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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (12): 1088-1091, 2003
ISSN 1028-8880

Pesticidal Activity of Pure Compound Annotemoyin-1 Isolated from Chloroform Extract of the Plant *Annona squamosa* Linn. Against *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae)

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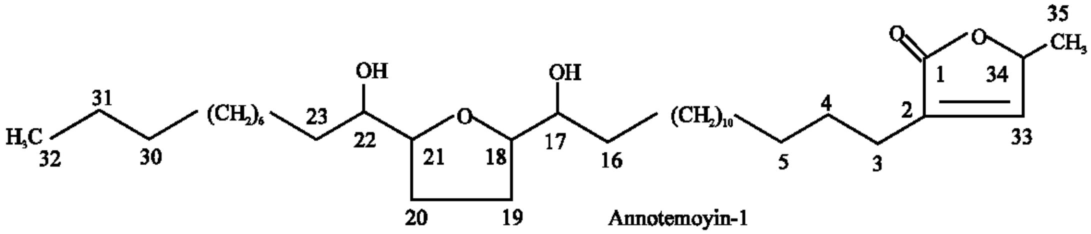
Abstract: The pure compound annotemoyin-1 isolated from the chloroform extract of the seeds of *Annona squamosa* Linn. was evaluated for its pesticidal activity against both adults and different instars of *Tribolium castaneum* (Herbst) under laboratory condition. The LD_{50} values of the pure compound were determined both for adults and different instars of larvae. The LD_{50} values of the compound were 579.67, 394.89, 243.10, 612.92, 366.95, 315.18, 636.12, 423.30, 333.67, 684.88, 449.28, 101.68, 742.69, 525.93, 199.41, 792.38, 609.08, 191.70, 827.43, 615.36, 221.13, 920.54, 693.10 and 429.42. These results demonstrated that the earlier instars were more sensitive to the compound than those of late instars as well as adults.

Key words: Pesticidal activity, annotemoyin-1, *Annona squamosa* Linn, *Tribolium castaneum* (Herbst)

Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) is a major pest of stored food grains and cosmopolitan distribution (Good, 1933). Both adults and larvae are able to exploit a wide variety of stored commodities (Ziegler, 1977). Their presence in the stored product results in both contamination and substantial economic damage due to loss of the product's market price and decreases nutritional value as well (Barkholder and Faustini, 1991)
Insecticides of plant origin, because of their high degree of tolerance by the mammals, are particularly desired for application against insect/pests of fodders, fruits, vegetables and stored grains (Jucobson,1975) and have reviewed the effectiveness of many plant derivatives for use against grain pests (Ketkar, 1975). Certain insects can exist under a wide range of conditions and can attack products at all phases of storage and distribution. More than 2000 species of field and storage pests annually destroy approximately one third of the world's food production, valued US 100 billion among which highest losses (43% of potential production) occur in developing Asian countries (Ahmed and Grainge, 1986). Chemical insecticides and other biological methods are commonly used to control insect infestation in stored products. The use of chemical insecticides to control insect infestation has been widely applied and is now causing concern because of environmental hazards, insect resistance and toxic to mammals. Recently, phytochemical pesticides have received much attention for their environmental friendliness. Therefore, they are less likely to contaminate the environment and less toxic to mammals (Freedman *et al*., 1979). In view of these fact, researchers have diverged their attention towards age-old practice of using phytochemicals, which would be of not hazardous, easy to use and specific in their action (Koul, 1982).
Annona squamosa Linn. (Family: Annonaceae) is well known in tropical and sub-tropical countries and it's fruit is edible. The seeds of the fruit have long been reported to have insecticidal properties (Chopra *et al*., 1956). *Annona squamosa* seed oil was reported to use by the farmers in Vietnam for protecting rice against leaf hoppers and plant-hoppers (Brady *et al*., 1978). Kawazu *et al*. (1989) reported that they isolated two compounds from the seed of *A. squamosa* which were found to be toxic to the eggs, larvae and adults of *Drosophilia melanogaster*. Ohsawa *et al.* (1990) reported that the ether extract of the seed of *A. squamosa, A. glabra, A muricata* and *A. reticulata* showed high contact toxicity against azuki bean weevils. They also mentioned that the extract of *A. squamosa* was particularly toxic against the diamond back moth, *Plutella xylostella.*
Some acetogenins have been isolated from the seeds of *A. squamosa* and it was found that Annonin I was more toxic than pyrethrum against *Phaedon cochlearial* and *P. xylostella* (Lieb *et al*., 1990). Annonin I was found to have respiratory inhibitory activity against *P. xylostella* (Londershausen *et al.,* 1991). Squamocin isolated from the seeds of *A. squamosa* was toxic to *P. xylostella* larvae (Fujimoto *et al*., 1988)
In view of this, the present work was undertaken to find out the efficacy of pure compound annotemoyin-1 of *Annona squamosa* on *Tribolium castaneum* (Herbst).



Annotemoyin-1

Materials and Methods

Isolation of compound: The matured seeds of the plant *Annona squamosa* Linn. were collected during the month of September-October, 2000 from the relevant areas of Rajshahi district and were identified by the Bangladesh National Herberium (Specimen No. 29, 544). The seeds were sun dried and pulverized into coarse powder and then stored in an airtight container. The plant materials were extracted in cold with absolute alcohol. After concentration, the absolute alcohol extract was fractionated with petroleum ether and chloroform. The chloroform soluble fraction was subjected to column chromatography. The pure compound annotemoyin-1 was purified by solvent washing followed by preparative thin layer chromatography (PTLC) and was characterized by Mass, ^{1}H-NMR, ^{13}C-NMR, HMBC, ^{1}H-^{1}H COSY 45 (Center for Phytochemistry, Southern Cross University, Australia) and IR and UV spectroscopy (Rajshahi University). The spectroscopic data were identical with the reported data for annotemoyin-1 isolated from the plant *Annona atemoya* by P. Duret *et al.* (1996).

Culturing procedures of the beetle: The insects used in this experiment were obtained from a culture of *Tribolium castaneum* (Herbst) maintained in glass jar containing food medium in an incubator at 30±0.5°C. A standard mixture of whole wheat flour and powdered brewers yeast in the ratio of 19:1 (Park and Frank, 1948) was used as food medium throughout the experiments. The food medium was sterilized in an oven at 120°C for 6 hours. Food was not used until at least 15 days after sterilization to allow its moisture content to equilibrate with the environment (Khan, 1981). The second, third, fourth, fifth and sixth instar larvae were obtained from the larval culture on the 3rd, 6th, 9th, 12th and 16th day from the hatching respectively, while the newly hatched larvae were used as first instar. The adults were collected by sieving the food medium using a 500 micrometer sieve.

Larvicidal/Insecticidal effects: The beetles were treated by surface film contact method on petridishes (μgm cm^{-2}) with the pure compound. The experiments were conducted at 30°C in an incubator without controlling light and humidity. The mortality of larvae at different instars as well as individual adult was assessed after 24, 48 and 72 h of treatment. Control group insects, in food media were treated with solvent only. The mortality was determined by counting survivors at the end of exposure period and the control mortality was adjusted dil using Abbotts formula and the results were subjected to probit analysis following the method of Busvine (1971). The mortality relationship was expressed as a median lethal dose (LD_{50}). In this investigation mortality in the control was not adjusted or corrected using Abbott (1925) formula because control mortality was zero in the said periods.

Results and Discussion

The LD_{50} values with 95% confidence limits, chi^2 values with 2 degrees of freedom and regression equation (Y) for larvae of different instars and adults mortality of *Tribolium castaneum* at different time intervals with pure compound, annotemoyin-1 have been shown in Table 1. The data in table 1 provided comprehensive evidence that the pure compound used in

Table 1: LD_{50} values and 95% confidence limits for LD_{50} values of pure compound annotemoyin-1 against different instars and adults of *Tribolium castaneum* (Herbst)

Sample	Life stage Adults/ larvae	Exposure time (h)	LD_{50} (μgm cm^{-2})	95% confidence limits Lower	Upper	χ^2value (df)
		24	579.67	399.88	840.31	0.182
	1st instar	48	394.89	237.11	657.65	0.181
		72	243.10	52.49	1125.84	1.413
		24	612.92	442.28	849.39	0.539
	2nd instar	48	366.95	172.21	781.89	0.030
		72	315.18	129.60	766.48	1.087
		24	636.12	488.89	827.68	0.042
	3rd instar	48	423.30	252.84	708.67	0.134
		72	333.67	152.25	731.27	0.206
		24	684.88	536.50	874.28	0.068
Annotemoyin-1	4th instar	48	449.28	276.63	729.68	0.209
		72	101.68	0.303	34094.49	1.050
		24	742.69	519.56	1061.64	0.065
	5th instar	48	525.93	79.08	729.66	0.870
		72	199.41	13.29	29990.81	0.042
		24	792.38	558.71	1123.76	0.192
	6th instar	48	609.08	501.03	740.42	0.555
		72	191.70	2.51	14584.27	0.215
		24	827.43	571.69	1197.56	0.016
	Male	48	615.36	493.23	767.74	0.819
		72	221.13	13.26	3687.21	0.216
		24	920.54	562.60	1506.21	0.024
	Female	48	693.10	565.59	849.34	0.790
		72	429.42	196.94	936.33	0.607

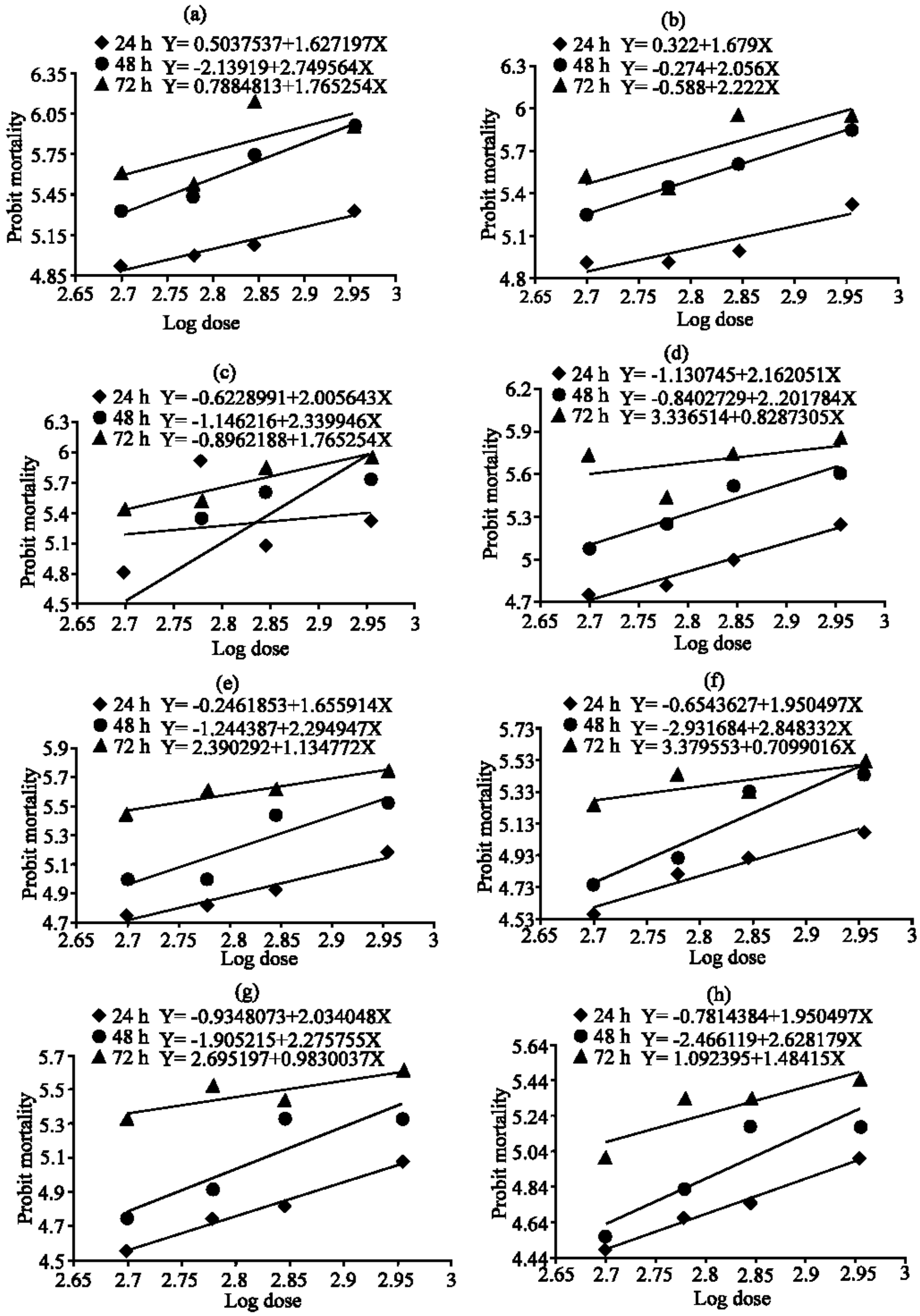


Fig. 1a-h: Regression line of probit mortality of *T. castaeneum* on log dose of pure compound, annotemoyin-1. a) 1st instar, b) 2nd instar, c) 3rd instar, d) 4th instar, e) 5th instar, f) 6th instar, g) Male and h) Female

the experiment was effective in controlling both larvae and adults of *Tribolium castaneum.* The chi^2 values in the probit analysis with 2 degrees of freedom indicated no significant heterogeneity. The pure compound caused the highest mortality of the 1st instar larvae in comparison with other larval instars (Table 1), which indicated that newly hatched larvae were the most susceptible to the compound. The 1st instar larvae (after 72 h exposure) were the most susceptible with lowest LD_{50} values (243.10 μgm cm^{-2}) whereas the female adult s (after 72h exposure) were less susceptible with highest LD_{50} values (429.42 μgm cm^{-2}). Thus the LD_{50} values of the pure compound were age dependent. This may clearly support others that insect age play an important role in influencing susceptibility (Mwangi and Mukiama, 1988). The graph (Fig. 1a-h) showed that the mortality of larvae as well as individual adults increased as the doses of the sample were increased. Both larval growth and adult population of *Tribolium casteneum* were significantly reduced in pure compound treated media in comparison with those of control. Although the mechanism of action is not known, but according to Miller *et al.* (1981) and Fox (1990), it may acts as chitin synthesis inhibitor. Due to its effect, the integument of larvae much less extensible which might have the effect of increasing internal pressure in the body, restricting movement and hampering feeding. This change to the cuticle elasticity may also be responsible for decreased larval growth (Schneiderman and Gilbert, 1964). The result of this experiment indicated that the pure compound annotemoyin-1 isolated from chloroform extract exhibited more potential pesticidal activity and it may be used as effective botanical pesticide against Tribolium. Use of plants for pest infestation control in stored grains, therefore, seems to offer desirable solutions, specially in developing tropical countries where plants are found in abundance throughout the year.

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