World Journal of Pharmaceutical research

Volume 3, Issue 2, 2091-2102. Research Article ISSN 2277 – 7105

EVALUATION OF INSECTICIDAL ACTIVITY FOR *ANNONA SQUAMOSA* L. BARK METHANOLIC EXTRACT AGAINST THE *LUCILIA SERICATA AND SITOPHILUS GRANARIUS* PESTS

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Article Received on
26 November 2013
Revised on 21 December 2013,
Accepted on 18 January 2014

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ABSTRACT

The use of insecticides of natural origin are therefore an important development in pest control as they have short residual action, low mammalian toxicity and reduced environmental pollution. The results exhibit the toxicity of *Annona* bark extracts against *Lucilia sericata* and *Sitophilus granarius*, respectively. The treatment of different concentrations of the bark extracts of plant with both pests exhibited relatively lower percent mortality after shorter duration (24h) than that at longer duration (48h). The methanol extract of *A. squamosa* was found to be quite effective against *Lucilia sericata* and *Sitophilus granarius* adults as nearly 100% mortality was observed at 600ppm. The LC_{50} values of *A. squamosa* extracts for *Lucilia sericata* flies was calculated to be 240 ppm and for *Sitophilus granarius* was calculated to be 280 ppm. The toxic effects of methanolic extract of *A. squamosa* was evaluated against *Lucilia sericata* and *Sitophilus granaries* by using the method of residual film technique. The results showed that the extract of *A. squamosa* at 200 µg/cm^2 possessed the lowest toxicity of 63.33% whereas at 1000 µg/cm^2 showed the highest toxicity of 93% in case of *Lucilia sericata.* The same mortality rate was found in case of *Sitophilus granaries* at concentrations 200 µg/cm^2 and 1000 µg/cm^2 i.e. 60% and 90% respectively.

Key words: *Annona squamosa, Sitophilus granarius, Lucilia sericata.*

INTRODUCTION

To minimize the use of synthetic pesticides and to avoid pollution of environment natural anti-feedent, deterrent and repellant substances have been searched for pest control[1]. At present pest control measures in storage rely heavily on the use of synthetic insecticides and fumigants[2]. Their indiscriminate use in storage, however, has sometimes led to a number of problems including toxic residues in food grains[3] and environmental pollution [4]. The use of insecticides of natural origin are therefore an important development in pest control as they have short residual action, low mammalian toxicity and reduced environmental pollution. Synthetic pesticides such as pyrethroids, carbamates and organophosphate are known for their known down effects but their incessant use has resulted in several environmental as well as biological problems. As Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control[5]. Insects often cause extensive damage to stored grains and grain products, amounting to 5-10% loss in temperate regions and 20-30% in the tropical regions [6]. In India, post harvest losses caused exclusively by insect pests are 12% [7]. The housefly has long been known to be a carrier of diseases. Among the most important are dysentery, cholera, typhoid, infantile or summer diarrhea, pink-eye, tuberculosis and smallpox. Besides, there are probably about 25 more diseases that may be transmitted by this vector.

Annona squamosa Linn. (Family; Annonaceae) commonly known as custard apple grown throughout India in rocky terrain with shallow and well drained soils. It is a small, semi-deciduous tree 3-7 m in height, with a broad, open crown or irregularly spreading branches, bark is light brown with visible leaf scars, inner bark light yellow and slightly bitter. Leaves occur singly, lanceolate or oblong lanceolate, pale green on both surfaces. Flowers greenish-yellow, fragrant, on slender hairy stalks, produced singly or in short lateral clusters. Fruit is round, heart shaped, ovate or conical, 5-10 cm in diameter, with many round protuberances; greenish-yellow when ripe, with a white, powdery bloom. The pulp is white, edible and sweetly aromatic. In each carpel is embedded a seed, oblong, shiny and smooth, blackish or dark brown, 1.3-1.6 cm long, numerous [8-9].

The bark decoction of *A. squamosa* is used to prevent diarrhoea, while the root is used in the treatment of dysentery. Leaf decoction is used for cold and to clarify urin and also used to treat hysteria and fainting spells. The fruits of are haematinic, cooling, sedative, stimulant, expectorant and maturant tonic. They are useful in treating anemia and burning sensation.

The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair. Fruit is used in making of ice creams and milk beverages. The bark and leaves contain annonaine, an alkaloid which is found to posses many of these properties[10]. Hypoglycemic and antidiabetic effect of *A. squamosa* was reported in the leaf extract[11]. The bark of plant contain a bioactive acetogenin with anticancer activity have been isolated[12]. Flavonoids from leaves [13]Aporphine alkaloids[14], glycoside[15] and squamoline were isolated from this plant. The seeds contain chemicals known as acetogenins, which are toxic to insects. Farmers in Vietnam use seed oil to control rice leafhoppers and plant hoppers[16]. The petroleum ether (40-60ºC) extract of *A. squamosa* seed was toxic to *Musca nebulo* adults as a contact poison[17]. However there is no report to indicate that *A. squamosa bark* extract possess insecticidal activity against housefly and grain pest, keeping these facts in view, the study was undertaken to investigate the insecticidal activities of methanolic extract under laboratory conditions.

Lucilia sericata can be a nuisance in and around our household. This is because many unsanitary places are prime places for this fly to reproduce. Flies have also been associated with food-borne diseases. The key feature to adaptation for *Lucilia sericata* is their wings. The wings are attached to the mesothorax. Flies can range many miles for breeding places. *Lucilia sericata* will have three pairs of legs and at the tip of each leg there will be a tiny pair of claws with pulvilli that help to stick to the surfaces.

The wheat weevil, *Sitophilus granarius* (grain weevil), occurs all over the world and is a common pest in many places. It can cause significant damage to harvested grains that are being stored and may drastically decrease yields. The females lay many eggs and the larvae eat the inside of the grain kernels. Adult wheat weevils are about 3–5 mm long with elongated snouts and chewing mouthparts. The adults are a reddish-brown colour and lack distinguishing marks. The life cycle takes about 5 weeks in the summer, but may take up to 20 weeks in cooler temperatures. Wheat weevils are a pest of many types of grain and may lay their eggs in wheat, oats, rye, barley, rice and corn.

MATERIALS AND METHODS

Collection and processing of plant sample

The stem bark of *Annona squamosa* was collected from the local area of Gondia district in Maharashtra. Identification and authentification of the crude drug was carried out at Botany Department, Nagpur University, India. The plant materials were properly cleaned and dried

for 5-7 days. The dried bark was coarsely powdered mechanically using commercial electrical stainless steel blender and stored in air tight container at room temperature.

Extraction and Phytochemical analysis of the extract

The dried plant materials were firstly extracted with petroleum ether (Ana.Gr.), to remove fatty materials and re-extracted with 90% methanol (Ana.Gr.) in a Soxhlet apparatus (Borosil, India). The extracts were concentrated at 50°C and the residue obtained was stored at 4°C. Qualitative phytochemical analysis of bark extract was carried out by the method of Mishra et al. In brief, the phytochemicals such as tannins, alkaloids, saponins, flavonoids, glycerides, terpenoids and phenols/polyphenols were qualitatively determined.

Test insect

Adult *Lucilia sericata* flies were collected from local areas using a sweep net *and Sitophilus granarius* was originally collected from flour mills in Gondia and reared in the laboratory at 26±2°C, 60±10% RH, photoperiod 12:12 (L:D). A standard mixture of whole-wheat flour with powdered dry sugar was used as food medium throughout the experimental period.

Preparation of experimental concentrations

Stock solution was prepared by dissolving 5 mg extract in 10 ml methanol and used for making further dilutions. The different extract concentrations such as 100, 200, 300, 400, 500 and 600 ppm were used for further study following trial runs with various concentrations of the extract for activity against *Lucilia sericata*. In case of *Sitophilus granarius* the extract concentrations were 100, 200, 300, 400, 500 and 600 ppm.

Mortality tests

Contact Biossay

Adult *Lucilia sericata* flies (30 Nos.) identified by shortening and thickening of size and shape, respectively. Glass beakers of 250 ml (6 Nos.) capacity were taken and labeled for different concentrations in addition to one for check (methanol) and one for control (water). Flies were dipped into the solution for three minutes and then transferred back in the rearing media. Each experiment was conducted in triplicates along with the control group. Mortality was recorded after 24h & 48h. LC_{50} was calculated using Karber's method[18]. In brief, the mortality with different concentrations of the extracts was recorded after 24 and 48 h. The LC_{50} value was determined as following

$$LC_{50} = LC_{100} - \frac{\Sigma \text{ Mean death} \times \text{Concentration difference}}{\text{No. of organisms per group}}$$

The experiment was repeated for two subsequent days. Same method of treatment was applied for *Sitophilus granarius*. To evaluate the insecticidal activity of *A. squamosa*, the insects were treated with 5% and 10% (equivalent to 1/20 and 1/10 of LC_{50} value) for 24 and 48h. The control group was exposed only with the equal volume of methanol, the solvent in which the extract was prepared.

Film residue method was also used to test the mortality of the adults of *Lucilia sericata* flies and *Sitophilus granarius* weevils. The extracted materials were weighed and dissolved in acetone for dosing. For calculating mortality five doses were used including control (water). (200,400,800 and 1000 µg/cm^2 concentrations). The doses were prepared by mixing the requisite quantities of extract with 1 ml acetone/ water. After mixing properly the liquid was dropped in a petri dish (9.5-cm diameter). After drying in an oven at 40 ºC, 30 adults of each species were released in each Petri dish. For each dose three replications were taken. The doses were calculated by measuring the weight of prepared product (µg) in 01 ml of water divided by the surface area of the petri dish and it was converted into *µg*/cm^2. Mortality was assessed after 24, 48 and 72 h of the treatment. The calculation of mortality rate was corrected for control mortality according to Abbott's formula[19]:

$$Mc = \frac{Mo}{100 - Me} \times 100$$

Where, Mo = Observed mortality rate of treated adults (%), Me = mortality rate of control (%), and Mc = corrected mortality rate (%)

The LD_{50} values were determined by probit analysis. The experiments were performed in the laboratory at 30ºC ± 0.5ºC.

Table 1: Toxicity testing for extract of *Annona squamosa* bark against *Lucilia sericata*

Concentration (ppm)	No. of live flies	No. of live flies	% Alive	% Alive	% Mortality	%Mortality
	24 h	48 h	24 h	48 h	24 h	48 h
0 (control)	30	30	100	100	00	00
0 (check)	30	30	100	100	00	00
100	27	23	90	77	10	23
200	22	19	73	63	27	37
300	15	11	50	37	50	63
400	12	07	40	23	60	77
500	08	05	27	17	73	83
600	05	00	17	00	83	100

Table 2: Toxicity testing for extract of *Annona squamosa* bark against *Sitophilus granarius*

Concentration (ppm)	No. of live weevil	No. of live weevil	% Alive	% Alive	% Mortality	% Mortality
	24 h	48 h	24 h	48 h	24 h	48 h
0 (control)	30	30	100	100	00	00
0 (check)	30	30	100	100	00	00
100	28	26	93	87	07	13
200	24	20	80	67	20	33
300	19	16	63	53	37	47
400	13	11	43	30	57	70
500	09	06	30	20	70	80
600	07	00	23	00	77	100

Table 3: The LC_{50} value of methanol extract of *Annona squamosa* bark against *Lucilia sericata* for 48 h

Concentration (ppm)	Concentration difference	No. of live flies	No. of dead flies	Mean death	Mean death × Conc. difference
0 (control)	00	30	00	00	00
0 (check)	00	30	00	00	00
100	100	23	07	07	700
200	100	19	11	10	1000
300	100	11	19	18	1800
400	100	07	23	22	2200
500	100	05	25	24	2400
600	100	00	30	27	2700
Total- 10800					

The LC_{50} value of the leaf extract of *Annona squamosa* for 48 h has been determined according to the arithmetic method of Karber (1931). The calculation was done as following:

$$LC_{50} = LC_{100} - \frac{\Sigma \text{ Mean death} \times \text{Concentration difference}}{\text{No. of organisms per group}}$$

$$LC_{50} = 600 - \frac{10800}{30} = 240 \text{ ppm}$$

Table 4: The LC_{50} value of methanol extract of *Annona squamosa* bark against *Sitophilus granarius* for 48 h.

Concentration (ppm)	Concentration difference	No. of alive weevil	No. of dead weevil	Mean death	Mean death × Conc. difference
0 (control)	00	30	00	00	00
0 (check)	00	30	00	00	00
100	100	26	04	05	500
200	100	20	10	09	900
300	100	16	14	13	1300
400	100	11	19	18	1800
500	100	06	24	23	2300
600	100	00	30	28	2800
Total- 9600					

The LC_{50} value of the leaf extract of *Annona squamosa* for 48 h has been determined according to the arithmetic method of Karber (1931). The calculation was done as following:

$$LC_{50} = LC_{100} - \frac{\Sigma \text{ Mean death} \times \text{Concentration difference}}{\text{No. of organisms per group}}$$

$$LC_{50} = 600 - \frac{9600}{30} = 280 \text{ ppm}$$

Table 5: The Mortality percentage of methanolic extract of *A. squamosa* bark against *Lucilia sericata* by film residue method.

Concentration ($\mu g/cm^2$)	No of Insect used	No of Insect dead		Total No of Insects dead	% of Average Mortality	% Corrected Mortality
		24 hr	48 hr			
200	30	17	21	19	63.33	63.33
400		19	25	22	73.33	73.33
800		23	27	25	83.33	83.33
1000		27	29	28	93.00	93.00
control		00	00	00	00	00

Table 6: The Mortality percentage of methanolic extract of *A. squamosa* bark against *Sitophilus granarius* by film residue method.

Concentration (µg/cm^2)	No of Insect used	No of Insect dead		Total No of Insects dead	% of Average Mortality	% Corrected Mortality
		24 hr	48 hr			
200	30	16	20	18	60.00	60.00
400		18	23	20.5	68.33	68.33
800		24	26	25	83.33	83.33
1000		26	28	27	90.00	90.00
control		00	00	00	00	00

Fig. 1: Mortality curve of *Lucilia sericata* for the determination of LC_{50} of A. squamosa bark extract.

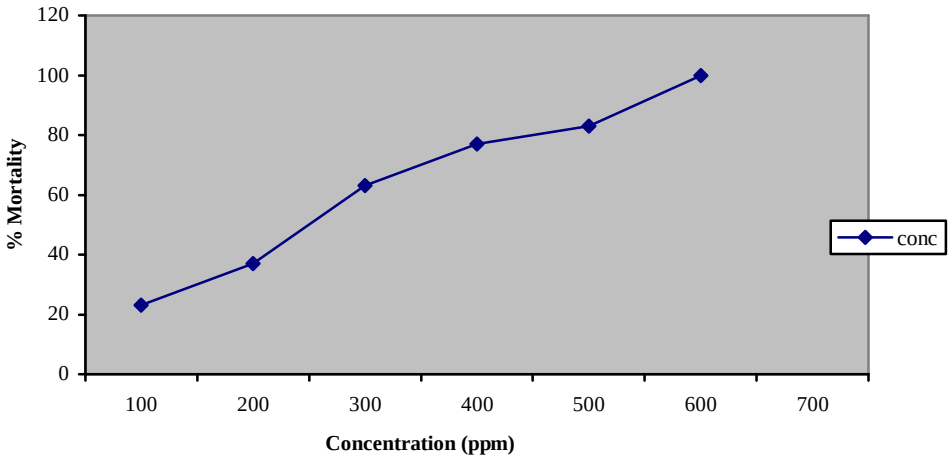


Fig. 2: Mortality curve of *Sitophilus granarius* for the determination of LC_{50} of A. squamosa bark extract.

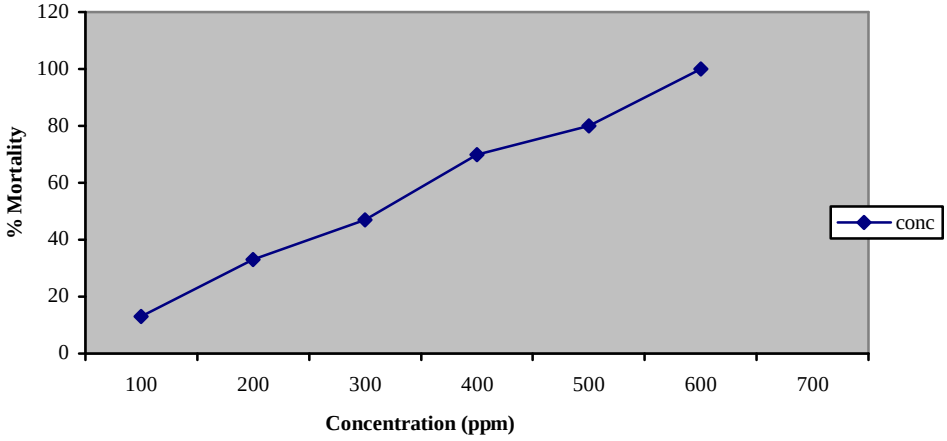
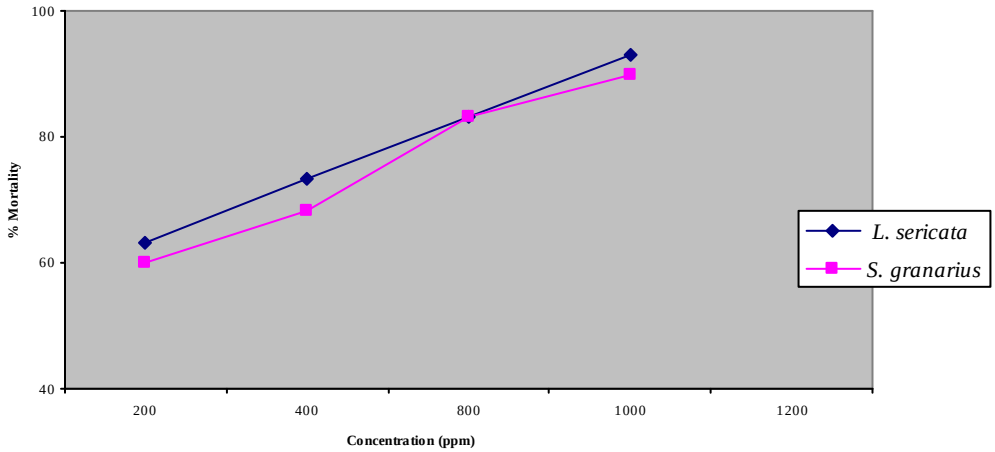


Fig. 3: Mortality curve of *A. squamosa* bark extract against *L. sericata* and *S. granarius* by Film residue method.



RESULTS AND DISCUSSION

The results presented in (Tables 1 and 3) exhibit the toxicity of *Annona* bark extracts against *Lucilia sericata* and *Sitophilus granarius*, respectively. The treatment of different concentrations of the bark extracts of plant with both pests exhibited relatively lower percent mortality after shorter duration (24h) than that at longer duration (48h). The exposure of the flies and weevils to the methanolic extracts caused significant mortality in a dose dependent manner. The methanol extract of *A. squamosa* was found to be quite effective against *Lucilia sericata* and *Sitophilus granarius* adults as nearly 100% mortality was observed at 600ppm. The mortality curves for the determination of LC_{50} values for the both pests are shown in Figures 1 and 2, respectively. The LC_{50} values of *A. squamosa* extracts for *Lucilia sericata* flies was calculated to be 240 ppm and for *Sitophilus granarius* was calculated to be 280 ppm (Tables 2 and 4). The methanolic bark extract of *A. squamosa* showed better insecticidal activity in case of both pests with weight loss and reduced size of the treated adults as compared to control ones when treated with first method. The morphological changes like reduced size, condensed appendages and failure to metamorphose were recorded due to the treatment of *the* extract. The results clearly indicated that the mortality of the pests in both the cases was dose dependent.

The toxic effects of methanolic extract of *A. squamosa* was evaluated against *Lucilia sericata* and *Sitophilus granaries* by using the method of residual film technique. The extract of *A. squamosa bark* at different four concentration were revealed for toxicity studies and for control The numbers of dead *Lucilia sericata* and *Sitophilus granaries* were counted after 24 and 48 hours at all doses 200,400, 800 and 1000 µg/cm^2 respectively. Then the percentages of

corrected mortality were calculated by using Abbott's formula and the results are shown in Table 4 and 5. The results showed that the extract of *A. squamosa* at 200 µg/cm^2 possessed the lowest toxicity of 63.33% whereas at 1000 µg/cm^2 showed the highest toxicity of 93% in case of *Lucilia sericata.* The same mortality rate was found in case of *Sitophilus granaries* at concentrations 200 µg/cm^2 and 1000 µg/cm^2 i.e. 60% and 90% respectively. The mortality percentage was directly proportional to the level of concentration of plant extract. Mortality curve of *A. squamosa* bark extract against *L. sericata* and *S. granarius* by film residue method are shown in figure 3. Further investigation on the identification of active ingredient from the extract, which is more effective, is utmost needed.

In the present study, the methanolic extract of *A. squamosa,* were quite effective against the adult housefly *Lucilia sericata* and weevils *Sitophilus granaries*. The extract drastically affected the adults in dose dependent manner. Previous investigations on annonaceous acetogenin, the bioactive principle of the plant family Annonaceae, have shown that it may have pesticidal or antifeedant properties. The phytochemical analysis of extract revealed presence of alkaloids, flavonoids, terpenoids and phenols. These phytochemicals may be responsible for the insecticidal nature of the extracts. Carbohydrates are the primary and immediate sources of energy. In stress condition, carbohydrate reserve is depleted to meet energy demand. The data obtained from the present study clearly indicate that *A. squamosa* bark extract was quite effective as insecticides for providing a better and excellent alternate for the control of *Lucilia sericata and the weevils Sitophilus granaries.* However, isolation of the active compounds from this plant and further trial assay in the field conditions is underway to explore its suitability for such application.

ACKNOWLEDGEMENTS

The authors express their gratitude to Prof. Dr. S.L. Bhongade, Principl, M.I.B.P. Gondia, for providing necessary facilities with financial assistance to one of the authors is gratefully acknowledged.The work was supported by a Special Research project of our Institute.

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