Toxicity of *Annona squamosa* Linn. seed oil extract on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

The toxicity of *Annona squasmosa* Linn. seed oil and two newly isolated compounds (annonastin and squamozin) was tested against the adults, eggs and larval stages of the FSS2 and CTC12 strains of *Tribolium castaneum* (Herbst). Malathion was used as a standard throughout the experiments. The CTC12 strain was found to be 8.38 times resistant to malathion for adult mortality. It did not show resistance to malathion for adult mortality. It did not show resistance to *A. squamosa* seed oil and two new compounds from seed oil. The CTC12 eggs mortality showed little resistance to the two compounds. Every stages of CTC12 larvae had showed very little resistance to malathion but not to *A. squamosa* seed oil and two new compounds.

Introduction

The Annonaceae is a large family of tropical and subtropical trees and shrubs comprising about 120 genera and more than 2000 species (Heywood 1978). The seeds of five annonaceous plants are reputed to have insecticidal actions: *Annona cherimola*, *A. glabra*, *A. reticulata*, *A. spinescens* and *A. squamosa* (McIndoo and Sievers 1924). *A. squamosa* seed oil was reportedly used by farmers in Vietnam for protecting rice against leafhopper and planthopper (Brady et al. 1978). The petroleum ether (40–60°C) extract of *A. squamosa* seed was toxic to *Musca nebulo* adults as a contact poison (Mukherjea and Govindo 1958). Pandey and Varma 1977 reported that the seeds of *A. squamosa* and *A. reticulata* contained an alkaloid, anonine, that was tested for its insecticidal properties against the pulse beetle. El-Nahal et al. (1989) reported that vapours of the essential oil of *Acorus calamus* have been found to be toxic to adults of *Callosobruchus chinensis*, *Sitophilus granarius* and *Sitophilus oryzae*. Most of the species of Annonaceae family have biologically active compounds (Rupprecht et al. 1990). Extracts of ground seeds from *A. squamosa* revealed insecticidal properties (Londershausen et al. 1991).

The occurrence of malathion resistance in *T. castaneum* has been reported in at least 12 countries (Dyte and Blackman 1972) and this has given an extra impetus to the search for alternative insecticides for the control of this stored product pest. Malathion is used in this insect. The dose–mortality relationship of an insecticide or toxic plant product is usually expressed as a median lethal dose (LD_{50}). This represents the dose of the chemical that produces death in half the test subjects after a certain exposure period. There is no published information concerning the toxicity of *A. squamosa* seed oil or any of its compounds to different stages of FSS2 (susceptible) and CTC12 (resistant to malathion) strains of *T. castaneum*. *A. squamosa* seed oil was extracted by petroleum ether (40–60°C). The two new compounds (annonastin and squamozin) were isolated from the seed oil fractions and these were purified and identified by different chemical processes which are not described here.

Materials and Methods

Toxicity to adults of FSS2 and CTC12 strains of *T. castaneum* H.

The required quantities of malathion (95%), *A. squamosa* seed oil and the two compounds were weighed individually on an electric balance, using a small aluminium foil boat. The chemicals and the foil boat were inserted into a clean, well-dried volumetric flask (10 mL). The required amount of distilled acetone was added to the volumetric flask to dissolve the chemicals. The flask was shaken well to ensure uniform mixing of the solution. Distilled acetone was used as a solvent throughout this mortality study because it evaporates quickly and has no toxic effect on insects after evaporation. Five serial dilutions of each chemical were made by adding the required amount of distilled acetone to a separate volumetric flasks for each dose. One control was maintained with distilled acetone only. Clean, well-dried petri dishes (9 cm inner diameter) were used for insecticidal treatments. Ten beetles (4–6 weeks old) irrespective of sex were kept in clean flat bottom glass vials for each replicates. Each treatment was replicated four times. One mL of solution from each treatment was applied by pipette to each petri dish. A lower concentration was applied first and the solvent was left to evaporate, leaving a residue of insecticide on the surface of the petri dish to which the insects would be exposed. Four glass rings (3 cm dia. and 1.5 cm height) were placed in each petri dish after evaporation of the solvent. Ten adults beetles were released into each glass ring and the petri dish was coated with a lid. The glass rings were covered with teflon (emulsification) to prevent the insects climbing out. All the petri dishes were then kept in the dark in an incubator for 24 hours at 30°C and 70% relative humidity.

Mortality of the test insects was recorded after 24 hours of the treatments by the residual film technique (contact method). Moribund insects were counted as dead. The insects which could not walk and failed to respond even after touching with a soft brush were considered dead. The mortality data obtained after 24 hours of the treatments were analysed statistically using probit analysis and linear regression (Busvine 1971). Mortality in the control was corrected using Abbott's formula (Abbott 1925).

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Toxicity to eggs of FSS2 and CTC12 strains of *T. castaneum* H.

Ten eggs (24 hours old) per replication were collected with a very soft brush under a microscope and placed individually in a flat-bottomed glass tube (75 × 19 mm) for the contact toxicity experiment. The toxic compound was mixed with food medium (Busvine 1971). The eggs were examined under a microscope (40×) to obtain fresh and undamaged eggs which were oval, very transparent and milky white in colour. For each strain, five different doses of malathion, seed oil and the two compounds were prepared from individual stock solution. Two mL of solution from each dose of each treatment was mixed with 2 g standard food medium (wholemeal flour and dry yeast, 19:1) by stirring.

The mixtures were then dried at 25°C in an incubator for 24 hours and mixed individually by electric blender (Mukherjea and Ramachandran 1989). Controls were distilled acetone and 2 g standard food. Four replicates were taken for each dose in each treatment. One mg of treated and untreated food media was taken separately for each individual egg into a glass tube and the mouth of the glass tube was covered with cotton wool. These tubes were placed in a dark incubator at 30°C and 70% relative humidity. The numbers of hatched and unhatched eggs were counted under the microscope every 24 hours. The unhatched eggs turned grey or pale yellow and were considered as dead. Hatched egg counts were confirmed by counting the number of larvae and empty egg shells. The mortality of eggs was determined by counting every 24 hours for 7 days the number of unhatched eggs, empty egg shells and lst instar larvae. The mortality data were analysed statistically by probit analysis and linear regression (Busvine 1971).

Toxicity to the various larval stages of FSS2 and CTC12 strains

Individual lst, 2nd, 3rd, 4th and 5th instar larvae of the FSS2 and CTC12 strains of *T. castaneum* H. were placed into individual flat-bottomed glass vials (5O × 25 mm) to measure the mortality of larvae by contact with toxic compounds mixed with food medium (Busvine 1971) and also by feeding (Kawazu et al. 1989). The required quantities of malathion, *A. squamosa* seed oil and the two compounds were weighed from stock solutions and dissolved in the required amounts of distilled acetone. Five serial dilutions were prepared from each stock solution for each larval instar by adding the required amounts of distilled acetone for each dose. Two mL of solution from each dose of each treatment for each instar larvae was added to 2 g standard food medium and mixed thoroughly by stirring. This mixture was dried at 25°C in an incubator for 24 hours and then mixed again by an electric blender for uniform mixing (Mukherjea and Ramachandran 1989). Two mg food medium of each dose from each treatment was inserted into glass tube with individual larvae for each individual treatment of lst, 2nd, 3rd, 4th and 5th instar larvae. Ten freshly emerged larvae of each instar were taken for each replication and four replications were used for each dose. The same number of each instar larvae was kept in untreated medium as a control. Mortality of each instar larvae was assessed after 24 hours of each treatment. The percentage mortality for each instar larvae of FSS2 and CTC12 strains of *T. castaneum* was analysed statistically by probit analysis and linear regression (Busvine 1971).

Results

Toxicity to adults of the FSS2 and CTC12 strains

The LD_{50} values, the ratios of LD_{50} values and 95% confidence limits for the LD5O's are shown in Table 1 for the mortality of the adults of FSS2 and CTC12 strains of *T. castaneum* with the treatments of malathion, *A. squamosa* seed oil and two new compounds. There was no significant heterogeneity for the chi^2 values (4.94, 6.42; 1.10,5.94; 1.42,1.72; 2.53,2.21). From the LD_{50} values it was found that, for adult beetles, malathion was more toxic than *A. squamosa* seed oil and the two compounds. The CTC12 strain was found to be mild resistant to malathion (Table 1). This might be due to the long period of non exposure of the whole population of the CTC12 strain to malathion treatments in the stock solution of this department. The CTC12 strain did not show resistance to *A. squamosa* seed oil or the two new compounds.

Toxicity to eggs of the FSS2 and CTC12 strains

The LD_{50} values, the ratios of the LD_{50} values and 95% confidence limits for the LD_{50}'s are shown in Table 1 for the mortality of eggs of the FSS2 and CTC12 strains following treatment with malathion, *A. squamosa* seed oil or the two new compounds. The chi^2 values did not show any significant heterogeneity (0.21, 0.27; 0.04, 0.03; 0.08, 0.50; 0.14, 0.04). The malathion treatments were more strongly ovicidal than seed oil to both the strains. The CTC12 strain did not show resistance to malathion for egg mortality but the same strain showed mild resistance to *A. squamosa* seed oil. This might be due to multiple resistance of the CTC12 strain to many chemi-

Table 1. The LD_{50} values, 95% confidence limits for LD_{50}s and ratios of LD_{50} values for adults and eggs mortality of the FSS2 and CTC12 strains of *T. castaneum* following different chemical treatments.

Chemical[a]	Stages	LD_{50} µg/cm2		LD_{50} ratio	95% confidence limit	
		FSS2	CTC12	FSS2:CTC12	FSS2	CTC12
					Lower–higher	Lower–higher
Malathion	Adult	0.08	0.67	1:8.38	0.072–0.109	0.59–0.90
Malathion	Eggs	1.94	2.42	1:1.25	1.42–2.450	1.77–2.53
Seed oil	Adult	57.89	86.68	1:1.50	48.0–75.0	77.90–116.4
Seed oil	Eggs	9688	59427	1:6.13	1694–17683	11051–70478
C-A	Adult	0.66	0.78	1:1.18	0.558–1.017	0.666–1.254
C-A	Eggs	1.152	2.51	1:2.18	0.855–1.449	1.30–3.720
C-B	Adult	0.79	1.00	1:1.25	0.688–1.017	0.834–1.254
C–B	Eggs	3.01	3.52	1:1.17	1.489–4.531	1.410–5.63

[a]C-A = Annonastin, C-B = Squamozin.

cal insecticides. The egg mortality of the two strains of *T. castaneum* indicated no resistance to the two new compounds isolated from *A. squamosa* seed oil fractions.

Toxicity to larvae of FSS2 and CTC12 strains

The LD_{50} values, the ratios for the LD_{50} values, 95% confidence limits for LD_{50} values and chi^2 values with three degrees of freedom for the mortality of lst, 2nd, 3rd, 4th and 5th instar larvae of the FSS2 and CTC12 strains following treatment with malathion, seed oil and two compounds from seed oil are shown in Tables 2, 3, 4, and 5, respectively. The chi^2 values with three degrees of freedom did not show any significant heterogeneity for either malathion or *A. squamosa* seed oil or the two new compounds to any of the larval stages of the FSS2 and CTC12 strains.

The LD_{50} values for lst instar larval mortality were found to be higher than those for 2nd instar of both the strains of beetles with malathion, *A. squamosa* seed oil and annonastin. This might be due to the relatively small amount of insecticide-treated food medium consumed by the larvae within the experimental period (24 hours) and also very small amount of medium consumed by the first instar larvae because at this stage the larvae are not very active. A low concentration of

Table 2. The LD_{50} values, 95% confidence limits for LD_{50}s and ratios of LD_{50} values for the mortality of different instars larvae of the two strains of *T. castaneum* following malathion treatments.

Larval instar	LD_{50} (ppm)		LD_{50} ratio	95% confidence limits		$\chi 2$ values
	FSS2	CTC12	FSS2:CTC12	FSS2 Lower-higher	CTC12 Lower-higher	FSS2–CTC12
lst	195.62	397.01	1:2.03	170.13–249.5	343.78–503	2.62–2.49
2nd	9.61	24.03	1:2.62	8.35–12.13	20.31–30.34	4.08–2.54
3rd	44.60	77.60	1:1.74	38.53–56.49	65.76–97.86	2.67-1.25
4th	37.14	78.41	1:2.11	31.11–47.89	67.84–98.95	2.10-2.18
5th	48.98	84.00	1:1.71	43.99–65.76	70.58–106.23	5.93–2.44

Table 3. The LD_{50} values, 95% confidence limits for LD_{50}s and LD_{50} ratios for the mortality of different instar larvae of the two strains of *T. castaneum* with *A. squamosa* seed oil treatments.

Larval instar	LD_{50} (ppm)		LD_{50} ratio	95% confidence limits		$\chi 2$ values
	FSS2	CTC12	FSS2:CTC12	FSS2 Lower-higher	CTC12 Lower-higher	FSS2–CTC12
lst	3912.27	3709.05	1:0.95	2956–4868	3037–4380	2.62–3.90
2nd	951.92	1224.49	1:1.29	784–1119	849–1599	4.65–5.93
3rd	5868	6137.94	1:1.05	4742–6994	4920–7355	2.62–2.52
4th	12652	13265	1:1.05	9814–15490	10141–16388	0.65–1.62
5th	11371	14643	1:1.29	8835–13908	10182–19104	1.27–1.41

Table 4. The LD_{50} values, 95% confidence limits, $\chi 2$ values and LD_{50} ratios for the mortality of different instar larvae of the two strains of *T. castaneum* with annonastin treatments.

Larval instar	LD_{50} (ppm)		LD_{50} ratio	95% confidence limits		$\chi 2$ values
	FSS2	CTC12	FSS2:CTC12	FSS2 Lower-higher	CTC12 Lower-higher	FSS2–CTC12
lst	1003.56	1091.02	1:1.09	818–1188	1088–1093	5.45–8.82
2nd	853.81	928–94	1:1.08	851–856	919–937	3.11–3.69
3rd	1308.80	1158.58	1:0.89	1029–1588	934–1384	5.20–4.33
4th	1054.46	1054–46	1:1	842-1266	1054–1064	3.87–3.87
5th	1457.11	1379.41	1:094	1137–1776	1365–1394	8.43–7.69

Table 5. The LD_{50} values, 95% confidence limits, LD_{50} ratios and χ^2 values for the mortality of the different instar larvae of the two strains of *T. castaneum* with squamozin treatments.

Larval instar	LD_{50} (ppm)		LD_{50} ratio	95% confidence limits		$\chi 2$ values
	FSS2	CTC12	FSS2:CTC12	FSS2 Lower-higher	CTC12 Lower-higher	FSS2–CTC12
lst	1061.62	1205.55	1:1.14	852–1270	1201–1210	2.00-1.28
2nd	1418.02	1479.90	1:1.04	1411–1424	1056–1780	7.18–7.18
3rd	1706.40	1784.55	1:1.05	1351–2061	1354–2058	4.27–4.27
4th	1428.54	1457.88	1:1.02	1142–1715	1443–1472	4.33–2.48
5th	1951.48	1790.19	1:0.92	1521–2382	1357–2223	8.43–11.19

malathion, seed oil and annonastin produced a higher percentage mortality in the second instar larvae than in the other instars of the FSS2 and CTC12 strains of *T. castaneum*. The reason might be that they were very active and young and consumed more treated medium than the first instar larvae. The second instar larvae were much more susceptible to malathion, seed oil and annonastin than were any of the other instars. This might be due to a relative small amount of insecticide-treated medium being consumed by older larvae in comparison to their body weight. There was no mortality in the control. Squamozin was found to be less effective than annonastin for the mortality of the adults, eggs and different instar larvae of the FSS2 and CTC12 strains of *T. castaneum*. Higher concentrations were required to produce mortality in the 3rd, 4th and 5th instars of the FSS2 and CTC12 strains when treated with malathion, *A. squamosa* seed oil and two new compounds, respectively, compared with the concentration required to produce equivalent mortality in 2nd instar larvae. This was because the mature larvae were more tolerant than the younger larvae and because the body weight of the mature larvae was higher than the younger larvae. This means that they can more readily metabolise the toxicant than can younger larvae.

Discussion

Toxicity to adults of the FSS2 and CTC12 strains

The LD_{50} values for adult mortality of FSS2 and CTC12 strains with malathion treatments by topical application were 0.04 and 0.31 µg/insect, respectively, and the ratio was 7.75 (Binns 1986). Bhuiyan (1985) stated that the LD_{50} values for malathion against adults of FSS2 and CTCl2 strains were 0.025 and 0.05 µg/cm^2, respectively and the resistance ratio was 2. Rajendran (1990) found that the LD_{50} values for malathion were 0.0019 and 0.0164 µg/cm^2, respectively, for FSS2 and CTC12 strains and the resistance ratio was 8.63. In the present experiment the LD_{50} values for FSS2 and CTC12 adults mortality with malathion treatment by the residual film method was found to be 0.08 and 0.67 µg/cm^2 and the ratio was 8.38. These differences between the LD_{50}, values for malathion to the same strains of the insect might be due to a difference in the age of the insects, temperature, relative humidity, photoperiod and method of application of insecticides during experiments carried out by different people at different times. From the present experiment it was found that the CTC12 strain did not show resistance to *A. squamosa* seed oil and the newly isolated compounds. Wool et al. (1992) reported that the caged *Cadra cautella* (almond moth) reduced its malathion resistance following either introduction of susceptible male population or long-term non-exposure of the whole population to insecticidal treatments. Mukherjea and Govindo (1958) reported that when the ether and petroleum insoluble resin obtained from *A. squamosa* was tested against *T. castaneum*, the average percentage mortality of adults beetles was 94.66 by ether extract and 99.33 by petroleum insoluble resin extract at a concentration of 1.0 to 0.125% (w/w), respectively, applied by direct spraying. An extract from the leaves of *Adhatoda vasca* was toxic to *T. castaneum* (Srivastava and Awasthi 1958). Visweswariah et al. (1971) reported that 22% mortality could be obtained at 10 mg per petri dish against *T. castaneum* by a solvent extract oil of *A. squamosa* seed powder. Qadri and Rao (1977) reported that the LD_{50} of *A. squamosa* seed extracted by ether was 141.30 µg/petri dish against *Callosobruchus chinensis*. The mortality of *T. castaneum* adults produced by *A. squamosa* seed oil was in agreement with previous findings (Visweswariah et al. 1971). As malathion is reported to be hydrolysed by the malathion specific enzyme 'malathionase' (Oppenoorth 1985), the low resistance to malathion in the CTC12 strain may be due to this enzyme.

Toxicity to eggs of the FSS2 and CTCl2 strains

A. squamosa L. seed oil and the two new compounds were found to have ovicidal activity for eggs of the FSS2 and CTC12 strains of *T. castaneum*. There are no other published reports on the ovicidal activity of malathion in FSS2 and CTC12 strains. The ovicidal activity of *A. squamosa* seed oil and two new compounds in the present experiment confirms a similar result obtained by Jacobson and Crosby (1971). However they did not specify the type of insect eggs used. The mode of action of fixed vegetable oils on *Callosobruchus maculatus* egg mortality revealed two possible mechanisms of action: (1) the oil exerts lethal action slowly by drastically reducing respiratory activity and the elimination of toxic metabolites as a result of the oil 'barrier effect'; and (2) direct toxic effects of the oil or oil constituents that possibly penetrate the eggs (Don-Pedro 1989). The LC_{50} of oleic acid (1.64 mL/kg) made it approximately 3 and 8 times more toxic against eggs of *C. maculatus* than groundnut oil and linoleic acid respectively. The ovicidal activities of the three fatty acids (lauric, oleic and linoleic) were found to be similar (Don-Pedro 1990). *A. squamosa* seed oil contains fatty acids e.g. oleic (18.7%), linoleic (55.20%), palmitic (14.7%), steric (10.70% and cerotic (0.9%) (Ghaneker and Ayyar 1927). It might be that the mode of action of all these fatty acids on eggs of *T. castaneum* H. is the same.

Toxicity to larvae of FSS2 and CTC12 strains

The LD_{50} values for lst. 2nd, 3rd, 4th and 5th instar larval mortality of the FSS2 and CTC12 strains by malathion treatments were found to be less than those for *A. squamosa* seed oil and the two new compounds. This proves that malathion is more toxic than any of the treatments used in the present experiments. The LD_{50} values for CTC12 larval instars mortality by malathion treatment indicated that the resistant parents produced resistant progeny, which is similar to the results of previous workers (White and Bull 1988). The larval instar mortality data for the CTC12 strains suggest no resistance to *A. squamosa* seed oil and two new compounds (Tables 3, 4 and 5).

It is well known that the larvae of the some stored-product beetles exhibit a higher tolerance to the contact insecticides than do adults (Parkin 1954; Lloyd and Hewlett 1958; Tyler and Binns 1977). Annona extractives had been claimed to act as contact and stomach poisons. Contact action was equivalent to that of rotenone or nicotine (Crosby 1971). Visweswariah et al. (1971) reported that the potent insecticidal principle was present in the form of an oily material which could be quantitatively extracted using petroleum ether, hexane, acetone benzene, alcohol and chloroform. They also mentioned that solvents other than petroleum ether gave comparatively poor yields of the insecticidal principle. Neoannonin was isolated from the ethyl acetate extract of the defatted seeds of *A. squamosa* and it showed larvicidal activity at 125–140 µg/2g diet for *Drosophila melanogaster* M. (Kawazu et al. 1989).

Binns (1986) reported that in all strains of *T. castaneum* (FSS2 and CTC12) the larvae showed a higher tolerance to malathion than did the adults. He also reported that the final instar larvae of CTC12 strain were more tolerant to malathion than those of the FSS2 strain. This agrees with the results of the present experiment (Table 2). In malathion treatments the

larvea of the FSS2 and CTC12 strains were 1.61 and 2.4 times more tolerant than the adults (Binns 1986). In the present studies it was found that the FSS2 and CTC12 larval stages were more tolerant than the adults of the same strains. The resistance ratio varied in the different larval instars because of differential tolerance among larval instars.

References

Abbott, W. S. 1925. A method for computing the effectiveness of insecticides. Journal of Economic Entomology, 18, 265–267

Bhuiyan, S.T. 1985. Toxicity of precocene II and some insecticides to *Tribolium castaneum* Herbst and *Drosophila melanogaster* Meig. Unpublished M.Sc. Thesis, 21–25

Binns, T.J. 1986. The comparative toxicity of malathion with and without the addition of triphenyl phosphate to adults and larvae of *Tribolium castaneum* Herbst (Coleopetra Tenebrionidae). Journal of Stored Products Research, 22(2), 97–101.

Brady, N.C.; Khush,G.S. and Heinrichs, E.A. 1978. Visit of the IRRI team to the Socialist Republic of Vietnam April 13–May 19. IRRI Annual Report 1978, 49.

Busvine, J.R., ed. 1971. A critical review of the technique for testing insecticides. C.B.A. England, 108–277.

Crosby, D.G. 1971. Minor insecticides of plant origin. In: Jacobson, M. and Crosby, D.G., ed., Naturally occurring insecticides. New York, Marcel Dekker Inc., 215–219.

Don-Pedro, K.N. 1989. Mode of action of fixed oils against eggs of *Callosobruchus maculatus* (F). Pesticide Science, 26, 107–115.

Don-Pedro, K.N. 1990. Insecticidal activity of fatty acid constituents of fixed vegetable oils against *Callosobruchus maculatus* (F) on cowpea. Pesticide Science, 30, 295–302.

Dyte, C.E. and Blackman, D.G. 1972. Laboratory evaluation of organophosphorus insecticides against susceptible and malathion resistant strain of *Tribolium castaneum* Herbst. Journal of Stored Products Research, 8, 10–39

El-Nahal, A.K.M.; Schmidt, G.H. and Risha, E.H. 1989. Vapours of Acorus calamus oil — a space treatment for stored product insects. Journal of Stored Products Research, 25(4), 211–216.

Ghaneker, R.V. and Ayyar, P.R. 1927. Part XI. The oil from the seeds of *Annona squamosa* L. Journal of Indian Institute of Science, 10A (2), 28–31.

Heywood, V.H. 1978. Flowering plants of the world. University Press. Oxford, 254–267.

Jacobson, M. and Crosby, D.G. 1971. Naturally occurring insecticides. Marcell Dekker Inc. USA, 216–219.

Kawazu, K.; Alcantara, J.P. and Kobayashi, A. 1989. Isolation and structure of Neoannonin, a novel insecticidal compound from the seeds of *Annona squamosa* L. Agriculture Biological Chemistry, 53(l), 2719–2922.

Lloyd, C.J. and Hewlett, P.S. 1958. The relative susceptibility to ptrethrum in oil of Coleoptera and Lepidoptera infesting stored products. Bulletin of Entomological Research, 49, 177–185.

Londershausen, M.; Leicht, W.; Lieb, F. and Moeschler, H. 1991. Molecular mode of action of Annonins. Pesticide Science, 33, 427–438.

McIndoo, N.E. and Seivers, A.F. 1924. Plant tested or reported to possess insecticidal properties. Bulletin of United States Department of Agriculture No. 120,1.

Mukherjea, T.D. and Govindo. 1958. Studies in indigenous insecticidal plants. Part II. *Annona squamosa* L. Journal of Scientific and Industrial Research (India). 17C, 9–15.

Mukherjea, S.N. and Ramachandran, R. 1989. Effects of azadirachtin on feeding, growth and development of *Tribolium castaneum* H. (Coleoptera : Tenebrionidae). Journal of Applied Entomology. 107(2),145–149.

Oppenoorth, F.J. 1985. Biochemistry and genetics of insecticides resistance. In. Kerkutl, G.A. and Gilbert, L.I. ed. Comparative Insect Physiology, Biochemistry and Pharmacology, 13, 731–774.

Pandey, G.P. and Varma, B.K. 1977. Annona (custard apple) seed powder as a protectant of mung against pulse beetle, *Callosobruchus maculatus* (F). Bulletin of Grain Technology, 15(2), 100–104.

Parkin, E.A. 1954. The susceptibility to DDT dust of coleoptera infesting stored product. Bulletin of Entomological Research, 44, 439–444.

Qadri, S.S.H. and Rao, B.B. 1977. Effect of combining some indigenous plant seed extracts against house hold insects. Pesticides (India), 11(12), 21–23.

Rajendran, C. 1990. Toxicology of selected plant products and insecticides in the red flour beetle, *Tribolium castaneum* Herbst and the fruit fly, *Drosophila melanogaster* Meig. Unpublished Ph.D. Thesis, 51–60.

Rupprecht, J.K.;Hui, Y.H. and McLughlin J.L. 1990. Annonaceous acetogenins: A review. Journal of Natural Product 53(2), 239–278.

Srivastava, A.S. and Awasthi, G.P. 1958. An insecticide from the extract of a plant, *Adhatoda vasica* Nees. Harmless to man. 10th International Congress of Entomology, 2, 245–246.

Tyler, P.S. and Binns, J.L. 1977. The toxicity of seven organophosphorus insecticides and lindane to eighteen species of stored product beetles. Journal of Stored Products Research, 1 3, 39–43.

Visweswariah, K. ; Jayaram, M. ; Krishnaprasad, N. K. and Majumder, S. K. 1971. Toxicological studies of the seed of *Annona squamosa* L. Indian Journal of Experimental Biology, 9(4), 519–521.

White, N.D.G. and Bull, R.J. 1988. Inheritance of malathion resistance in a strain of *Tribolium castaneum* Herbst (Coleoptera : Tenebrionidae) and effects of resistance genotypes on fecundity and larval survival in malathion treated wheat. Journal of Economic Entomology, 81(l), 381–386.

Wool, D., Brower, J.H. and Belsky, N.K. 1992. Reduction of malathion resistance in caged almond moth, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) populations by the introduction of susceptible males. Journal of Stored Product Research, 28(l), 55–59.