



## GROWTH INHIBITORY ACTIVITY OF *ANNONA SQUAMOSA* AGAINST POLYPHAGOUS PEST *SPODOPTERA LITURA* (F)

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### ABSTRACT

The present study was conducted to evaluate the insect growth inhibitory effect of seed extract of the medicinal plant, *Annona squamosa* L. against *Spodoptera litura* F., under laboratory conditions. The four solvent seed extracts of *A. squamosa* namely, methanol, ethyl acetate, hexane and chloroform at 0.5, 1, 1.5, 2 and 2.5% concentrations were tested against third instar larvae of *S. litura*. The results showed that, among the four solvent extracts, methanol solvent extracts of *A. squamosa* at 2% and 2.5% concentration was found to be effective. It has been observed that methanol extract at 2.5% resulted in 97.5% mortality with no malformations in the developmental stages. The result indicated that the response of larval mortality and deformities in the developmental stages was dose dependent.

**Key words:** *Annona squamosa* seed, insect growth inhibitory activity, methanol, ethyl acetate, hexane, chloroform, *Spodoptera litura*, larval pupal intermediates, pupal mortality, pupal adult malformities, pupation, adult emergence

In tropical and subtropical regions, the devastating tobacco caterpillar *Spodoptera litura* F. (Noctuidae: Lepidoptera) has been reported on 112 cultivated plants belonging to 40 families, causing monetary losses of 25.8-100% in many economically valuable crops such as cotton, pulses, and vegetable crops (Natikar and Balikai, 2015). Synthetic pesticides have been used by farmers to combat *S. litura*, but this pest has become resistant to the majority of them (Tong et al., 2013). A comprehensive strategy for managing *S. litura* is required, including botanicals to address these problems. The sweetsop *Annona squamosa* L. grown well in tropical and sub-tropical nations notably, India is a potential biopesticides. Acetogenin, a polyketide group found in annonaceous plants, has insecticidal characteristics and can be utilized to control lepidopteran insect pest (Vyas, 2011). There are numerous natural pesticides to eradicate the *S. litura* insect population that have toxicological effects (Chandler et al., 2011). The *A. squamosa* seed has reported to have toxicological properties against cabbage pests and its safety to the natural enemies like *Chrysoperla carnea* and *Orius insidiosus* (Leatemia and Isman, 2004). In this context, the growth inhibitory activity in *S. litura* to four solvent extracts of *A. squamosa* seeds were assessed in a preliminary study for the development of potential biopesticide formulation.

### MATERIALS AND METHODS

The seeds were collected from ripened *A. squamosa* fruits and shade dried for about four weeks with 10-12% moisture content. The shade-dried seeds were ground and sieved with 40-60 mesh sieve to obtain fine powder and stored it in air-tight containers. From the seed powder, extraction was done using methanol, ethyl acetate, hexane and chloroform by following the maceration method (Azwanida, 2015) 10 g of seed powder was soaked in 100 ml of respective solvent in a conical flask and allowed to stand for three days with frequent agitation in a magnetic stirrer at 800 rpm. After extraction, the extracts were filtered and resultant filtrate was allowed to evaporate in a Rotary vacuum evaporator at 30°C to obtain the viscous concentrate. Larvae were mass cultured with the artificial diet (Urs and Subramanya, 1974) at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore (11°1'N, 76°55'E).

A solution of formaldehyde 0.1% was used to sterilize the rearing trays and other equipment. The freshly emerged larva was transferred to the artificial diet containing tray using camel hair brush (number 00) and allowed to feed and the stock culture was maintained continuously for the bioassay. The effect of different concentrations of four solvent extracts of

*A. squamosa* seeds on growth inhibitory activity of *S. litura* were observed. The artificial diet prepared for *S. litura* and was supplemented with the different concentrations of four solvent extracts of *A. squamosa* (0.5, 1.0, 1.5, 2.0, 2.5%) and diet without seed extract was used as untreated check. Azadirachtin 1% EC (2ml/ℓ) was used as treated check. The diet was weighed and cut into pieces of 3 g each and placed in plastic container. The ten numbers of third instar larvae of *S. litura* was pre-starved for 4-6 h and released in plastic container having treated diet and allowed to feed. Each concentration was replicated four times. The temperature and humidity conditions were maintained at 25± 2°C and 65± 5% respectively throughout the experiment. The developmental stages of larvae, pupal and adults were recorded. The experiments were conducted under completely randomized design (CRD) and replicated four times. Observations were made on the, larval mortality, larval-pupal intermediates, pupal mortality, pupation, pupal-adult malformations and number of adults emerged were recorded. The collected data were subjected to arc-sine transformation subjected to one-way ANOVA. For comparison of means, Tukey's Honestly Significance Difference (HSD) ( $p < 0.05$ ) test (Tukey, 1977) was performed. All the statistical analyses were performed with the IBM SPSS® 22.0, statistical program.

### RESULTS AND DISCUSSION

The results of the present study are presented in (Fig. 1 to 4). Larva fed with methanol extract treated artificial diet, at higher concentration (2.5%) exhibited complete larval mortality while lower concentrations 2, 1.5, 1 and 0.5% concentration exhibited larval-pupal intermediates of 5, 10, 17.5 and 22.5%, respectively. Similarly, pupal-adult intermediates were 2.5, 5, 10 and 12.5% in methanol seed extract at the concentrations of 2, 1.5, 1 and 0.5%, respectively. At 2, 1.5, 1 and 0.5% concentration, the methanolic extract caused pupal mortality of 7.5, 7.5, 5 and 2.5%, respectively.

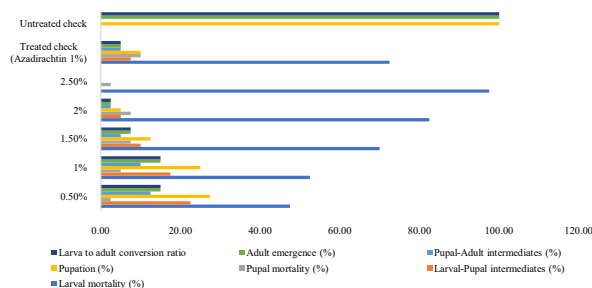


Fig. 1. Insect growth regulator (IGR) activity of methanol extracts of *A. squamosa* against *S. litura*

Regarding pupation and adult emergence, 5% pupation and 2.5% adult emergence was observed at 2% concentration (Fig. 1.) The results of larvae treated with ethyl acetate extract presented in Fig. 2 reveal that 27.5% larval-pupal intermediates were observed at 0.5% while 2.5% caused 2.5% of larval-pupal intermediates. The larva to adult conversion ratio was 1:0.03 at 2.5% concentration, which was very low compared to other concentrations. The hexane extract of *A. squamosa* at 2.5% showed 82.5% of larval mortality while lower concentrations of 2, 1.5, 1 and 0.5% showed larval-pupal intermediates of 10, 12.5, 22.5 and 30%, respectively. Concerning pupation and adult emergence, 7.5% pupation and 5% adult emergence was observed at 2.5% (Fig. 3). Check azadirachtin showed 72.5% larval mortality, 7.5% larval-pupal intermediates, 5% pupal-adult intermediates, 10% pupal mortality, 10% pupation, 5% adult emergence with ratio of larva to adult conversion being 1:0.05. This outcome was statistically comparable with the chloroform extract of *A. squamosa* at 2.5% which exhibited the larval pupal intermediates from 10 to 35%, pupal-adult malformation 5 to 15% at different concentrations. The pupation and adult emergence ranged from 10 to 37.5% and 5 to 22.5%, respectively when treated with chloroform extract of *A. squamosa* (Fig. 4.).

The present study results revealed larval mortality at higher concentration with minimum of larval-pupal and pupal-adult malformation while lower concentration exhibited maximum deformities. Maximum larval mortality occurred at 2.5% methanol extract of *A. squamosa*, which is in line with Prijono et al. (1997) who observed 100% mortality of *Crociodomia binotalis* with 0.8% acetone extracts of *A. squamosa* seeds two days after treatment. The ethyl acetate, hexane and chloroform extracts of *A. squamosa* showed 90, 82.5 and 70% mortality of larvae at 2.5%. Nenotek et al. (2022) reported that the methanol extract of *A. squamosa* seeds caused 50% mortality at 0.04% and 90% mortality at 0.16%, with *C. pavonana*; and Anosom

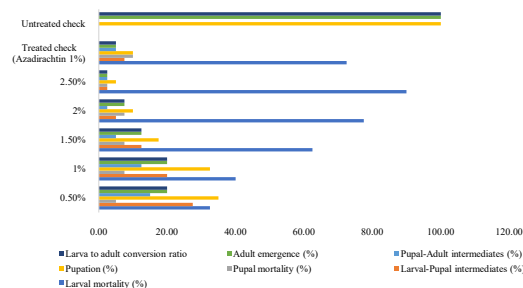


Fig. 2. Insect growth regulator (IGR) activity of ethyl acetate extracts of *A. squamosa* against *S. litura*

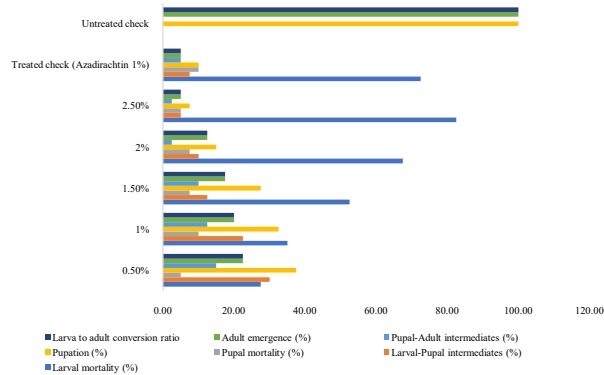


Fig. 3. Insect growth regulator (IGR) activity of hexane extracts of *A. squamosa* against *S. litura*

1%EC (*A. squamosa*) was found to significantly shorten the adult life span, fertility, and egg hatchability of *Spodoptera frugiperda* at a sublethal concentration of 2.71 mg/l (Pavana et al., 2023). The maximum larval-pupal and pupal-adult malformations was recorded in ethyl acetate (27.5 and 15%), hexane (30 and 15%) and chloroform (35 and 15%) extracts at the concentration of 0.5%. Likewise, Mahmoud and Hassan (2022) also reported pupal and adult malformations in *Spodoptera littoralis* when treated with acetone extract of *A. squamosa* seed. Deshmukhe et al. (2010) reported that cold ethyl alcohol extract of *A. squamosa* caused 1.66 and 3.33% of larval-pupal intermediates at 1 and 5%. The azadirachtin treated larvae produced 7.5% larval-pupal intermediates and 5% pupal-adult malformation. Summarwar et al. (2016) observed abnormalities with *S. litura* larvae, pupae and adults when exposed to extracts of *Azadirachta indica*, *Catharanthus roseus*, and *Ocimum sanctum* leaf. Azadirachtin was implicated in the development of the malformation in the wings of *S. litura* (Rao and Subramanyam, 1987). Martinez and Van Emden (2001) recorded a 100% mortality at higher concentration (1ppm) and lower concentration (0.1 ppm) caused growth disruption and abnormalities on *S. littoralis*. Sosa et al. (2019) observed 80% pupal mortality and wing deformities in adults of *S. frugiperda* when sprayed with *Vernonanthura nebularum* plant extracts. Velmani et al. (2019) evaluated the proteinase inhibitor, derived from *Adenanthera pavonina* on *S. litura* and noticed 30% of pupal-adult intermediates. Similarly, Root and seed extracts of *Withania somnifera* were tested against *S. litura* and *Pericallia ricini* at doses of 5, 10, 15, and 20  $\mu\text{g}/\text{ml}$  which revealed an abnormal pupae, delayed pupal-adult ecdysis, reduced pupation and adult emergence (Gaur et al., 2019). Shaalan et al. (2005) reported that many botanicals have an effect on insect development, growth, and adult emergence.

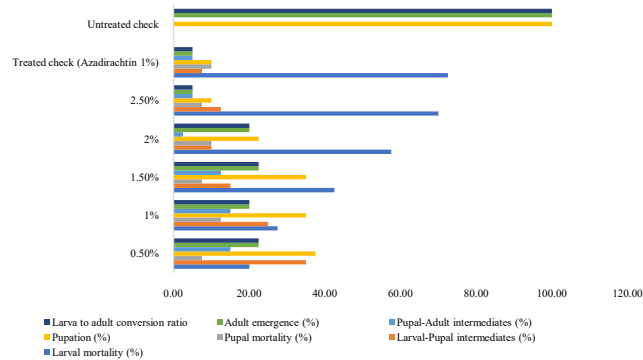


Fig. 4. Insect growth regulator (IGR) activity of chloroform extracts of *A. squamosa* against *S. litura*

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#### AUTHOR CONTRIBUTION STATEMENT

R.M: conceived, design of manuscript, conducted the experiment, analyzed the data, interpreted the data and drafted the manuscript. R.V: design the manuscript, scrutinized the experiment and reviewed the manuscript. S.J., D.U and V.P: Reviewed and commented on the experiment and manuscript for critically important intellectual content. All authors read and approved the final version of manuscript.

#### CONFLICT OF INTEREST

No conflict of interest.

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