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Insecticidal potential of ethanol and hexane solvent seed extract of *Annona squamosa* against *Spodoptera litura* Fab.

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Abstract
Larvicidal activity of Hexane and ethanol seed extracts of *Annona squamosa* were studied against third instar larvae of *Spodoptera litura* at different concentration (5, 10, 15, 20 and 25 mg/ml. The plant seeds were dried, powdered and extracted in soxhlet apparatus in hexane and ethanol solvent for 24 hrs. The third instar larvae of *S. litura* were exposed to various concentration and percent mortality were recorded after 96hrs.

The larvicidal activity of seed extract of *A. squamosa* were (LD 10= 5.91 mg/ml, LD 50= 13.9878 mg/ml) in hexane and (LD10= 11.72 mg/ml, LD 50= 22.48 mg/ml) in ethanol respectively. Results revealed that the mortality was increased with increasing in concentration of the plant extracts.

The hexane solvent extract of *A.squamosa* showed higher larvicidal property against third instars larvae of *S. litura*. Statistical variance, 95% confidence limits and regression equations are presented.

Keywords: *Spodoptera litura*, *Annona squamosa* and mortality

Introduction

The tobacco caterpillar, *Spodoptera litura* (Fab.) is one of the serious and dominant polyphagous pest of cotton, soya bean and other major crops which caused much damage and affect agricultural productivity (Balaraju *et al*., 2011) [7]. It is also one of the most economically important insect pests of 51 countries including India, Japan, China, and other countries of Southeast Asia infesting 112 species of plants belonging to 44 families including groundnut, cotton, chilly, tobacco, castor and soyabean (Chari and Patel,1983) [9]. In India *S. litura* feeds on 74 species of cultivated crops and some wild plants (Rao *et al.*, 2008) [25]. The average crop losses worldwide due to pests and diseases are 60% of potential production.

Chemical pesticides have been used for many years in controlling pests as they give high mortality rate in less time. The huge amount of synthetic pesticides is applied in the fields of cotton and other economically important crops to protect them from insect attack. However, the indiscriminate use of synthetic pesticides resulted in many problems such as resistance of pest to pesticides, resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affect human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment. With a greater awareness of hazards associated with the use of synthetic pesticides there has been an increase need to explore suitable alternative method of pest control. Farmers use different plant material to protect their crops from pest infestation. Natural products in their crude form or plant extract provide unlimited opportunities as biopesticide.

In recent years research efforts are reported on development of insecticides of plant origin. Botanical insecticides are ecofriendly and environmentally safer alternative method for crop protection (Mansour *et al*., 2011; Kabili *et al*., 2012; Abbad and Basheli, 2013) [1, 17, 21]. Plant derivatives are highly toxic to many insect species and more than 2000 plant species are known to possess some insecticidal properties (Kaushik *et al*., 2009). Some of the plants from Meliaceae, Rutaceae, Asteriaceae, Labiatae, Convolvulaceae and Pedaliaceae are promising sources of insecticide based property (Schutterer, 1990, Isman, 1995 Sujatha *et al.*, 2010) [16, 28, 29]. Thangarasu *et al.*, (2015) [31] evaluated the role of different extracts of *Abrus precatorius* for their ovicidal activity, oviposition deterrent activity, antifeedant activity and larvicidal activities against various life stages of selected agricultural field pest *S. litura*. This paper reports the results of research on the effect of *Annona squamosa* plant extracts against the tobacco cutworn, *Spodoptera litura.*

The custard apple family (Annonaceae) is a large family of almost exclusively tropical trees and shrubs comprising some 130 genera (Cronquist, 1993) [10] and 2300 species (Mass *et al.*, 2001) [22], with a worldwide distribution.

(Leatemia and Isman., 2004; Seffrin *et al.*, 2010) [4, 20, 26] studied the biopesticidal activity of aqueous seed extracts of *A. squamosa* on cabbage looper, *Trichoplusia ni*. Leatemia and Isman., (2004) [4, 20] obtained the biopesticide from crude ethanolic seed extracts of *Annona muricata*, *A. squamosa* (Annonaceae), *Lansium domesticum* and *Sandoricum koetjape* (Meliaceae) against the *Spodoptera litura* (Noctuidae) Kamaraj *et al*., (2010) [18] studied the larvicidal activity to determine the efficacies of hexane, chloroform, ethyl acetate, acetone and methanol extracts of ten medicinal plants including *Annona squamosa* against fourth instar larvae of malaria vector, *Anopheles stephensi*.

Therefore, the present study was undertaken to evaluate the effect of hexane and ethanol extracts of *Annona squamosa* seed against the tobacco cut worm, *Spodoptera litura.*

Material and Methods

Plant Collection and Extraction

Seeds of were collected from local area and were properly identified from taxonomist. The seeds were shade dried. The dried seed material were powdered in domestic grinder and stored in air tight container in refrigerator till further use. From the stock 100 g of powdered was extracted with 500 ml of ethanol and hexane using Soxhlet apparatus for 24 hrs separately.

Insect Culture

The eggs of *S. litura* (NBAII-MP-NOC-02: *S.litura*) were purchased from National bureau of Agriculture Insect Resources Bangalore and were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching, the larvae were reared on normal diet with castor leaf, *Ricinus communis*. Third instars larvae were used for further study to minimize handling effects.

Insecticidal bioassay

Third instars larvae of *S. litura* were used for the insecticidal assay. Fresh Castor leaf was taken in each acrylic plastic jar and was exposed to several doses of hexane and ethanol extracts of *A. squamosa*. The dose was prepared by mixing the extract with respective solvent and was sprayed on castor leaves. One jar of control containing only fresh castor leaf sprayed with respective solvent was maintained. The treated castor leaves were allowed to evaporate the solvent. 10 newly emerged III instars larvae were released in each experimental and control acrylic plastic jar containing castor leaves. Three replications were conducted. The percent mortality was calculated after 96 h and the observed data was subjected to probit analysis (Finney, 1947; Busvine, 1971) [8, 13].

Results

The toxic effect of *A. squamosa* seed extracts was evaluated against *S. litura*. The numbers of dead *S. litura* were counted after 24, 48, 72 and 96 h at different doses (5, 10, 15, 20, and 25 mg/ml) of hexane and ethanol crude extract. The total percent mortality was observed after 96 h, and then the corrected mortality was calculated using Abbott's formula (Abbot, 1925) [2] and the results are presented. The results showed that, the mortality increases with increase in concentrations of all doses (Figure and Tables).

The results of probit analysis for the estimation of LD_{10}, LD_{50}, variance, 95% confidence limits and regression equation at 96h for the mortality of third instar larvae of *S. litura* are presented in Table – 2.

The insecticidal bioassay in hexane solvent extracts of *Annona squamosa*, LD_{10} = 5.91 mg/ml and LD_{50} = 13.98 mg/ml and in ethanol extract of *Annona squamosa*, LD_{10} = 11.72 mg/ml and LD_{50} = 22.48mg/ml. Among the various estimate of regression based probit analysis, the $\chi 2$ values for the regression coefficients showed homogeneity to the data.

Table 1: Percent mortality of *Spodoptera litura* treated with hexane and ethanol seed extracts of *Annona squamosa*.

Sr. No.	Dose in mg/ml	Mortality after 96 hrs. (Hexane)	Mortality after 96 hrs. (Ethanol)
1.	Control	-	-
2.	5	10	-
3.	10	30	-
4.	15	40	20
5.	20	70	40
6.	25	90	60

Table 2: LD_{10}, LD_{50} values with variance, 95% confidence limits and probit analysis parameters for larvae of *Spodoptera litura* after 96h of treatment.

Solvent	LD_{10}	LD_{50}	Variance	95% CL		Regression equations	$\chi 2$
				Lower	Upper		
Hexane	5.91	13.98	0.0035172	1.02946	1.2619	Y= 3.4290x+1.0717	1.5892
Ethanol	11.72	22.48	0.0024425	1.2550	1.4487	Y=4.56x+1.1651	0.1586

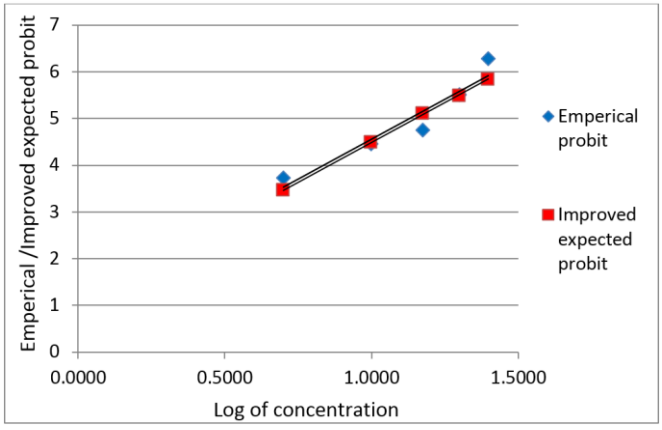

Fig 1: Regression and provisional lines for *Spodoptera litura* exposed to hexane seed extract of *Annona squamosa* after 96 h

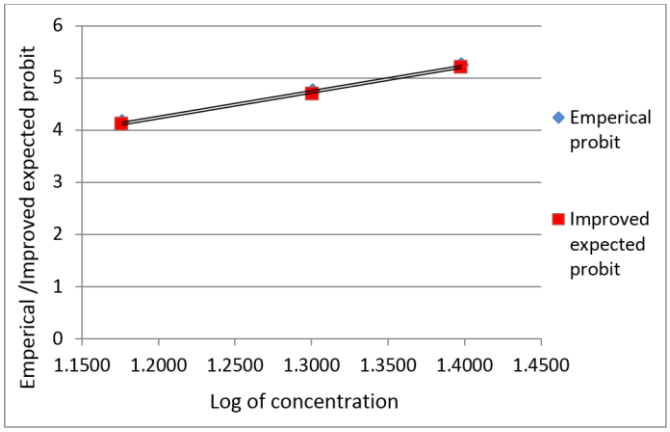

Fig 2: Regression and provisional lines for *Spodoptera litura* exposed to ethanol seed extract of *Annona squamosa* after 96 h

Discussion

Spodoptera litura is one of severe agricultural pest. In insect-plant interactions, insects often have unique adaptation to their host plants in locating and selecting the plants by the use of chemical, visual and mechanical cues (Schoonhoven *et al*., 1998) [27]. According to Mustaparta, (2002) [24], unsuitable plants are avoided by detection of other chemical cues; such chemical substances may have repellent or toxic properties against insects. Based on this principle, botanical pesticides are invented and utilized for control of insect pests. Crude extracts from the leaf, stem, root and seeds of various plant species have been reported to possess antifeedant, insecticidal, and/or growth inhibitory properties Ekesi., (2000) [11]. Hummel bruner and Isman., (2001) [14, 15] reported that synergistic effects of complex mixtures (crude extracts) of phytochemicals are also thought to be important in plant defenses against insect herbivores.

In the present investigation, the toxicity of ethanol and hexane seed extract of *Annona squamosa* was tested against *S. litura*. In our study mortality increased with increase in concentration at all the doses up to 96 hrs of exposure.

Similar to the present investigation, several studies documented the insecticidal activity of *Annona squamosa* on different pests. Rajguru and Sharma., (2012) [23] studied the efficacy of crude aqueous extracts of eight plant species against *Spodoptera litura* larvae at 25, 50, 75 and 100% concentrations and found that leaf extracts of *Acacia arabica* and *Annona squamosa* caused 76.66% and 83.33% larval mortality respectively at 25% concentration within 3 days of treatment. Babu *et al.,* (1998) [6] reported about 60% mortality in *S. litura* larvae with 5% concentration of *A. squamosa* crude oil. They also reported seed extract of *A. squamosa* to be highly toxic even to 5th instar larvae (Babu *et al.,* 1999) [5]. Khalequzzaman and Sultana, (2006) [19] studied the insecticidal activity of the seed extracts of custard apple, *Annona squamosa* L. in petroleum spirit, ethyl acetate, acetone and methanol against Raj, CR 1, FSS II and CTC-12 strains of the red flour beetle, *Tribolium castaneum* (Herbst). Their result showed that the highest toxicity was for petroleum spirit extract (LD50= 0.03µg cm-2) in Raj strain and the lowest toxicity was for methanol extract (LD50=15.697µg cm-2) in FSS II strain for larva and in adults petroleum spirit extract showed highest toxicity (LD50= 58.697µg cm-2) in CTC 12 strain and the lowest toxicity (LD50=22004.710µg cm-2) was for acetone extract in CR 1 strain. Leatemia and Isman, (2004) [4, 20] studied the biopesticidal activity of crude ethanolic seed extracts of *Annona muricata*, *A. squamosa (Annonaceae), Lansium domesticum* and *Sandoricum koetjape* (Meliaceae) against *Spodoptera litura*, they reported that extracts of *A. squamosa* inhibited larval growth in a dose-dependent manner and were significantly more active (20-fold) than those of *A. muricata. A. squamosa.* Kamaraj *et al*., (2010) [18] studied the larvicidal activity to determine the efficacies of hexane, chloroform, ethyl acetate, acetone and methanol extracts of ten medicinal plants including *Annona squamosa* against fourth instar larvae of malaria vector, *Anopheles stephensi* and they found highest larval mortality in leaf acetone of *Adhatoda vasica*, bark ethyl acetate of *Annona squamosa*, methanol leaf and flower of *Cassia auriculata*, leaf ethyl acetate of *Hydrocotyle javanica*, methanol leaf and seed of *Solanum torvum* and leaf hexane extracts of *Vitex negundo* against the fourth instar larvae of *An. stephensi* and *Cx. Quinquefasciatus. Annona squamosa.*

In many countries, plant derived products are being used by the farmers from ancient times and it triggered the scientists to search for ecofriendly insecticides from plant kingdom. Several hundred plants have been reported as insect repellents, antifeedants, attractants, insecticides, ovicides and oviposition deterrents (Arnason *et al*., 1992; Ewete *et al*., 1996) [3, 12]. Antifeedants offer first line of crop protection against notorious insects. According to Isman., (2002) [15] any substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent. In general, antifeedants have profound adverse effects on insect feeding behavior was reported by Hummel (2001) [14].

Due to the toxic effect of ethanol extract of *Annona squamosa* maximum number of treated larvae died in comparisons to ethanol used in the present investigation. Similarly, Leatemia and Isman, (2004) [4, 20] reported that high concentrations of extracts caused high mortality of larvae even though only very small portions of the leaf discs were consumed they also reported that crude extracts of plants often consist of complex mixtures of active principles. Telang, (2003) [30] reported that Apart from insecticidal activities, larval-pupal intermediates, pupal and malformed insects are formed, and these unhealthy adults are short lived and infertile. Isman, (2002) [15] reported that botanical antifeedants and insecticidal agents can play a significant role as part of an Integrated Pest Management.

The finding of the present investigation revealed that, the seed extract of *Annona squamosa* possesses remarkable insecticidal activity against *Spodoptera litura*. The LD_{10}=5.91mg/ml, LD_{50}=13.98 mg/ml in ethanol and LD_{10}=11.72 mg/ml, LD_{50}=22.48 mg/ml in ethanol is reported. The study needs further investigation to find out active ingredients responsible for insecticidal properties of *A. squamosa* and to reach any final recommendations.

Conclusion

The result of this study has confirmed that the *Annona squamosa* have explored the potential biopesticide and plant protecting activity against cut worm, *Spodoptera litura*.

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