Efficacy of pithraj (*Aphanamixis polystachya*) seed extracts against stored-product pests

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Abstract

Crude seed extracts of pithraj (*Aphanamixis polystachya*) were evaluated for their repellency and contact toxicity to *Tribolium castaneum*, *Callosobruchus chinensis* and *Sitophilus oryzae*; and feeding deterrency to *T. castaneum* and *S. oryzae*. Seed volatiles were also evaluated for their repellency to *T. castaneum*, *C. chinensis* and *S. oryzae*. The results shows that crude extracts and seed volatiles have strong repellent effects of *T. castaneum*, and *S. oryzae*, but relatively weak effects on *C. chinensis*. Crude extracts were moderate feeding deterrents to *T. castaneum* and highly deterrents to *S. oryzae*. Laboratory studies showed that pithraj seed extracts have pesticidal effects on three tested insects. Extracts were highly toxic to *C. chinensis* and *T. castaneum*, and moderately to *S. oryzae*.

Introduction

More than 20000 species of field and storage pests annually destroy approximately one-third of the world's food production, valued at more than $100 billion among which highest losses (43% of potential production) occur in developing Asian countries (Ahmed and Grainge 1986). In USA and Canada, 20–26% of stored wheat was infested by stored-product pest (White et al. 1985). In India, losses caused by insets accounted for 6.5% of stored grains (Raju 1984). The loss of food grain during storage due to various insect pests is a very serious problem. Climate and storage conditions, especially in the tropics, are often highly favourable for insect growth and development. control of these insects by chemical insecticides has serious drawbacks (Sharaby 1988). The indiscriminate use of chemical pesticides has given rise to many obvious serious problems, including genetic resistance by pest species, toxic residues, increasing costs of application, storage environment pollution, hazards from handling etc. (Ahmed et al. 1981; Khanam et al. 1990).

With the advent of synthetic pesticides, research on plant-derived pesticides diminished. The synthetic pesticides, however, have come under increasing attack in recent years due to persistence in the environment, insect resistance and high mammalian toxicity. On the other hand, plant-derived pesticides are more readily biodegraded. Therefore, they are less likely to contaminate the environment and may be less toxic to mammals (Freedman et al. 1979). In view of these facts, workers for the last two decades or so have diverted their attention towards age-old practices of using phytochemicals, which would be non hazardous, easy to use and specific in their action (Koul 1982).

Extracts of plants have been used by humans for control of insects since before the time of the ancient Romans. In many areas of Africa and Asia, locally available plants and minerals are being widely used to protect stored products against damage by insect infestation, as an alternative to synthetic pesticides (Golob and Webley 1980; Su et al. 1982; Zehrer 1984; Ahmed and Koppel 1985; Khalique et al. 1988). Many plants manufacture chemicals that protect them against insect depredation and the extract from these plant affects metabolism and response in species other that those attacking the plant from which the chemical was derived. Use of plants for pest infestation control in stored grains therefore seems to offer desirable solutions, especially in developing tropical countries where plants are found in abundance everywhere throughout the year.

Research on stored-product insect-pest control with plant materials contributes to the study of natural products chemistry. Although few investigators have studied the anti-insect properties of natural products on stored-product pests, such research offer a good opportunity to open a new era of natural control. Therefore, the present research has been undertaken to find out the efficacy of extracts of pithraj, *Aphanamixis polystachya*, against three major stored-product insects.

Preparation of plant extracts

The plant used for the present research works is known as pithraj, *Aphanamixis polystachya* Wall and Parker (family Meliaceae), is a perennial tree (flowering plant) with broad green leaves and is a native to the South and Southeast Asia. This plant has two other scientific names viz. *Amoora ruhituka* Wright and Arn (Islam 1984; Islam 1985) and *Ricinocarpodendron polystachyum* (Razzaque et al. 1983). Pithraj seeds were dried, then ground in an electric grinding machine. The extracts were made in two different ways. (i) 75g lots of ground seeds were extracted in Soxhlet procedure with redistilled petroleum ether (b.p. 40–60°C) for 6 hours. Similarly, another two 75g seeds were extracted, one with 95% ethanol and another with acetone, for the same period. The petroleum ether and acetone solvents were evaporated in a rotary vacuum evaporator under reduced pressure, yielding the petroleum ether (PSE) and acetone (ASE) extract. The ethanol solvent was completely evaporated under reduced pressure and the extract redissolved in petroleum ether solution. This mixture was stirred for 20 minutes, filtered and evaporated as before to yield an ethanol-pet ether extract (ESE). (ii) 75 g of ground seeds were extracted with redistilled petroleum ether in above way. The marc was then extracted successively with acetone, 95% ethanol and with methyl alcohol, for the same periods. Solvents in each extract were evaporated in a rotary vacuum evaporator under reduced pressure, yielding petroleum ether (PSX), acetone (ASX), ethyl alcohol (ESX) and methyl alcohol (MSX) extracts.

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Aeration technique for seed volatiles

An aeration apparatus was used to trap airborne volatiles of pithraj seeds in their natural blends. The volatiles released were trapped on charcoal. The apparatus had three sections: (a) a prefilter (70 × 3 cm) filled with activated charcoal which was used to remove volatile contaminations from the air, (b) a glass chamber (65 cm × 7.5 cm) and (c) a volatile collecting tube (16 × 1.3 cm) filled with activated charcoal. The charcoal was activated by heating the glass tube containing charcoal with a bunsen burner in a stream of dry nitrogen flowing at ca 25 mL/minute.

All the glassware was washed in decon 90, rinsed with distilled water and finally dried overnight in an oven. The crushed seeds of pithraj were introduced into the glass chamber. The whole unit was connected to a water pump with a continuous airflow at 25 mL/minute. The water pump drew a stream of filtered air through the glass chamber containing the seeds leading the volatile compounds to absorb in the collection tube for 24 hours. Then the trapped volatiles in activated charcoal (charcoal tube) were extracted by solvent desorption method with 10 mL of dichloromethane. The aeration extract was later concentrated to 1 mL by gently passing dry nitrogen over it.

The test insects

Three different species of major stored-product insects were used for the experiment: red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) and rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *T. castaneum* was reared in the diet mixture of wholemeal wheat flour and brewer's yeast (ratio 19:1), *C. chinensis* in whole mung bean and the *S. oryzae* in rice grain (Bashmati variety). All three species of insects were reared in rectangular jars (size 14 × 10.5 × 30 cm), in the laboratory at 27 ± 2°C temperature and 75 ± 5% relative humidity and with alternating light and dark periods of 12 hours.

Bioassays

All the insect response assays were conducted in petri dishes (9 × 1.3 cm) placed into modified locust cages (38 × 38 × 65 cm). The cages were made airtight by lining the mesh base with polyethylene and sealing the corners with masking tape. The relatively humidity in the cage was kept at 75 ± 5% by using a standard solution of sodium chloride (Greenspan 1977).

Repellency tests by filter papers

Repellency tests were conducted for all three species according to the standard method number 3 with some modifications (McDonald et al. 1970). Substrates were prepared by cutting filter-paper circles (Whatman No. 40), 9cm in diameter, in half. Pithraj extracts were redissolved in a known amount of solvent to provide a concentration of 10 mg/mL. One millilitre solution of each plant extracts was applied to the half circle of filter paper s uniformly as possible with a pipette, in preparation of the treated substrate containing 0.16 mg/cm^2 extract. Then the treated half circles were air-dried to evaporate the solvent completely. One full-circle was then remade by attaching a treated half to an untreated half circle of the same dimension by transparent adhesive tape and placed in petri dishes (one circle in one petri dish) in four different directions to avoid any incidental stimulus affecting distribution of insects. Ten insects were released on the centre of each filter-paper circles and a cover placed on the petri dish. For each concentration of plant extract, four replications were used. Counts of the insects present on each strip were made at one hour intervals up to 5 hours. The averages of counts were converted to express 'percentage repulsion (PR)' by the following formula:

$$PR = (N_c - 5) \times 20$$

Where, N_c is the number of insects present in the control half.

Positive values (+) indicated repellency and negative values (–) attraction. Data were analysed by analysis of variances (ANOVA) after transforming them into arcsin √percentage values. The average values were then categorised according according to the following scale:

Class	Repellency rate (%)
0	>0.01 to <0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

The concentration seed volatile from the aeration technique was also used for repellency tests.

Feeding deterrent activity tests

The potency of the antifeedant effect of pithraj seed extracts against *Tribolium castaneum* and *Sitophilus oryzae* was determined using the wheat wafer disk bioassay described by Nawrot et al. (1986). Wafer disks (4.1 cm in diameter) were used as the test food. The disks were saturated by dipping into ether solvent only (control or untreated disks CC) or with 10 mg/mL solution of extracts (treated disks T) and then air-dried overnight and weighed before giving them to the 10 red flour beetles or 20 rice weevils for next 5 days as a sole food source.

Feeding of insects was recorded under three conditions: (i) on pure food, composed of two untreated disks CC (control), (ii) on food with a possibility of choice between one treated (T) and one untreated (C) disk (choice test) and (iii) on food with two treated (TT) disks (no-choice test). Each treatment was replicated 5 times. After completion of the experiment, the disks were reweighed. Despite the drying, the wheat wafer disks were still observed to increase in weight as a result of water suction from the surrounding humidity artificially created for normal growth and development of the insects during bioassay. Therefore, a correction procedure was applied. Disk weight loss, which was the estimate of the amount of food consumed (FC), was calculated by the formula of Serit et al. (1992):

$$FC = IW_s - [(FW_s \times IW_b)/FW_b)]$$

where, IW = initial weight of the disk after treated with extract or solvent.

FW = final weight of the wafer disk.

$_b$ = blank disk (treated by the solvent only and no insect was applied on this disk) and

$_s$ = treated and control (treated by solvent only) disks which were given to insects for consuming.

According to the amount of the food consumed in the control (CC), choice (CT) and no-choice (TT) tests, three feeding deterrent activity coefficients were calculated by the use of following formulas:

1. The absolute coefficient of deterrency A =(CC–TT/CC+TT) × 100
2. The relative coefficient of deterrency R = (C–T/C+T) × 100
3. The total coefficients of deterrency T= A + R

The values of the total coefficient served as an index of antifeeding activity expressed on a scale between 0 and 200. The index zero (0) indicates an inactive compound and 200 indicates an activity maximum. Antifeedants having index of 150–200 were marked with ++++; 100–150 +++; 50–100++ and 0–50 +. All experimental data were analysed by Analysis of Variance (ANOVA).

Contact toxicity

For contact toxicity by topical treatment studies, laboratory tests were conducted according to the standard method number 1 slight modifications (McDonald et al. 1970). Stock solutions were prepared by dissolving 100 mg of extract in 1 mL of solvent (petroleum ether, acetone, ethanol or methanol). Lower concentrations (60, 40, 20 and 10 mg/mL) were obtained by dilution of the stock solution with solvent. Insects were chilled for periods of 10 minutes to immobilise them and them picked up individually (by small suction tube). Using capillary tube, 1 μL of solution (100, 60, 40, 20 or 10 μg/insect) was applied to the dorsal surface of the thorax of the insect. Fifty unsexed insects, in 5 replicates of 10 insects each, were treated at each dose. In addition, the same numbers were treated with solvent only as control. After treatment, insects were transferred to 9 cm diameter Petrie dishes placing 10 insects/Petrie dish containing regular food. Insects were examined daily and those that did not move or respond to gentle touch were considered as dead. Insect mortalities were recorded at 24, 48 and 72 hours after treatment and corrected by Abbott's formula (1987) and then analysed by ANOVA and mean values adjudged with Duncan's Multiple Range Test (Duncan 1951). Mean lethal doses (LD_{50}) were calculated by probit analysis (Finney 1971). Results were expressed as micrograms per insect (μg/insect).

Results

All of the extracts from extraction method I (EM-1), were tested on *Tribolium castaneum* only, whereas extracts from extracted method II (EM–II) were tested on *T. castaneum*, *C. chinensis* and *S. oryzae*.

Repellency effects

In extraction method I (EM -1), all the three tested extracts (PSE, ASE & ESE) showed strong repellent effects on red flour beetle (Figure 1). Among them, ASE performed the highest average percent repellency on the beetle (repellency class V), though the differences were not statistically significant ($P < 0.05$).

In extraction method II (EM-II), the pithraj seed extracts showed strong repellent effects on red flour beetles, moderate effects on rice weevils but very poor effects on pulse beetles. Among the tested four extracts on red flour beetles (Figure 2), ASX showed highest average repellent effects (100%), followed by MSX (98%), ESX (95%) and PSX (92%), though their differences were not statistically significant ($P > 0.05$). Although the rate of repellency differed with the extract types, but all of them belongs to repellency class V. In case of pulse beetles, very poor levels of repellent effects were observed in all four extracts. Among the tested extracts, MSX showed highest repellency (44%, class-III), followed by ESX (30%, class-II), ASX (26%, class-II0 and PSX (19%, class-1), though their differences were not statistically significant ($P> 0.05$). However, In rice weevils, seed extracts showed moderate to high repellent effects. Among the tested extracts, both ASX and MSX showed highest repellency (67%, class-IV), followed by ESX (57%, class-III) and PSX (48%, class-III) but their differences were not statistically significant ($P > 0.05$).

The average repellency of the pithraj seeds volatiles on insects were given in Figure 2. The results showed that volatile extracts also have repellent effects on three tested insects. Among the insects, red flour beetles were significantly repelled (63%) by the volatiles, followed by rice weevils (28%) and pulse beetles (11%).

Feeding deterrent activity tests

The data, on the feeding deterrent activity (EM-I) of tested three extracts (PSE, ASE & ESE), for extraction method I are given in Figure 1. The present study revealed that all the extracts inhibit feeding activity of *T. castaneum*. Among them, ASE showed the most effective feeding deterrent capability (total coefficient of deterrency = 87.99), on the other hand ESE showed least effect (48.82).

From EM-II, the data on the feeding deterrent activities of pithraj seed extracts are given in Figure 3. The results revealed that extracts inhibited the feeding activities moderately in red flour beetles and highly in rice weevils. Among the tested four extracts, ESX showed the highest feeding deterrent activity (total coefficient of deterrency = 69.89) on red flour beetles, followed by ASX (69.66) and PSX (68.82). On the other hand, MSX showed least effect (50.64), but the mean differences were not statistically significant ($P>0.05$). In case of rice weevils (table 6), ASX showed the highest total feeding deterrent activity (159.50), followed by ESX (142.10) and PSX (132.32), where as MSX showed least effect (122.60). However, the mean differences were statistically insignificant ($P > 0.05$).

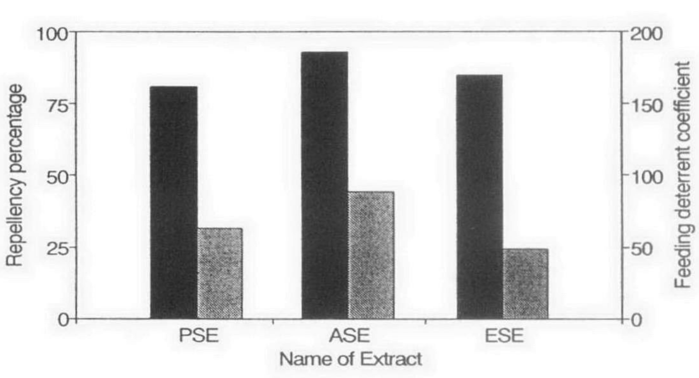

Fig. 1. Average repellent and feeding deterrent effects of extracts to adult red flour beetles. Repellency rate ■ ; Feeding coefficient ▒ .

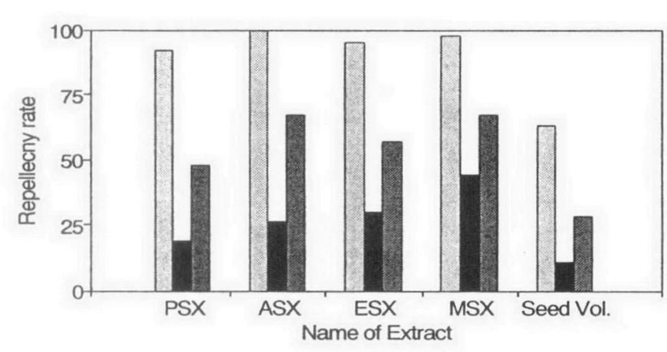

Fig. 2. Comparative repellency of four seed extracts and volatiles to adult beetles and weevils by treated filter paper method. Red flour beetles □; pulse beetles ■; rice weevils ▒.

Contact toxicity

Pithraj seeds extracts (EM-I) are moderately toxic to *T. castaneum* producing 42–55% adult mortality within 72 hours of treatment at the dose of 100 μg/insect (Figure 4). From the result, topical application of ESE showed the statistically best effect (55.1 ± 3.6% mortality) among the tested extracts, where as ASE showed the least effect (42.9 ± 3.3%). Lower doses of any extract did not showed promising results in direct contact toxicity test. Probit analysis revealed that ESE was most toxic to *T. castaneum* whose LD_{50} was 90μg/insect whereas it was 233.55 μg/insect in case of ASE.

Of the doses tested for EM-11, 100 μg/insect showed highest contact toxicity to red flour beetles (Figure 5). The results indicated that ethanol extract (ESX), at 100 μg/insect dose, caused the best mortality effect (79.1±0,5%) on *T. castaneum* followed by ASX (32.7 ± 0.3%) and PSX (66.8 ± 019%). Mortality data, obtained for *S. oryzae* indicated that ESX, at 100 μg/insects dose, possessed moderate toxic activities (mortality 37 ± 0,7 %) at 72 HAT, followed by ASX (22 ± 0.6%), MSX (22 ±1.2%) and PSX (20 ±1.0%). However, the observed mortality rate in *S. oryzae* for pithraj extracts application was lower in comparison to the other two insects.

Probit analysis, for extracts from EM I, revealed that ESE was most toxic to *T. castaneum* whose LD $_{50}$ was 90 μg/insect whereas it was 233.55 ug/insect in case of ASE (Figure 4).

The probit statistics, estimate of LD_{50} for extracts of EM-II, are presented in Figure 5. Form the probit analyses, comparison of LD_{50}'s among the extracts showed that Ethanol (ESX) was most toxic (LD_{50} 32 μg/insect) to *T. castaneum* (Figure 10), followed by ASX (169 μg/insect) where as MSX was the least toxic (364 μg/insect), followed by ASX (169 μg/insect) where as MSX was the least toxic (364 μg/insect). In tests with *C. chinensis*, comparison of LD_{50}'s among the extracts showed that ESX was highly toxic (LD50 10 μg/insect), followed by ASX (12 μg/insect), MSX (40 μg/insect. However, in case of *S. oryzae*, ESX was most toxic (213 μg/insect) and MSX (1792 μg/insect).

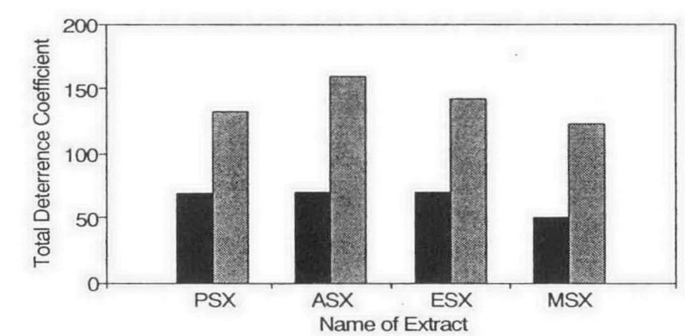

Fig. 3. Total feeding deterrent coefficients of seed extracts to adult beetles and weevils. Red flour beetles ■; rice weevils ▒.

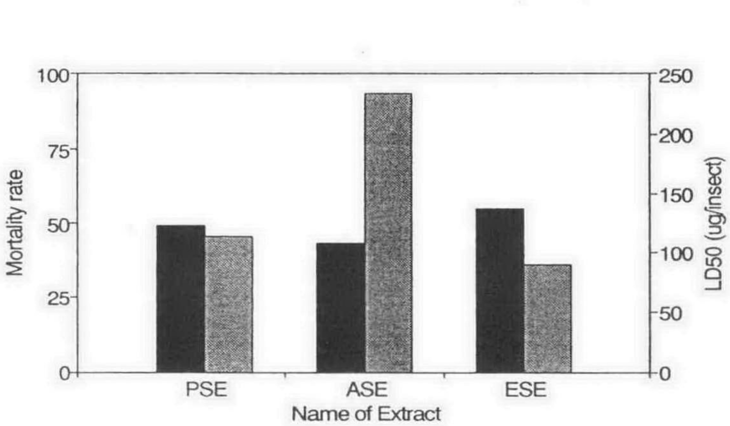

Fig. 4. Average toxic effects and LD_{50} values of extracts to adult red flour beetles by topical application method. Mortality rate ■; LD_{50} ▒

Discussion

Both the pithraj seed extracts and seed volatiles showed the repellent effects on red flour beetles, pulse beetles and rice weevils, to some extent, when evaluated by the filter paper repellency method. At 0.16 mg cm^2 dose, the repulsion effects of all extracts (among PSE, ASE and ESE or among PSX, ASX, ESX and MSX) were not statistically different from each other (at $P < 0.05$). But the repulsion effects of seed volatiles were significantly different on three tested insects. When the repellency rate of extracts were compared among the three insects, the highest rate was observed in tests with red flour beetles, followed by rice weevils. Where as both the seed extracts and seed volatiles showed weak repellent effects on pulse beetles. The phenomenon might be related to the non-feeding behaviour of pulse beetles adults during their adult period.

It was observed from the feeding deterrent tests that at 10 mg/mL dose, seed extracts showed moderate deterrent effects on red flour beetles but high deterrent effects on rice weevils. These results indicate the possible future use of pithraj seed extracts as feeding deterrents for rice weevils. Islam (1984) reported the lower feeding deterrent effects of pithraj on Angoumois moth. This result is in agreement with his results. The absolute and relative coefficients represent the no-choice and choice tests. When the insects had no opportunity to choose between treated and control disks (no-choice test), adults consumed either treated (small amount) or control (large amount) disks, which produced low absolute coefficient values; whereas when they got the opportunity to choose between treated and control disks (choice test), the adults directed their food consumption to untreated ones, which produced high relative coefficient values.

The contact toxicity of pithraj seed extracts was high for red flour beetles, highest for pulse beetles and moderate for rice weevils. Mortality percentages were directly proportional to the level of concentration. Among the three insects tested, pulse beetles were most susceptible to seed extracts, whereas rice weevils were less susceptible. Khanam et al. (1990) reported the lethal effect of pithraj seed coat extracts on *Tribolium confusum*. Among the tested extracts, ASE (in case of EM-1) and ESX (in case of EM-II) showed the highest toxic

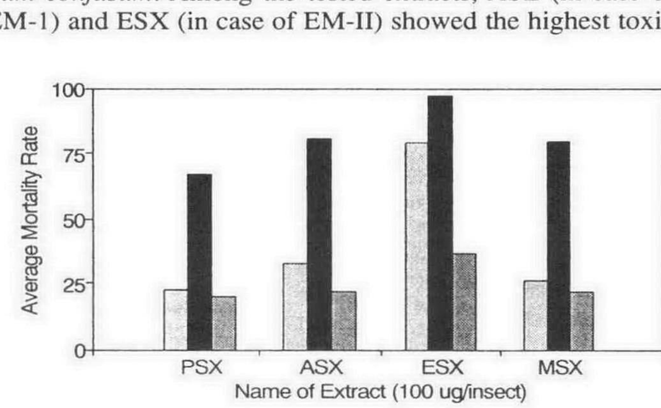

Fig. 5. Comparative toxicity of four seed extracts to adult beetles and weevils by direct contact method. Red flour beetles ░; pulse beetles ■; rice weevils ▒.

effects and lowest LD_{50} values, on red flour beetle and all insects, respectively. On the basis of the present data analysis, higher concentrations contributed more significantly to the efficacy of extracts on the mortality of insects and they appeared to be the most important factors in the degree of control obtained with pithraj seed extracts.

Conclusion

The obtained results for Extraction method I (EM-I) showed that crude extracts (PSE, ASE and ESE) of pithraj seeds have strong repellent effects, moderate antifeedant and topical (direct-contact) effects but less residual toxic effects on *Tribolium castaneum*. Crude seed extracts of pithraj (PSX, ASX, ESX and MSX), from EM-II, were evaluated for their repellency, contact toxicity and oviposition deterrency to red flour beetles, pulse beetles and rice weevils; and feeding deterrency to red flour beetles and rice weevils. Seed volatiles were also evaluated for their repellency to red flour beetles, pulse beetles and rice weevils. The results obtained so far showed that crude extracts and seed volatiles have strong repellent effects on red flour beetles and rice weevils, whereas weak effects on pulse beetles. Crude seed extracts were moderately feeding deterrent to red flour beetles and highly deterrent to rice weevils. Present laboratory studies revealed that pithraj seed extracts have pesticidal effects on three tested insects. Extracts were highly toxic to pulse beetles and red flour beetles, and moderately to rice weevils. ASE (EM-I) and ESX (EM-II) were most toxic among the tested extracts and showed lowest LD_{50} values. Finally, considering the above results, it may be concluded that the initial efficacy tests described here indicate a potential use of pithraj extracts in storage pest management systems.

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