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Effect of leaf and seed extract of custard apple (*Annona squamosa* L.) on ovicidal activity against *Spodoptera litura* F.

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Abstract
The ovicidal activity of *Annona squamosa* L. leaves and seed extracts with organic solvents 0.1-1.0% acetone and ethanol respectively were evaluated against *Spodoptera litura* under laboratory conditions. An experiment was conducted to find out ovicidal activity of leaf and seed extracts of *Annona squamosa* against eggs of *S.litura*. The highest ovicidal activity was found in 1% ethanol seed extract (72±0.1)) and leaf extract (52±1.0) at 96 hours after treatment. Acetone leaf and seed extract exhibited low percentage of ovicidal activity in *Spodoptera litura*.

Keywords: *Annona squamosa*, *Spodoptera litura*, ovicidal activity, lepidoptera

1. Introduction
Plants have evolved a range of adaptations to increase their survival and reproduction by minimizing the impact of phytophagous insects. Plants defend themselves from herbivores with the help of secondary metabolites produced by them and these secondary chemicals can act as repellents or toxins to herbivores and affect their behavior, growth, or survival. Volatile plant signals attract natural enemies of the herbivore insect pests [1]. Over the past 30 years, the search for plants with novel insecticidal constituents has been intensive. Many leads from numerous plant species have been identified [2].
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 [3].
The tobacco cutworm,*Spodotera litura* Fabricius (Lepidoptera: Noctuidae), is a polyphagous pest that feeds on more than 150 different host plants and widely distributed throughout tropical and subtropical regions [4]. *Spodotera litura* has emerged as a serious and dominant pest on many agricultural crops causing enormous losses in India [5]. The use of plant extracts to manage *S. litura* as an eco-friendly management strategy in organic farming is the need of the day. The objective of this study was to examine the ovicidal activity of *Annonna squamosa* L. leaf and seed extract against *S.litura*

2. Material and Methodology
2.1. Plant collection: The plant leaves of *Annona squamosa* L. was collected from Nippani, Karnataka. The fruits were collected from local market and seeds were separated. The collected plant materials were washed, shade dried under the room temperature (27 ± 2 ^{0}C) and powdered using electric blender. 50 gm powder was macerated with 150 ml of acetone and ethanol sequentially for a period of 48 hours each and filtered. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at 4 ^{0}C.

2.2 Collection of larvae and maintenance: Eggs and early larval instars of *S.litura* were collected from Citrus fields. The eggs were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching, the neonate larvae were reared on leaves of citrus and castor.

The room temperature (25± 2 ^{0}C) with 15–11 hour light dark photoperiod and 75±5% relative humidity were maintained and this process was repeated throughout the study period.

2.3 Ovicidal activity: The egg masses of *S.litura* were counted with the aid of a hand lens (10x) and egg mass (pre counted – 100) was dipped in 0.1-1.0% concentration of acetone and ethanol leaf and seed extracts of *A. squamosa*. Aqueous leaf extract (1.0%) was used as control experiment. The numbers of eggs hatched in control and treated were recorded and percentage of ovicidal activity calculated. The experiment was conducted at room temperature 28 ±2 ^{0}C and 75 ±5 R.H. The results were recorded at an interval of 12, 24, 48 and 96 hours after treatment. A total of three trials were carried with five replicates per trial. The data obtained were subjected to angular transformation.

$$\text{Percent ovicidal activity} = \frac{\text{\% of eggs hatched in control} - \text{\% of eggs hatched in treated}}{\text{\% of eggs hatched in control}} X100$$

3. Results and Discussion

The ovicidal activity was determined against *S.litura* at 0.1-1.0% concentrations of selected solvents acetone and ethanol were tested under laboratory conditions and the hatch rates were assessed 12- 96 hours post treatment by using the protocol of Abbott's formula (1925) [6].

Leaf extract in acetone (0.8 and 1.0%) exhibit ovicidal effect after 96 hours of treatment period (46±2.0). At lower concentration of *A. squamosa* leaf extract (0.1- 0.6) ovicidal effect is relatively less (Table 1).

An ovicidal effect of *A. squamosa* leaf extracts increases with concentration and treatment period. Maximum ovicidal activity was recorded in 1.0% ethanol leaf extract (52±1.0) of *A. squamosa* after 48 and 96 hours treatment period. At lower concentrations also an ethanol leaf extract exhibited significant ovicidal activity after 96 hours treatment period (Table. 2) as compared to control.

Annona squamosa seed extract in acetone at 1.0 and 0.8% was proved to be significant as percent ovicidal effect (58±1.0 and 49±00) in *Spodoptera litura* after 96 hours of treatment period (Table 3). Ethanol seed extract of *A. squamosa* at 1.0% concentration was proved to be an excellent ovicidal source on *S. litura* (Table 4).

Maximum ovicidal activity was recorded in 1.0 and 0.8% ethanol seed extract (72±0.1) of *A. squamosa* after 48 and 96 hours treatment period. Ethanol seed extract at higher concentration was revealed to be significant as ovicidal in *S. litura*. Our results agreed with Arivoli and Tennyson [7] in which the hexane extracts of *Cleistanthus collinus* (85.16%) was reported. These finding correlates with the findings of Kathirvelu [8] who reported that the hexane, chloroform, chloroform, acetate extracts of *Couroupita guianensis* showed ovicidal activity against *S. litura.* Similarly, fractions eluted using hexane: ethyl acetate from chloroform extract of *Clerodendrum phlomidis* showed maximum ovicidal activity against *Earias vittella* [9].

Elumalai *et al*. [10] noticed ovicidal activity in plant oils of *Zingiber officinale, Ocimum bassilicum, Cyperus scariosus, Pimpinella anisum, Nigella sativa, Rosmarious officinalis* and *Curcuma longa* against *S. litura*.

Hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract of *Tinospora cardifolia* showed ovicidal activity against the eggs (0-6hrs) of the *H. armigera* at 100mg/L concentration [11]. These finding also correlates with the results of Gokce *et al*, [12] and *Vitex negundo* leaf hexane extract exhibited 60.7% ovicidal activity against *Hyblaea puera* [13, 14].

Table 1: Effect of different concentrations of *Annona squamosa* L. leaf extract (Acetone) on ovicidal activity of *Spodoptera litura*.

Observation (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of leaf extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
12	6±0.3	6±0.3	10±0.1	10±0.8	16±0.0	16±0.0	6±0.3
24	9±1.2	9±1.2	12±0.2	12±0.2	24±1.0	24±2.0	9±1.2
48	15±0.2	15±0.2	19±0.1	25±1.0	38±0.4	43±2.1	9±1.2
96	15±0.0	15±0.2	19±0.1	27±0.0	40±0.1	46±2.0	9±1.2

Values are mean of five replicates of three trials. ± = Standard deviation

Table 2: Effect of different concentrations of *Annona squamosa* L. leaf extract (Ethanol) on ovicidal activity of *Spodoptera litura*.

Treatment Period (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of leaf extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
12	7±0.1	7±1.4	16±0.2	17±0.8	17±0.1	19±1.2	6±0.3
24	16±0.2	16±0.2	23±0.0	27±1.4	33±0.1	41±1.1	9±1.2
48	28±1.0	28±1.0	35±0.0	40±0.3	46±1.4	52±1.0	9±1.2
96	28±1.0	28±1.0	35±0.0	44±0.6	44±0.6	52±1.0	9±1.2

Values are mean of five replicates of three trials. ± = Standard deviation

Table 3: Effect of different concentrations of *Annona squamosa* L. seed extracts (Acetone) on ovicidal activity of *Spodoptera litura*.

Treatment Period (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of seed extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
12	6±0.1	6±0.1	13±0.4	13±0.0	16±0.0	18±0.2	6±0.3
24	14±1.1	14±1.1	15±0.0	15±0.2	30±1.1	30±0.0	9±1.2
48	18±1.2	18±0.2	26±0.1	31±1.0	49±0.0	58±1.0	9±1.2
96	18±1.2	20±0.0	20±0.0	31±1.0	49±0.0	58±1.0	9±1.2

Values are mean of five replicates of three trials. ± = Standard deviation

Table 4: Effect of different concentrations of *Annona squamosa* L.seed extracts (Ethanol) on ovicidal activity of *Spodoptera litura*.

Treatment Period (hrs)	Ovicidal Activity (%)						
	Treatment Concentration (%) of leaf extract						
	0.1	0.2	0.4	0.6	0.8	1.0	Control
12	9±0.0	9±0.0	20±0.6	21±2.0	21±2.0	22±0.1	6±0.3
24	16±0.2	16±0.2	27±0.2	27±1.4	37±0.1	48±1.6	9±1.2
48	28±1.0	30±2.2	43±1.0	47±0.1	47±0.1	67±0.0	9±1.2
96	28±1.0	31±1.0	43±1.0	48±0.0	48±0.0	72±0.1	9±1.2

Values are mean of five replicates of three trials. ± = Standard deviation

4. Conclusion

The present study clearly indicates that an ethanol seed extract at higher concentration and longer treatment period showed the highest ovicidal activity against the eggs of *S. litura*. Similarly ethanol leaf extract was found to be significant source of ovicidal activity in *S. litura*. Further study is necessary to identify the active principle(s) responsible for the activity and to develop a new formulation to control the agricultural pests.

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