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STUDIES ON NEEM PLANT AS REPELLENT AGAINST STORED GRAIN INSECTS

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Abstract. The extracts of various plants were tested against stored grain insects; the 'neem' plant possessed maximum repellent property. The maximum repellency was exhibited by seeds compared with leaves, flowers and fruits of 'neem' plant. It was further observed that the adults of *Rhyzopertha dominica* F. were less affected. The flour beetles fed on neem-extract-impregnated flour failed to reproduce and their feeding activity, determined by using P^{32} labelled-flour, was greatly reduced.

Insect infestation of stored grains and their products is a serious problem throughout the world. The fumigation has been recommended and is being used in advanced countries; it is only applicable if extensive storage facilities and appropriate funds are available. It is economical at large scale applications and is not feasible during transportation and storage at farmer's level. As an alternative the use of insect resistant bags, impenetrable by stored grain insects, has been suggested.

Attempts have been made to make a practicable insect resistant bag by using various constructing materials[1–5] and other compounds as repellent or toxicant to stored grain insects.[6] Most commonly used repellents are the synergised pyrethrins, allethrin or methoxychlor,[1] Dichlorovos,[7,8] but only pyrethrins are approved for commercial use. Some organic compounds like piperonyl butoxide[1] and carbaryl[4] have also been used as barriers for inward migration of insects. Similarly extracts of various plants immune to insect attack have been tested for their repellency and toxicity to various insect species.[9,10] The work described here reports the results of biological evaluation of three locally occurring plants which are known to be immune to insect attack for their repellency against some of the stored grain insects. These plants have been tested for their toxicity against various insect species.[11] The extract of leaves of neem was found to be nontoxic against German cockroaches, milk weed bugs,[11] armyworms, pea aphids, celery leaf tiers and two spotted-spider mites[12] but slightly toxic to American cockroaches.[11] However, repellent property has been reported against *Acrididae*[13] and grasshoppers when extract of neem leaves was sprayed on coffee and cabbage plants under laboratory conditions.[14] Chauvin *et al.*[15] extracted a carotenoid-like substance, 'Meliatin' as antifeedent and a phagorepellent fraction called 'melintriol' against desert locust.[16]

Materials and Methods

Culture. The three species of stored grain insect, *Tribolium castaneum* Hbst., *Trogoderma granarium* Everst., and *Rhyzopertha dominica F.* were reared at a controlled temperature (29±1°C) and humidity (55% R.H.) under low crowding conditions. The culture of *T. castaneum* was maintained on whole wheat flour with 5% yeast, whereas the other two species were reared on broken wheat (Mexi–Pak).

Extract Application and Test Arena. Twenty gram material was homogenized in water or ethanol (50%) and extracted repeatedly. The extract was concenterated to 50 ml by rotary evaporation at 37°C. The extract was applied to a filter paper strip (Whatman No. 1; 10×5 cm dimension) by dipping in the extract solution for 1 min, then the strip was dried. The treated strip was attached edge to edge to an untreated strip of the same dimensions by cellotape on the reverse side. The test arena was made by placing glass cylinder open from both sides, 6.5 cm in internal diameter and 4 cm high, on the joined filter paper strips. In each test four cylinders were used, while filter paper strips were placed in different directions to avoid any incidental stimulus affecting distribution of insects. The insects were released on the centre of the arena and the cylinder was covered with glass slab. Counts of the insects present on each strip were made after intervals. All the tests were carried out in dark and the counts were made in red light. The average of counts were converted to express 'percentage repulsion' by doubling the difference between the percentage of insects counted on the untreated half and the 50% distribution expected if only untreated paper were used.

Labelling of Flour. For feeding studies the flour was labelled with the extract and radio labelled with P^{32}. The extract and $H_3P^{32}O_3$ in required quantity were added to the flour at the rate of 2 ml/g flour, which resulted to a paste, which was freeze-dried for 24 hr and fluffy flour was obtained. The final radioactivity in the flour was 25 μc/g.

Experimental and Results

Preliminary tests were conducted by using untreated paper strips to determine the minimum number of insects for each arena to be used and minimum time

interval at which uniform distribution of beetles was recorded. In various tests, 10, 20, 30, 40 or 50 flour beetles were introduced to the test arena and their distribution was recorded after interval of 15, 30, 60, 120 or 240 min. The distribution of 50:50 insects on each side of arena did not level off until 30 min, and was significant at all the levels of insect numbers. Since it was convenient to record the position of 10 beetles, therefore, for further experiments a time interval of 1 hr and 10 insects per arena were used. The test arena was standardized by treating the filter-paper strips with the extract of secretory glands of flour beetles, which served as a standard repellent.[12] The secretory glands of 3000 flour beetles were excised out from the abdomen and extracted in 50 ml methanol.

Repellency Test of Different Plants. Twenty gram leaves of each of 'neem', *Melia azadirachta* (L.) 'ak' *Calotropis procera* Ait. and gardenia, *Gardinia jasminoides* Ellis were extracted in 50 ml water and tested against adults of *T. castaneum*. The maximum repellent property was exhibited by 'neem' leaves (80%), while 'ak' leaves were least repellent (10%, Table 1). Further studies were undertaken with the 'neem' plant in details.

Repellent Property in Different Parts of Neem Plant. The water and 50% ethanolic extracts of leaves, flowers, endocarp and exocarp of fruits and seeds of equal weights, (20 g each) were tested against the adults of red flour beetles. The repellency was observed in all the parts of the neem plants tested, significant at 0.1% level (Table 2). Since all the plant parts exhibited repellency, the concenterated extracts were diluted logestically and tested for repellency till no significant effect was observed in that dilution. The results have been computed in probit repellency and are given in Fig. 1(a and b). From the figure it can be inferred that although all plant parts tested exhibited repellency, the seeds and endocarp of the fruit possessed more of repellent substances.

Repellency Against Different Species of Stored Grain Insects. The extract of seeds was also tested against larvae of *Tribolium castaneum* and *Trogoderma granarium* and adults of *Rhyzopertha dominica* similarily as above. The results (Fig. 2a and b) indicate that these species were also repelled though the adults of *Rhyzopertha dominica* were not repelled at lower concentrations. From this it can be inferred that adults of *R. dominica* are resistant to repellent as compared to other species tested.

Effect on Feeding. The flour was impregnated with the water extract of the seeds at the dose of 400, 800 and 1600 p.p.m. (dry weight of 20% extract in flour) and labelled with P^{32}. Sixty 15-days old adults of flour beetles were placed on 5 g labelled flour at each dose level alongwith control. The control flour was also labelled with P^{32}. The beetles were allowed to feed for 1, 2, 4 or 8 days, then taken off, cleared for

TABLE 1. THE REPELLENCY EXHIBITED BY THE EXTRACTS OF DIFFERENT PLANTS AGAINST *Tribolium castaneum* HBST.

Plant	No. of beetles on treated-half	Repulsion (%)
Neem	1.0±0.2	80
Gardenia	2.5±0.3	50
Ak	4.5±1.2	10

TABLE 2. THE REPELLENCY OBSERVED IN VARIOUS PARTS OF NEEM PLANT AGAINST *Tribolium castaneum* HBST.

Neem plant		Water extract		Alcohol (5%)	
		A*	Repulsion (%)	A*	Repulsion (%)
Leaves		1·0±0–2	80	2·5±1·2	50
Flowers		1·8±2·0	64	2·5±0·5	50
Exocarp	Fruit	2·3±0·5	54	1·3±0·5	74
Endocarp	Fruit	1·3±0·5	74	1·3±0·6	74
Seeds		1·0±0·6	80	1·5±0·6	70

*The mean number of beetles counted on the treated-half of arena. ± S.E.M.

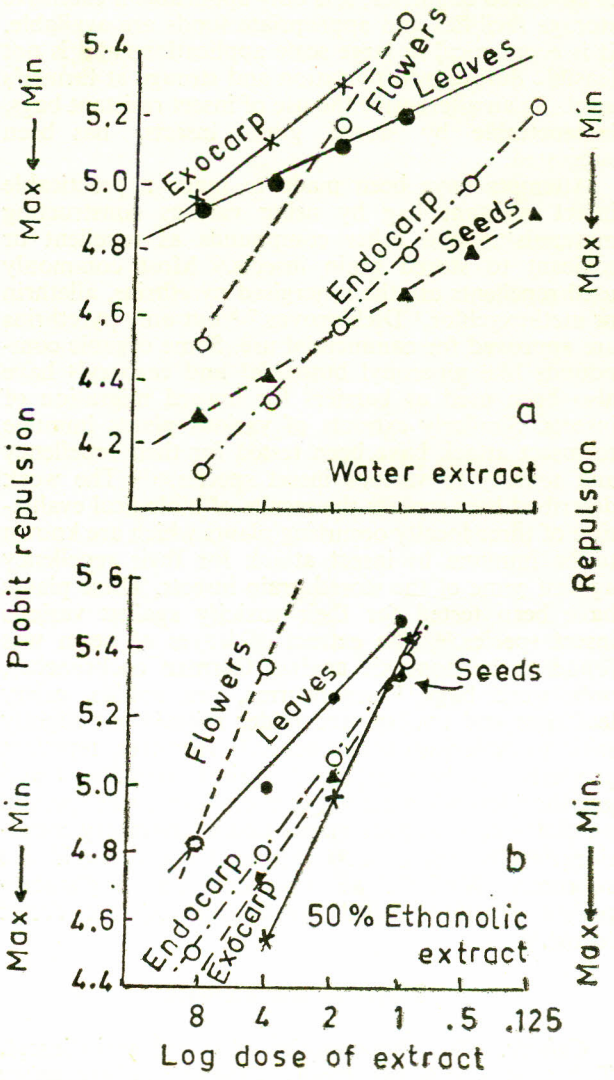

Fig. 1. The repellency of various parts of neem plant against red flour beetle, *T. castaneum*.

adhered flour, washed with aceton 3–4 times and counted with Panax G.M. counter for the presence of radioactivity. The counts were corrected for background and decay. The amount of radioactivity present represented the amount of flour ingested during that period (Table 3). These results reveal that the extract has an inhibitory effect on the feeding beside repulsion. The inhibition in feeding was dose-dependent. Although the beetles did consume some of the extract impregnated flour, the amount was far less than controls. At higher level, i.e. 1600 p.p.m. after 8 days beetles ingested same amount as controls did in one day. This compulsary feeding may be result of starvation.

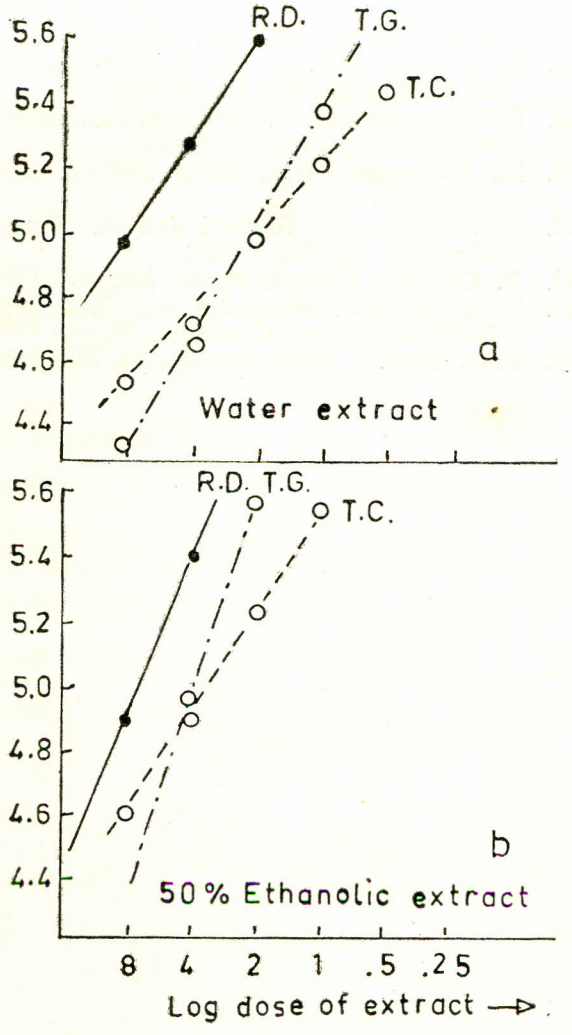

Fig. 2. The repellency of neem seeds against various species of stored grain insects. TC, *Tribolium castaneum* (larvae); TG, *Trogoderma granarium* (larvae); RD, *Rhyzopertha dominica* (adults).

Effect on Reproduction. The flour was impregnated with the extract of seeds at the dose of 400, 800 or 1600 p.p.m. in the same way as in feeding studies. Twenty randomly selected beetles were placed on 8 g flour in 3 replicates at each dose level alongwith control for oviposition. After 3 days interval the beetles were sieved and given fresh flour, whereas the sieved flour was incubated for emergence of larvae. The effect on reproduction was studied for 15 days. The progeny produced in each vial was recorded after 15 days of oviposition. The data (Table 4) reveal that beetles failed to reproduce in the seed's extract labelled flour. The reproduction was totally inhibited at higher dose level, i.e. 1600 p.p.m. whereas at lower levels produced only 3.8 and 0.38% of controls progeny at 400 and 800 p.p.m. respectively.

Discussion

The results of these preliminary investigations suggest that 'neem' plant has the repellent property and its seeds and leaves have potentials to be used for protecting stored grains from insect attack if applied to the bags or earthen pots used in this country by local farmers. It is further evident that if the grains are sprayed with the seed's extract, even on infestation, the flour beetles will fail to consume stored grains and their reproduction will be inhibited and the chances of further build up of their population will be eliminated. As the 'neem' plant is quite common in this country, its application would be much cheaper even

TABLE 3. COUNTS DETERMINED PER BEETLE (*T. castaneum*) FED ON RADIOLABELLED FLOUR (control as well as treated with the extract of seeds).

Dosage	Days			
	1	2	4	8
Control	125	1483	2147	3143
400 p.p.m.	19	188	185	729
800 p.p.m.	9	96	74	880
1600 p.p.m.	5	45	61	147

TABLE 4. THE PROGENY PRODUCED BY 20 RED FLOUR BEETLES (*T. castaneum*) IN CONTROL FLOUR AND FLOUR TREATED WITH THE EXTRACT OF SEEDS.

Dosage	Days					
	0–3	3–6	6–9	9–12	12–15	Total
Control	22.3±2.6	51.7±9.6	83.3±6.8	103.7±9.7	103.0±4.5	364.3±8.1
400 p.p.m.	3.7±2.3	4.0±3.1	3.7±0.9	1.7±0.9	1.0±0.6	14.0±7.1
800 p.p.m.	0.0	0.3	0.0	0.0	0.3±0.3	0.7±0.7
1600 p.p.m.	0.0	0.0	0.0	0.0	0.0	0.0

at small scale applications. As this plant is already in use for medicinal purposes, it is expected that it will not have any ill effects on mammals or man. Nevertheless, further studies are needed for its toxicity to mammals and its persistance over long time intervals. The studies on chemistry and application to the bags are in progress.

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