Bioefficacy of cold ethyl alcohol extract of *Annona squamosa* against *Spodoptera litura* Fabricius.

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ABSTRACT

The tobacco caterpillar, *Spodoptera litura* Fabricius has emerged as a serious and dominant pest on many agricultural crops causing enormous losses. The use of plant extracts to manage *S. litura* as an eco friendly management strategy in organic farming is the need of the day. A cold ethyl alcohol extract of seeds of *Annona squamosa* at different concentrations from 0.5 per cent to 25 per cent was administered to lab-reared; pre starved fourth instar larvae, topically as well as through food. The fourth instar larvae treated topically with the 25 per cent extract exhibited a total larval mortality of 61.66±1.66 per cent and the total per cent mortality was 80.0±0 during its development to the adult stage. The larvae which were administered the plant extract through their food showed total mortality of 76.66±4.41% at 25 per cent concentration, of which 61.66±1.66 per cent was at the larval stage itself. Larval-pupal and pupal-adult deformed stages were observed during the development but the adults were normal. Mortality was observed in the larvae and pupae. LC_{50} and LC_{90} values for topical application was 21.52 and 47.87 respectively .The same for leaf application was 25.75 and 53.70. The seed extract also exhibited phagodeterrent properties.

Key words: Biological insecticide, *Spodoptera litura*, crop pests, *Annona squamosa*, lethal concentration

INTRODUCTION

Among the polyphagous pests, the tobacco caterpillar, *Spodoptera litura* Fabricius. has emerged as a serious and dominant pest causing enormous losses to crops like pulses, cotton, oilseeds, vegetables etc. The avoidable yield loss has been estimated as 70 per cent due to this pest on black gram grown in the fallows in Andhra Pradesh (Murugesan and Dhingra, 1995). The fully grown caterpillars of the tobacco cutworm, *S. litura* are most voracious feeders and can cause extensive damage by defoliation. In India, *S. litura* has been reported as an increasingly important pest during the rainy seasons causing heavy yield loss throughout India (Amin,1983). It has the ability to develop resistance to many conventional insecticides used for its control.

The present studies involve the application of cold ethyl alcohol extract from the seeds of *Annona squamosa* to study its effect on the survival rate of the insect at various stages of development. This botanical was used in the tests as it is economic, easily available, biodegradable and safe for humans and also safe on other non-target organisms. It is ecofriendly and can be used as one of the components in organic farming.

MATERIALS AND METHODS

Annona squamosa (Annonaceae) cultivated in gardens in the whole of India for its sweet and delicious fruits. Its original home is West Indies. The plant has a woody stem from the ground (shrub). Leaves alternate, in two rows, oblong, dull green on the upper side and pale with a bloom below. When crushed they have an aromatic smell. Flowers are borne singly. Fruit represents the aggregate of many fleshy carpels (Pfleiderer, 1990)

Seeds are acrid and poisonous. The bark, leaves and seeds contain the alkaloid, anonaine.Six other aporphine alkaloids have been isolated from leaves and stem.Corydine, roemerine, norcorydine, norysocoridine, glaucine may also be present. In India pounded seeds are used for killing lice in hair (care is taken to keep it away from the eyes as it is known to cause blindness-the seeds are a powerful irritant of the conjunctiva). Fruit enriches the blood, is a sedative and relieves vomiting . An infusion of the leaves is considered efficacious in prolapses ani of children; and the bruised leaves with salt make a cataplasm to induce suppuration. The astringent bark is used as an antidiarrhoeic cure in Cambodia. (Kirtikar and Basu, 1987). Importance of *A. squamosa* crude extracts in pest manage ment was emphasized very recently by seffrin *et.al* 2010.

Freshly laid egg batches of *S. litura* were collected from the castor fields at the University of Agricultural Sciences, Dharwad, Karnataka. The eggs were sterilized with 0.05 per cent NaOCl and then with distilled water, transferred to a fresh castor leaf and allowed to hatch under laboratory conditions. The larvae were reared in the laboratory with sterilized earthen pots of 2 l capacity. The mouths of the pots were covered securely with a clean sterilized white muslin cloth. The larvae were fed on fresh castor *(Ricinus communis* L.*)* leaves, and maintained at a temperature of 26 ± 6^0C , and relative humidity of 65 ± 5 %, and photoperiod of L10:D14. The laboratory reared pre-starved fourth instar larvae were used as test insects.

Plant extracts

The shade dried seeds of *Annona squamosa* were pulverized in to a fine powder in an electric mixer grinder and sieved through a muslin cloth. This powder was used for preparing solvent extract within 24 hours. 500 grams of the botanical powder was soaked in 500 ml of absolute alcohol (ethyl) and kept overnight. The mixture was stirred with a magnetic stirrer frequently. The solution was then filtered through an ordinary filter paper. The alcohol was allowed to evaporate from the filtrate at room temperature. The plant extract paste was dissolved in 50 ml of acetone. This has served as a stock solution. As the fourth instar larvae cause the maximum damage to the foliage, fourth instar larvae were selected for topical as well as leaf application tests. Under each type of application, different concentrations of the stock solution *i.e*, 0.5, 1.0,5, 10, 15, 20 and 25 per cent concentrations were used. Acetone served as a treated control and untreated control was also taken. Each treatment comprised 20 larvae kept in 20 separate sterilized earthen pots of 1 liter capacity each and were fed on fresh castor leaves. Two ml extract was sprayed on the larvae (topical application) and on similar sized castor leaves with an atomiser. An ordinary cold-air blow dryer was used to hasten the process of drying of the extract on the body/leaves. The experiments were replicated thrice. Observations were made to assess the Total per cent mortality, per cent mortality in each stadium, feeding deterrence and deformities in the adults. The per cent mortality was corre cted using Tukey's honestly significant test. LC_{50} and LC_{90} were calculated using probit analysis (Finney, 1971).

RESULTS AND DISCUSSIONS

In the topically treated group, feeding was totally stopped by the larvae for the initial 20 - 25 minutes of application, for all the seven concentrations. A total Feeding depression

Table 1. Bioefficacy of cold alcohol seed extract of *A. squamosa* on development of *S. litura* following topical application on fourth instar larvae.

Concentrations (in %)	Per cent larval (IV, V andVI instar) mortality	Per cent pre-pupal (shrunken stage) mortality	Per cent mid -pupal (larval-pupal intermediate) mortality	Per cent pupal` mortality	Per cent total mortality
0.5	21.66±1.66^{e}	6.66±1.66^{b}	0^{b}	0^{a}	28.33±1.66^{d}
1	23.33±1.66^{e}	8.33±1.66^{b}	1.66±1.66^{ab}	0^{a}	33.33±1.66^{d}
5	38.33±1.66^{d}	10.00±00^{b}	3.33±1.66^{ab}	0^{a}	51.66±1.66^{c}
10	48.33±1.66^{c}	10.00±00^{b}	0^{b}	0^{a}	58.33±1.66^{b}
15	56.66±1.66^{ab}	16.66±1.66^{a}	3.33±1.66^{ab}	0^{a}	78.33±3.33^{a}
20	51.66±1.66^{bc}	16.66±1.66^{b}	5.00±2.88^{ab}	1.66±1.66^{a}	75.00±00^{a}
25	61.66±1.66^{a}	8.33±1.6^{c}	8.33±1.66^{a}	1.66±1.66^{a}	80.00±00^{a}
Carrier (acetone control)	6.66±1.66^{f}	0^{c}	0^{b}	3.33±1.66^{a}	10.00±00^{e}
Absolute control	0^{f}	0^{c}	0^{b}	1.66±1.66^{a}	6.66±1.66^{e}
SE	1.59	1.27	1.55	0.91	1.27
"F" test	136.98	16.80	3.57	1.52 NS	381.25
CD (0.05)	4.82	3.88	4.72	NS	3.88
CD (0. 01)	6.69	5.38	NS	NS	5.38

Means followed by the same letters do not differ significantly from each other at P < 0.05 by Tukey's honestly significant test

LC_{50} = 21.52 LC_{90} = 47.87

might have been caused by behavioural effects (Jeyabalan and Murugan, 1997).Faecal pellets were normal for all the seven treatments. In the leaf application group, this behavior persisted for the initial 35 - 50 minutes in all the seven treated subgroups. Feeding, when it was resumed, was slow and intermittent in the next three hours. Pellets were extremely moist and pasty in the larvae fed with the leaf applied with 5, 10, 15, 20, and 25, per cent leaf extract. This may be because of loss of peritropic membrane of the digestive tract in the larvae.

In the topically treated group (Table 1) more than 50 per cent mortality was recorded for 5 to 25 per cent concentrations. Maximum total mean per cent mortality was 80.0 ± 0, of which 61.66 ± 1.66 per cent was at the larval stage itself was observed at 25 per cent concentration. This indicated the direct knock-down action of the plant extract. No deformities in adults were observed in the topically treated or the orally treated group. Deformities and death observed at the larval, mid-pupal and pupal stages may be due to inhibition of chitin synthesis as observed in *S .litura* treated with diflubenzuron, a chitin synthesis inhibitor (Nelson and Venugopal, 2006). The LC_{50} and LC_{90} were 21.52 and 47.87 respectively. Results from Table 2 (leaf application) showed that more than 50 per cent total mortality was observed for 5, 10, 15, 20, and 25 per cent concentrations. Maximum total mean per cent mortality was 76.66 ± 4.41 at 25 per cent concentration. Highest mortality at the larval stage was 61.66 ± 1.66 per cent for 25 % concentration, at pre-pupal stage it was 13.33 ± 1.66 per cent for 20 % concentration at the larval-pupal intermediate stage it was 5.0 ± 2.88 per cent (for 5 % concentration) and at the pupal stage no mortality was observed at all. This supports our suggestion that seeds of *Annona squamosa* have knock-down/direct insecticidal properties against *S.litura*. LC_{50} and LC_{90} values for leaf application were 25.75 and 53.70 respectively. Larval mortality could be attributed to direct insecticidal action (as a contact poison) or due to feeding inhibition or gustatory repellency or impairment in the food assimilation. The abdominal segments in some of the deformed and dead pupae were very much elongated and were never more than four segments in number. Some dead pupae showed incomplete chitinisation in the thoracic and cephalic region. Death in the pupal stage is ascribed to the slow action of plant products on growth stages of insect or due to the enhanced activity of plant constituents when assimilated in insect tissues. Similar effects were documented by Senthamizhselvan and Muthukrishnan (1992) in *S. exigua*. The incomplete

Table 2. Bioefficacy of cold alcohol seed extract of *Annona squamosa* on development *S. litura* following leaf application on fourth instar larvae.

Concentrations (in %)	Per cent larval (IV,V&VI instar) mortality	Per cent pre-pupal (shrunken stage) mortality	Per cent mid -pupal (larval-pupal intermediate) mortality	Per cent pupal` mortality	Per cent total mortality
0.5	23.33±1.66^{e}	8.33±1.66^{a}	1.66±1.66^{a}	0^{b}	33.33±1.66^{f}
1	31.66±1.66^{de}	11.66±1.66^{a}	1.66±1.66^{a}	0^{b}	45.00±00^{e}
5	38.33±1.66^{cd}	8.33±1.66^{a}	5.00±2.88^{a}	0^{b}	51.66±1.66^{de}
10	46.66±1.66^{bc}	11.66±1.66^{a}	0^{a}	0^{b}	58.33±1.66^{cd}
15	53.33±3.33^{ab}	10.00±2.88^{a}	0^{a}	0^{b}	63.33±1.66^{bc}
20	55.00±2.88^{ab}	13.33±1.66^{a}	1.66±1.66^{a}	0^{b}	70.00±00^{ab}
25	61.66±1.66^{a}	10.00±00^{a}	1.66±1.66^{a}	0^{b}	76.66±4.41^{a}
Carrier (acetone control)	5.00±00^{f}	0^{b}	0^{a}	3.33±1.66^{a}	8.33±1.66^{g}
Absolute control	5.00±00f	0^{b}	0^{a}	1.66±1.66^{a}	6.66±1.66^{g}
SE	2.07	1.76	1.55	0.58	1.42
“F” test	85.32	6.0	1.12 NS	4.00	221.62
CD (0.05)	6.30	5.36	NS	1.78	4.32
CD (0. 01)	8.74	7.44	NS	NS	6.00

Means followed by the same letters do not differ significantly from each other at $P < 0.05$ by Tukey’s honestly significant test

LC_{50} = 25.75 LC_{90} = 53.70

chitinisation in pupae suggests that the botanical used may have caused an inhibition in chitin synthesis. Death at the larval, pre-pupal and larval-pupal intermediate (mid-pupal) stage occurred due to a strong moult inhibition; they were unable to shed off their skin completely. Deformities and death in larval, larval-pupal intermediate and pupal stages may be due to change in the ecdysteroid titre as demonstrated by Leuschner (1972) against coffee bug when it was treated with methanol extract of Neem leaves. Similar morphogenetic effects of *Azadirachtin* rich fractions against *Spodoptera litura* were observed by Nelson and Venugopal (2006). As no significant difference in the per cent mortality was recorded between control and acetone carrier groups, we may suggest that acetone did not exhibit toxic effect on the test insect. The knock-down effect may be attributed to the action of the natural product by interfering respiration, leading to asphyxiation, or causing muscle paralysis or affecting the nervous system (Patole *et al*, 2008).

The present study indicates that both topical and leaf applications of cold alcohol extract of *Annona squamosa* seeds are highly effective in controlling the lepidopteran pest, *S. litura* by causing a significant per cent mortality at the larval, larval-pupal intermediate and pupal stages under laboratory conditions. This plant product is also eco-friendly, easily available and economically viable. Biopesticides are considered to be safe to natural enemies and free from any residue problem on the crop and in the environment. (Mukherjee and Singh, 2006). Considering the overall performance, the cold alcohol extract from seeds of *Annona squamosa* may be utilized in the management *of S .litura* after evaluating its effects against *S. litura* under field conditions.

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