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INSECTICIDAL ACTIVITY OF *ANNONA SQUAMOSA* L. SEED EXTRACTS AGAINST THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)

M Khalequzzaman* and Shajia Sultana

Institute of Biological Sciences, University of Rajshahi, Rajshahi 6205, Bangladesh

Abstract

Insecticidal activity of the seed extracts of custard apple, *Annona squamosa* L. in petroleum spirit, ethyl acetate, acetone and methanol against Raj, CR 1, FSS II and CTC-12 strains of the red flour beetle, *Tribolium castaneum (*Herbst) was studied. The seeds were dried, powdered and extracted in Soxhlet`s apparatus in the solvents below 60°C. Extractions were applied on larvae and adult beetles in film-residue methods and mortality was recorded after 24 h. For larval bioassay the highest toxicity was recorded for petroleum spirit extract (LD_{50}= 0.03μg cm^{-2}) in Raj strain and the lowest toxicity was for methanol extract (LD_{50}=15.697μg cm^{-2}) in FSS II strain. In adults petroleum spirit extract offered highest toxicity (LD_{50}= 58.697μg cm^{-2}) in CTC 12 strain and the lowest toxicity (LD_{50}=22004.710μg cm^{-2}) was for acetone extract in CR 1 strain. LD_{50}, 95% confidence limits and regression equations are presented.

Key words*: Tribolium castaneum*, *Annona squamosa*, custard apple, LD_{50}.

Introduction

The insecticidal and acaricidal properties of a number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control (Hedin and Hollingworth 1997). Especially remarkable are tropical plants, from which hundreds of products of secondary metabolism with insecticidal properties have been extracted (Hiiesaar *et al.* 2001). They are environmentally less harmful than synthetic pesticides and acting in many insects in different ways.

Custard apple (*Annona squamosa* L.) extracts have shown promise for pest control against a range of insect pests. Laboratory and field tests showed that extracts from custard apple kernels were effective against crop pests like spotted stem borer, *Chilo partellus* (Swin.); leafhopper, *Nilaparvata lugens* (Stal.), *Spodoptera litura* (Fab.), *S. frugiperda*, *Helicoverpa armigera* (Hubner), hairy caterpillar, *Spilosoma obliqua* (Wk.); Brinjal spotted leaf beetle, *Henosepilachna vigintioctopunctata* (Fabr.); cotton boll worm, *Dysdercus koenigii* (Fab.); semi-looper *Achaea janata* Linn. and Aphids (Saito *et al*. 1989, Rao *et al*. 1990, Bhagawan *et al*. 1992, Ghatak and Bhusan 1995b, Hiremath *et al*. 1997, Bhatnagar and Sharma 1997, Mathew *et al*. 1999, Raman *et al*. 2000, Sonkamble *et al*. 2000, Bhuiyan *et al*. 2001).

Among stored grain pests, extracts of custard apple was tested on *Callosobruchus chinensis* Linn. (Tripathy *et al*. 2001, Kotkar *et al*. 2002, Misra 2000); *C. analis* (Juneja and Patel 1994); *C. maculatus* (Dharmasena *et al*. 2001); *Rhizopertha dominica* Fab. (Patel and Valand 1994), *Sitophilus oryzae* (L.) (Mishra *et al*. 1992), *Tribolium castaneum* (Herbst) (Malek and Wilkins 1994,1995, Hussain *et al*. 1995) and *Corcyra cephalonica* Staint. (Ghatak and Bhusan 1995a).

Published reports shows that the previous works on *T. castaneum* was done only on feeding deterrence or on the larvicidal effects. These promising attributes led to evaluate the potential use of *A. squamosa* seed against *T. castaneum* .

* To whom all correspondence should be addressed.

Materials and Methods

Test insect: Four strains of *T. castaneum* viz. Raj, CR 1, FSS II and CTC-12 were collected from the Crop Protection and Toxicology Laboratory, Rajshahi University, where the stocks were maintained since 1990. Raj strain was originally collected from flour mills at Rajshahi, Bangladesh and other three strains were collected from Crop Protection Lab., Department of Agriculture and Environmental Science, University of Newcastle upon Tyne, U K. Mass cultures were maintained in glass jars (1000ml) and subcultures were in beakers (500ml) with food medium and kept in an incubator at 30±0.5°C. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1 was used as food medium throughout the experimental period.

Seed extractions: During 2002 custard apple ripe fruits were procured from local market of Rajshahi, and seeds were separated from them after washing with tape water. Seeds were dried in shade and crushed in a hand grinder. Extractions were done in a Soxhlet`s apparatus with four solvents using the process described by Feuerhake and Schmutterer (1982) below 60°C. The solvents were petroleum spirit, ethyl acetate, acetone and methanol used serially on the same stock of seeds. After completing extraction, the mixed solvent was removed from the extract with a vacuum rotary evaporator.

Mortality tests: Film residue method (Busvine 1971) was used to test the mortality of the larvae and adults of *T. castaneum*. The extracted materials were weighed and dissolved in acetone for dosing. For larval bioassay the experiments were done in glass vials (10 ml, 2.7 mm dia.) in which 0.2 ml of each dose was dropped and air-dried. Then 2.0g of food was added and ten first instar larvae were released in each vial in four replicates for each dose. A control batch was maintained with the same number of larvae. The doses for all strains were similar and they were 48.530, 4.853, 0.485 and 0.048 μg cm^{-2} for petroleum spirit extract, 61.080, 6.108, 0.610 and 0.061 μg cm^{-2} for ethyl acetate extract, 41.63, 4.163, 0.416 and 0.041 μg cm^{-2} for acetonic extract, and 26.04, 2.604, 0.260 and 0.026 μg cm^{-2} for methanolic extracts.

Adult bioassays were done in petri dishes (90 mm). One ml of each dose was dropped separately, covering uniformly the whole area of the petri dish. After air dried four plastic rings (30 mm diam) were placed inside the petri dish and 10 adult beetles were released within each ring. The rings within the petri dish were served as replications. The doses were chosen after an ad-hoc bioassay done before the actual experiments. The doses for Raj, FSS II and CTC-12 strains were similar and they were 4367.99, 436.79, 43.68 and 4.37 μg cm^{-2} for petroleum spirit extract; 5497.95, 549.80, 54.98 and 5.50 μg cm^{-2} for ethyl acetate extract, 3746.64, 374.66, 37.47 and 3.75 μg cm^{-2} for acetone extract; and 2343.98, 234.40, 23.44 and 2.34 μg cm^{-2} for methanol extract. In case of CR 1 strain the doses were 10060.30, 1006.03, 100.60 and 10.06 μg cm^{-2}; 14147.52, 1414.75, 141.48 and 14.15 μg cm^{-2}; 12268.60, 1226.86, 122.69 and 12.27 μg cm^{-2}; and it was 3109.08, 310.91, 31.09 and 3.11μg cm^{-2} in the same orders of extractions respectively.

Larval and adult mortality was recorded after 24 h of treatment. The mortality percentage was corrected using Abbott`s formula (Abbott 1925). The observed data was subjected to probit analysis (Finney 1947, Busvine 1971).

Results

The calculated LD_{50}, 95% confidence limits and regression equations are presented in Table 1. In larval bioassay the LD_{50} of petroleum spirit extract of seed is 0.031, 0.024, 0.045 and 0.069 μg cm^{-2} for Raj, CR1, FSS II and CTC 12 strains respectively, which are lowest in comparison to the other extracts. Thus, it proved to be the most toxic to *T. castaneum* larvae. The methanolic extract was least toxic for Raj (LD_{50}=4.038 μg cm^{-2}) and FSS II (LD_{50}=15.697 μg cm^{-2}) strains, whereas for CR 1 the lowest toxicity was recorded in acetonic (LD_{50}=0.26 μg cm^{-2}) and for CTC 12 strain in ethyl acetate (LD_{50}=0.392 μg cm^{-2}) extracts.

Table 1. LD_{50}, 95% confidence limits, regression equations and χ^2- values of the seed extracts in different solvents treated on larvae and adults of *T. castaneum*.

Strains	Solvent	LD_{50} µg/cm^2	95% CL		Regression equations	χ^2 (df)
			Lower	Upper		
Larval bioassay						
Raj	Pet. spt.	0.031	0.006	0.150	Y = 4.7354 + 0.54558X	0.769 (2)
	EtOH	0.632	0.315	1.265	Y = 3.6248 + 0.76372X	1.342 (2)
	Acetone	0.591	0.285	1.224	Y = 3.7375 + 0.71240X	0.462 (2)
	Methanol	4.038	1.727	9.440	Y = 3.3092 + 0.64873X	0.625 (2)
CR 1	Pet. spt.	0.024	0.004	0.130	Y = 4.7878 + 0.54786X	1.108 (2)
	EtOH	0.041	0.008	0.202	Y = 4.6719 + 0.5334X	1.095 (2)
	Acetone	0.263	0.124	0.555	Y = 3.9432 + 0.7443 X	0.214 (2)
	Methanol	0.071	0.028	0.179	Y = 4.4077 + 0.6942X	0.262 (2)
FSS II	Pet. spt.	0.045	0.012	0.167	Y = 4.6040 + 0.6072X	0.082 (2)
	EtOH	0.398	0.163	0.972	Y = 4.0214 + 0.6113X	0.696 (2)
	Acetone	0.115	0.040	0.327	Y = 4.3621 + 0.6019X	1.392 (2)
	Methanol	15.697	3.011	81.821	Y = 3.6589 + 0.41961X	0.503 (2)
CTC-12	Pet. spt.	0.069	0.023	0.213	Y = 4.45160 + 0.6506X	0.130 (2)
	EtOH	0.392	0.057	2.683	Y = 4.5592 + 0.2761X	0.338 (2)
	Acetone	0.095	0.031	0.285	Y = 4.4127 + 0.5995X	0.072 (2)
	Methanol	0.172	0.002	0.110	Y = 4.8933 + 0.4519X	0.437 (2)
Adult bioassay						
Raj	Pet. spt.	198.57	116.065	339.735	Y = 2.0045 + 1.3035X	1.235 (1)
	EtOH	614.26	392.760	960.681	Y = 0.9501 + 1.4524 X	2.857 (1)
	Acetone	1000.40	556.200	799.358	Y = 1.7822 + 1.0725X	3.371 (1)
	Methanol	135.25	85.580	213.741	Y = 1.9832 + 1.41558X	3.008 (1)
CR 1	Pet. spt.	316.85	175.067	573.474	Y = 2.3069 + 1.07685X	1.719 (1)
	EtOH	2837.04	1892.606	4252.741	Y = –1.1179 + 1.7718X	3.062 (1)
	Acetone	22004.71	7148.368	67736.66	Y = 0.8865 + 0.94725X	1.205 (1)
	Methanol	207.47	31.859	1351.006	Y = 3.0606 + 0.83701X	3.517 (1)
FSS II	Pet. spt.	85.46	56.6137	129.008	Y = 1.6968 + 1.70991X	1.810 (1)
	EtOH	1947.52	383.078	9900.901	Y = 2.3492 + 0.80584 X	0.522 (1)
	Acetone	389.09	97.788	1547.731	Y = 1.51261 + 1.3464X	3.634 (1)
	Methanol	326.33	177.626	599.527	Y = 2.61212 + 0.9499X	2.671 (1)
CTC-12	Pet. spt.	58.77	37.788	98.060	Y = 2.86467 + 1.2069X	3.793 (1)
	EtOH	193.66	93.836	399.666	Y = 2.9661 + 0.8893X	0.481 (1)
	Acetone	3996.57	1182.55	13506.85	Y = 2.4657 + 0.7036 X	0.020 (1)
	Methanol	98.79	42.374	230.329	Y = 3.59937 + 0.7021X	2.133(1)

In adult bioassay again petroleum spirit extract was the most toxic (LD_{50} is 198.57, 316.85, 85.46 and 58.77 µg cm^{-2} for Raj, CR1, FSS II and CTC 12 strains respectively). The acetonic extract was least toxic for Raj (LD_{50} = 1000.40 µg cm^{-2}), CR 1 (LD_{50} = 22004.71 µg cm^{-2}) and CTC 12 (LD_{50} = 3996.57 µg cm^{-2}) strains. In case of FSS II ethyl acetate extract was the least toxic (LD_{50} = 1947.52 µg cm^{-2}). Four solvents extracted compounds and their toxicity also acted differently on strains of *T. castaneum.*

For larvae the LD_{50} is more or less uniform in petroleum spirit extract of the seeds of *A. squamosa*. Ethyl acetate and acetonic extracts showed a slight and methanolic extarct in some cases showed major variation in toxicity with the strains. In adults toxicity varied widely with the strains of beetle used. This could be due to the resistance capability of the strains to plant extracts. The insignificant χ^2 values for the regression coefficients suggest no heterogenity of the data.

Discussion

The seeds of *A. squamosa* were reported to have insecticidal and abortifacient properties. The crude oils from seeds of *A. squamosa* at 2.5 percent concentrations significantly reduced the leaf damage caused by larvae (Babu *et al*. 1998) and petroleum ether extract of seeds reduced the weight and length of *S. litura* (Boreddy and Chitra 2001). Acetone extracts from fresh and stored leaves were toxic to adult *C. maculatus*, whereas the ethanol extracts were not active (Dharmasena *et al*. 2001). But in the present study seed extracts in acetone was less active against adults of *T. castaneum*. Sonkamble *et al*. (2000) recorded seed extract at 1.5 per cent concentration the highest mortality in *H. armigera* (43.33%) and 36.66 per cent mortality at 1% concentration in *S. litura*. Larval development of FSS II and CTC-12 strains of *T. castaneum* significantly affected by the seed oil of *A. squamosa* (Malek and Wilkins 1995) and the weight and development of pupae and adults in both strains was significantly affected by seed oil treatments (Malek and Wilkins 1994). Adults *T. castaneum* were repelled by contact with food medium treated with 2 and 5 g leaf dust/10 g flour, for *A. squamosa* (Hussain *et al*. 1995).

Extracts of seeds of *A. squamosa* had repellent and antioviposition properties when applied to *Ceratitis capitata* (Epino and Chang 1993). In laboratory studies, topically applied seed extract of caused a steady decline of all free amino acids in freshly emerged 5^{th}-instar larvae of *Dysdercus koenigii* (Reddy *et al*. 1993). The depletion in free amino acids had an adverse effect on protein turnover, resulting in delayed metamorphosis (Bhagawan *et al.* 1992).

Kawazu *et al*. (1989) isolated two compounds from the seed, which were found to be toxic to fruit fly. The petroleum ether extract of the seeds of this species yielded 13 known adjacent, and 4 non-adjacent bis-tetrahydrofuranic acetogenins, and the compounds squamocin and squamostatin A (Sahai *et al*. 1994). Four non-adjacent bis-tetrahydrofuranic acetogenins, named squamostatins-B to -E, were isolated from the petroleum ether extract of *A. squamosa* seeds by Fujimoto *et al*. (1994). Flavonoids isolated from aqueous extracts showed antimicrobial activity against all the common microbial contaminants of pulses and 80% insecticidal activity against *C. chinensis* at a concentration of 0.07 mg ml^{-1} (Kotkar *et al*. 2002). Adults of *T. castaneum* were repelled by contact with food medium treated with 2 and 5 g custard apple leaf dust/10 g flour (Hussain *et al*. 1995).

The present experiments showed that *T. castaneum* larval mortality was the most in petroleum spirit extract of seed, whereas ethyl acetate and acetone extracts were moderately toxic and methanol extract was the least toxic for Raj and FSS-II strains, but it remained toxic for CR-1 and CTC 12 strains. In adults acetone extracts showed less toxic to all strains used. Petroleum spirit extract remained most toxic for FSS-II and CTC 12 strains, whereas methanol extract was most toxic for other two strains i. e., Raj and CR-1 strains.

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