Journal of Chemical Ecology, Vol. 19, No. 11, 1993

DETERRENT AND INSECTICIDAL EFFECTS OF EXTRACTS OF PITHRAJ, *Aphanamixis polystachya* (MELIACEAE), AGAINST *Tribolium castaneum* IN STORAGE

F.A. TALUKDER* and P.E. HOWSE

Department of Biology, University of Southampton Bassett Crescent East, Southampton SO9 3TU, England, U.K.

(Received February 22, 1993; accepted May 27, 1993)

Abstract—Laboratory experiments were carried out to investigate the efficacy of seed extracts of pithraj, *Aphanamixis polystachya* (Wall & Parker), a locally grown plant in Bangladesh, against the red flour beetle, *Tribolium castaneum* (Herbst.). Results of three different bioassays showed that crude extracts of pithraj seeds have strong repellent effects and moderate feeding deterrent and insecticidal (direct-contact) effects on adult *Tribolium castaneum*.

Key Words—Pithraj, *Aphanamixis polystachya*, Meliaceae, repellent, feeding deterrent, *Tribolium castaneum*, red flour beetle, Coleoptera, Tenebrionidae.

INTRODUCTION

To alleviate insect pest problems in storage, synthetic pesticides are generally recommended. These may have drawbacks, including toxicity to nontarget organisms, development of pest resistance, and environmental pollution. Hence, there is a worldwide interest in the development of alternative strategies, including the use of new types of insecticides derived from a reevaluation of age-old, traditional botanical pest control agents (Heyde et al., 1984). In the rural areas of South Asia, including Bangladesh, farmers traditionally mix leaves, bark, seeds, roots, or oils of certain plants with stored grains to keep them free from insect attacks. Such techniques have been inherited as part of the traditional culture (Saxena et al., 1988). However, few studies have been conducted in Bangladesh on the traditional use of botanicals against stored products pests

*To whom correspondence should be addressed.

(Anonymous, 1985; Islam, 1984, 1987; Islam, 1985; Khanam et al., 1990, 1991). The present study was undertaken with a locally grown plant, pithraj, *Aphanamixis polystachya* Wall & Parker (synonym *Amoora ruhituka* Wright & Arn.), used traditionally by farmers to protect stored products from insect attacks. We tested pithraj against the red flour beetle, *Tribolium castaneum* (Herbst), a major stored products pest in Bangladesh, to determine the repellent, feeding deterrent, and insecticidal action of the seed extracts.

METHODS AND MATERIALS

The seeds of pithraj, *Aphanamixis polystachya* (Meliaceae), used in this study, were collected from Bangladesh, and the red flour beetle, *Tribolium castaneum*, was obtained from the Blades Biological Company, Kent, England. The beetle was reared in glass jars (14 × 10.5 × 30 cm) on a diet of wholemeal wheat flour mixed with brewer's yeast in a 19:1 ratio. The cultures were started with 100–120 adult beetles of each sex. Five pairs of adults about 5–6 days old were placed in 13.9 × 2.8-cm Petri dishes containing the rearing media. The dishes were sealed and a maximum of seven days were allowed for mating and oviposition. Then the parent stocks were removed and the egg-containing media were transferred to media in the breeding containers. The jars were covered with pieces of cloth fastened with rubber bands to prevent contamination and the escape of insects. The experiments were conducted in the laboratory of the Department of Biology, University of Southampton, England. The rearing and experimental conditions were 12-hr light and dark periods at 27 ± 2°C and 70 ± 1% relative humidity.

Extraction

Seeds of *Aphanamixis polystachya* were air-dried, ground, and then extracted in a Soxhlet apparatus separately with petroleum ether (at 55°C), acetone (at 65°C), and 95% ethanol (at 80°C), for 6 hr in each case. The petroleum ether and acetone solvents were evaporated in a rotary vacuum evaporator at 40°C under reduced pressure yielding the petroleum ether (PSE) and acetone (ASE) extracts. The ethanol solvent was completely evaporated, and the extract was redissolved in petroleum ether solution, stirred for 20 min and then filtered. From the filtered solution, solvent was evaporated to obtain the ethanol extract (ESE).

Insect Responses to Plant Extracts

Repellency Tests Using Filter Papers. Repellency was tested by Standard Method Number 3 described by McDonald et al. (1970), with some modifications. Substrata were prepared by cutting in half Whatman No. 40 filter-paper disks (8 cm diam.), dipping them in the 0.5% and 1.0% solutions of pithraj extracts for 1 min, and then air-drying them overnight at room temperature. Each treated disk half was then attached lengthwise, edge-to-edge, to an untreated disk half with cellulose tape and placed in a Petri dish. The orientation of the seam was changed in each replicate to avoid the effects of any external directioned stimulus affecting distribution of the test insects. Ten adults (6–7 days old) were released in the middle of each filter-paper circle and a cover was placed on the Petri dish. For each concentration of plant extract, four replications were made. Individuals that settled on each filter paper disk half were counted at hourly intervals for 5 hr. The average of the counts was converted to express the percentage repulsion (R) as follows:

$$R = 2 \times (C - 50)$$

where C is the percentage of insects on the untreated half. Positive values expressed repellency and negative values attractancy. Data were analyzed using analysis of variance after correction of the percentage repulsion by the use of Abbott's (1987) formula and transformation into arcsin $\sqrt{}$percentage values. The averages were then assigned to different classes using the following scale:

Class	Percent repulsion
0	>0.01 to <0.1
I	0.1–20
II	20.1–40
III	40.1–60
IV	60.1–80
V	80.1–100

Feeding Deterrence Test. The potency of the feeding deterrence effect of pithraj seed extracts against *Tribolium castaneum* was determined by using the wheat wafer disk bioassay described by Nawrot et al. (1986). Wheat wafer disks (4.1 cm diam.) obtained from the Poor St. Claire's Convent, Southampton, England, were used as the test food. The disks were saturated by dipping in either solvent only (control disks K) or in 1.0% solution of PSE, ASE, or ESE (treated disks E). The disks were then air-dried for 30 min and weighed before presenting them to the 10 adults (6–7 days old) over the following five-day period, during which they were the sole food source.

Feeding of the insects was recorded under three conditions: (1) on pure food, composed of two control (KK) disks, (2) on food with a choice between one treated disk (E) and one control disk (K) (choice test), and (3) on food with two treated disks (EE) (no-choice test). Each treatment was replicated five times. After the completion of the experiment, the disks were reweighed and, based on the amount of food consumed in control (KK), choice (KE), and no-choice (EE) test, three coefficients for feeding deterrent activity were calculated as follows:

1. Absolute deterrence coefficient:

$$A = (KK - EE/KK + EE) \times 100$$

2. Relative deterrence coefficient:

$$R = (K - E/K + E) \times 100$$

3. Total deterrence coefficient:

$$T = A + R$$

Values of the total deterrence coefficient served as an index of feeding deterrence activity expressed on a scale between 0 and 200. The index zero (0) was an inert compound and 200 a highly deterrent compound. Feeding deterrents having an index of 150–200 were marked ++++; 100–150, +++; 50–100, ++, and 0–50 +. Data were analyzed by analysis of variance.

Topical Application. Contact toxicity of pithraj extracts was tested by topical treatment, using Standard Method Number 1 described by McDonald et al. (1970) with slight modifications. Stock solutions were prepared by dissolving 100 mg of PSE, ASE, or ESE extract in 1 ml of either petroleum ether or acetone solvent. Lower concentrations (60, 40, 20, and 10 mg/ml) were obtained by dilution of the stock solution with the solvent. Insects were chilled for a period of 10 min, and then the immobilized insects were picked up individually with a small suction tube. Using a capillary tube, 1 μl of the extract solution (100, 60, 40, 20, or 10 μg per insect) was applied to the dorsum of each insect. Fifty unsexed insects, in five replicates of 10 each, were treated at each dose. Control insects were treated with solvent. Treated and control insects were transferred to Petri dishes (10 insects/dish). The insects were examined daily, and those that did not move or respond to gentle touch were considered dead. Dead insects were recorded at 24, 48, and 72 hr after treatment and corrected mortality rates were calculated using the formula of Abbott (1987). Concentration–mortality data were analyzed by probit analysis (Finney, 1971).

RESULTS

Repellency Effects

All three extracts of pithraj strongly repelled the red flour beetle (Table 1). ASE was the most repellent (88 and 93% repellency at 0.5% and 1.0% concentration, respectively) to the beetle, although the repulsion differences were not statistically significant ($P < 0.05$). Although repellency varied with concentration, all extracts belonged to class V repellency. In all cases, repellency decreased with time. The rapid drop in repellency could be attributed to the closed condition of the test arena in the Petri dishes, leading to saturation of the test arena with volatiles from the pithraj seed extracts.

Feeding Deterrence Effects

All three pithraj extracts inhibited the feeding activity of *T. castaneum* (Table 2); ASE was the most deterrent, while ESE was the least effective. There were no significant differences among feeding deterrent activities of the PSE,

TABLE 1. AVERAGE REPELLENCY OF PITHRAJ SEED EXTRACT TO *T. castaneum* ADULTS USING TREATED FILTER PAPER TEST[a]

Extract	Conc of extract	Average repellency (%) at hours after treatment					Mean	Repellency class
		1	2	3	4	5		
Petroleum ether	0.5%	95	95	95	75	60	84	V
	1.0%	95	85	95	70	60	81	V
Acetone	0.5%	100	85	95	85	75	88	V
	1.0%	85	95	100	95	90	93	V
Ethanol	0.5%	95	95	90	80	70	86	V
	1.0%	80	80	90	85	90	85	V
F value		NS[b]	NS	NS	NS	NS	NS	

[a]Original data were transformed into arcs in $\surd$ percentage values during ANOVA test.
[b]NS = not significant.

TABLE 2. FEEDING DETERRENT ACTIVITY COEFFICIENTS OF PITHRAJ SEED EXTRACTS AGAINST *T. castaneum* ADULTS[a]

Extract	Coefficient of deterrency			Efficacy of Extract
	Absolute	Relative	Total	
Petroleum ether	−9.98	73.10	63.12	+ +
Acetone	16.92	71.07	87.99	+ +
Ethanol	−12.91	61.73	48.82	+
F value	NS[b]	NS	NS	

[a]Original data were transformed into arcs in $\surd$ percentage values during ANOVA test.
[b]NS = not significant.

ASE, and ESE extracts, but PSE and ASE were ranked more repellent (++) than ESE (+) by the method used.

Topical Application

The pithraj seed extracts were moderately toxic to *T. castaneum*; 42–55% adults died within 72 hr of treatment at a dose of 100 μg/insect (Table 3). ESE applied topically was more toxic than ASE or PSE. At lower doses, the extracts showed no direct contact toxicity. Probit analysis showed that ESE was most toxic to *T. castaneum*, whose LD_{50} was 90 μg/insect, whereas it was 234 μg/insect in the case of ASE (Table 4).

Figure 1 gives the regression lines $y = 2.38 + 1.28x$ for PSE, $y = 1.92 + 1.30x$ for ASE, and $y = 0.95 + 2.07x$ for ESE, which were calculated by probit analysis (Finney, 1971). The steepness of the slopes indicated that *Tribolium castaneum* adults are moderately susceptible to pithraj seed extracts.

TABLE 3. TOXICITY OF PITHRAJ SEED EXTRACTS APPLIED TOPICALLY TO *T. castaneum* ADULTS[a]

Extract	Dose (μg/insect)	% average mortality at hours after treatment ± SE[b]		
		24	48	72
Petroleum ether	10	6 ± 0.8 b[c]	7 ± 1.1 b	11 ± 1.1 b
	20	14 ± 1.3 ab	12 ± 1.3 b	14 ± 1.9 b
	40	14 ± 1.3 ab	23 ± 2.1 ab	29 ± 2.0 ab
	60	18 ± 1.8 ab	25 ± 2.2 ab	35 ± 1.6 ab
	100	34 ± 3.7 a	45 ± 4.3 a	49 ± 3.5 a
Acetone	10	4 ± 0.8 b	6 ± 0.8 b	7 ± 1.1 b
	20	4 ± 0.8 b	6 ± 1.3 b	9 ± 0.9 b
	40	6 ± 1.3 b	8 ± 1.8 b	11 ± 1.5 b
	60	10 ± 1.7 b	10 ± 1.7 b	13 ± 1.6 b
	100	38 ± 2.7 a	40 ± 2.8 a	43 ± 3.3 a
Ethanol	10	4 ± 0.8 c	4 ± 0.8 c	5 ± 0.6 c
	20	6 ± 0.8 c	8 ± 0.6 c	9 ± 0.9 c
	40	6 ± 0.8 c	10 ± 1.0 c	10 ± 0.6 c
	60	34 ± 1.3 ab	42 ± 1.2 ab	50 ± 0.8 ab
	100	42 ± 3.1 a	52 ± 3.7 a	55 ± 3.6 a

[a]Original data corrected by Abbott's (1987) formula and then transformed into arcs in √ percentage values before ANOVA and DMRT (Duncan, 1951) test.
[b]SE = standard error of mean.
[c]Values followed by the same letter within a column are not significantly different at the 0.05 level by DMRT (Duncan, 1951).

TABLE 4. PROBIT ANALYSIS FOR CONTACT TOXICITY AT 72 H AFTER TOPICAL APPLICATION OF PITHRAJ EXTRACTS TO *T. castaneum* ADULTS[a]

Extract	Insects (*N*)	LD_{50} (μg/insect)	95% fiducial limit	Slope ± SE[b]
Petroleum ether	250	113.0	67.0 − 190.0	1.28 ± 0.27
Acetone	250	234.0	115.0 − 472.0	1.30 ± 0.32
Ethanol	250	90.0	42.0 − 192.0	2.07 ± 0.33

[a]Values were based on five replicates of 10 insects each.
[b]SE = standard error.

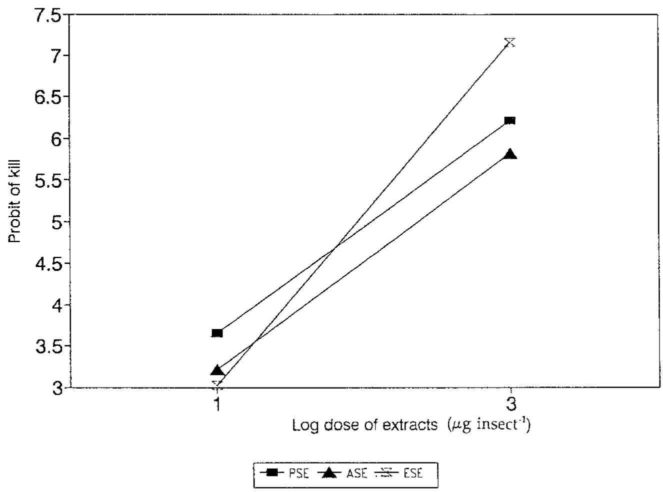

FIG. 1. Relationship of probit kill of *T. castaneum* adults to log concentration of pithraj seed extracts. At least 50 insects were tested for each concentration.

DISCUSSION

Repellents, feeding deterrents, and insecticides of natural origin are rational alternatives to synthetic insecticides. These compounds are naturally produced in many plants and play a vital role as resistance factors in plant defense systems. Crude extracts of pithraj seeds have been shown here to have strong repellency, moderate feeding deterrence, and some measure of contact toxicity to *Tribolium castaneum*. Nawrot et al. (1986) reported that among the stored grain pests, *Tribolium* spp. react more strongly to antifeedants and the adults were more sensitive than the larvae. Islam (1984) reported weak feeding deterrent effects of *A. polystachya* (referred to as *Amoora ruhituka* Wright & Arn.) on *Dicladispa* *armigera* and repellent effects on *Sitotroga cerealella* and *Spilosoma obliqua*. Islam (1985) found that seed extracts of pithraj (*Amoora ruhituka* Wright & Arn.) also deterred feeding by *Epilachna 12-punctata*. Islam (1987) gives data on the mortality effects of seed extracts of *A. polystachya* (referred to as *Amoora ruhituka*) on the larvae of *Callosobruchus chinensis*. Khanam et al. (1990, 1991) reported that seed coat extracts of pithraj (*A. polystachya*) had a deleterious effect on the growth and development of *Tribolium confusum* and, in another experiment, they found that pithraj seed extracts were toxic to the adults of *Sitophilus oryzae*. In the present experiment, the rapid decrease of repellency with time may be explained by the findings of Jilani and Saxena (1990), who found that the repellency of compounds with low molecular weights and high volatility decreased rapidly over time. The present results also demonstrate the lower extraction capability of petroleum ether, acetone, and ethanol from pithraj seeds. The present study has shown that pithraj seed extracts have strong repellent but moderate feeding deterrent and direct-contact effects on *T. castaneum* adults. The study also confirms the validity of traditional use of pithraj against stored-grain pests.

REFERENCES

ABBOTT, W.S. 1987. A method of computing the effectiveness of an insecticide. *J. Am. Mosq. Control Assoc.* 3:302–303.

ANONYMOUS. 1985. Annual report for 1985. Bangladesh Rice Research Institute, Gazipur. 271 pp.

DUNCAN, D.B. 1951. A significance test for differences between ranked treatments in an analysis of variance. *V. J. Sci.* 2:171–189.

FINNEY, D.J. 1971. Probit Analysis, 3rd Ed. Cambridge University Press, Cambridge. 333 pp.

HEYDE, J.V.D., SAXENA, R.C., and SCHMUTTERER, H. 1984. Neem oil and neem extracts as potential insecticides for control of hemipterous rice pests, pp. 377–390, *in* Proceedings, 2nd International Neem Conference, Rauischholzhausen, 1983.

ISLAM, B.N. 1984. Pesticidal action of neem and certain indigenous plants and weeds of Bangladesh, pp. 263–290, *in* Proceedings 2nd International Neem Conference, Rauischholzhausen, 1983.

ISLAM, B.N. 1987. Use of some extracts from Meliaceae and Annonaceae for control of rice hispa, *Dicladispa armigera*, and the pulse beetle, *Callosobruchus chinensis*, pp. 217–242, *in* Proceedings 3rd International Neem Conference, Nairobi, 1986.

ISLAM, K.S. 1985. Studies on the growth disrupting effects of plant extracts and isolates of neem, chinaberry and pithraj in *Epilachna 12-punctata* Muls. MSc(Ag) thesis. Department of Entomology, Bangladesh Agricultural University, Mymensingh. 50 pp.

JILANI, G., and SAXENA, R.C. 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). *J. Econ. Entomol.* 83:629–634.

KHANAM, L.A.M., TALUKDER, D., KHAN, A.R., and RAHMAN, S.M. 1990. Insecticidal properties of Royna, *Aphanamixis polystachya* Wall. (Parker) (Meliaceae) against *Tribolium confusum* Duval. *J. Asiat. Soc. Bangladesh, Sci.* 16:71–74.

KHANAM, L.A.M., KHAN, A.R., and TALUKDER, D. 1991. Use of some indigenous plant materials in controlling *Sitophilus oryzae* (L.). *J. Asiat. Soc. Bangladesh, Sci.* 17:75–78.

MCDONALD, L.L., GUY, R.H., and SPEIRS, R.D. 1970. Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored-product insects-I. Marketing Research Report No. 882. Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 8 pp.

NAWROT, J., BLOSZVK, E., HARMATHA, J., NOVOTNY, L., and DROZDZ, B. 1986. Action of antifeedants of plant origin on beetles infesting stored products. *Acta Entomol. Bohemoslov.* 83:327–335.

SAXENA, R.C., JILANI, G., and KAREEM, A.A. 1988. Effects of neem on stored grain insects, pp. 97–111, *in* M. Jacobson (ed.). Focus on Phytochemical Pesticides, Vol. 1: The Neem Tree. CRC Press, Boca Raton, Florida.