in pulp[2] is likely to help us to address the question.

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ACKNOWLEDGEMENT. We thank Mr N. Banik of Embryology Unit, ISI for valuable assistance in the laboratory.

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Synergistic effect of Cry1Ac and Cry1F δ-endotoxons of *Bacillus thuringiensis* on cotton bollworm, *Helicoverpa armigera*

Insecticidal δ-endotoxins of *Bacillus thuringiensis* (Bt) have acquired great significance in recent years because of their specificity to target insects, toxicity at very low concentration and environment friendly nature[1]. Genes coding for Bt δ-endotoxins have been deployed in a wide range of transgenic crop plants with considerable success[2]. One of the major concerns in field level application of Bt transgenic plants is development of resistance in insects towards δ-endotoxins upon continuous selection pressure[3]. Various strategies have been suggested to prevent or delay the resistance development among which gene pyramiding/mixture is an important measure[4]. A combination of Bt genes coding for δ-endotoxins which differ in their mode of action, receptor binding and sequence homology needs to be worked out in relation to each target insect. Recently we have reported the toxicity of eleven lepidoptera specific δ-endotoxins of Bt towards *Helocoverpa armigera*, an important polyphagous pest on cotton, chickpea, pigeonpea, tomato, sunflower,