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## **Effect of Crude Methanol Leaf Extracts of *Andrographis paniculata* (Burm.f) Nees on Larvae of *Helicoverpa armigera* (Hübner)**

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### **Abstract**

Plant extracts, especially botanical insecticides, have gained prominence because of their use as potential plant protecting agents. Biological activity of solvent extracts of *Andrographis paniculata* Ness. (Acanthaceae) were evaluated using the larvae of gram pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae), Antifeedant and larvicidal activity of methanol crude, petroleum ether, methanol fraction and ethyl acetate fraction of leaf extracts of *A. paniculata* were estimated in the present study. Preliminary screening of the extracts was tested at a concentration of 1,000 ppm. The larval mortality was observed after 24h of exposure. All extracts exhibited moderate larvicidal activity. However, highest larval mortality was observed in crude methanol extract (1.9, 3.2, 8.2, 19.3, 59.0 and 127.9) followed by the methanol fraction (3.1, 4.2, 9.4, 20.6, 63.6 and 162.6) of leaf extract of *A. paniculata* against the I, II, III, IV, V and VI instar larvae of *H. armigera* respectively. Petroleum ether extract of *A. paniculata* leaf was least active against the larvae of *H. armigera*. The results suggest that crude extract of *A. paniculata* leaves can be used as biopesticide for the control of *H. armigera*.

**Keywords:** *Andrographis paniculata*, Antifeedant, Biopesticide, *Helicoverpa armigera*, Larvicidal.

### **Introduction**

India is basically an agro-based country; more than 80% of Indian population depends on agriculture. Indian economy is largely determined by agricultural productivity. Insect-pests are known to cause significant damage to crops and affect agricultural productivity. The environmental hazards posed by synthetic pesticides

provide an impetus for investigations into some ecofriendly and biorational alternatives (Subashini *et al.*, 2004). In central and north India, it is the major pest affecting cotton. *H. armigera* has a long history of resistance to conventional insecticides. Variety of chemical insecticides and pesticides are used to control *H. armigera*. However, harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives. Economic loss due to this pest in India accounts for 5,000 cores (Manjunath *et al.*, 1985). The monetary loss due to feeding by larvae and adult insects alone contributes to billion dollars per annum (Jacobson, 1982).

*Helicoverpa armigera*, commonly known as cotton bollworm or American bollworm, is a major polyphagous noctuid pest in Asia, causing heavy damage to agricultural, horticultural and ornamental crops (Talekar *et al.*, 2006). In central and northern India, it is the major pest affecting cotton. The larvae feed extensively on cotton plant parts including the newly emerging bolls causing severe loss of crop. Larvae of *H. armigera* feed on the leaves initially and later bore into the pods and seeds with its head thrust into, while rest of the body lies outside. Hence, a large number of *H. armigera* larvae in cotton and other vegetables survive to adults that may disperse widely, producing progeny that damage high-value crops (Cabanillas and Raulston, 1995, Michael and Donald, 1996). It has been reported to cause significant damage in different parts of the world (Setiawati *et al.*, 2000, Fakrudin *et al.*, 2004).

However, colonization of new host by *H. armigera* induces selection of adaptive characters and genetic differentiation in population (Rice, 1987; Diehl and Bush, 1989). Since, *H. armigera* can survive on alternate host it is characterized by high mobility and fecundity. Further, it has been reported to develop resistance to synthetic insecticides used in its management (Ramasubramaniam and Regupathy, 2004). Even the impact of certain plant products and *Bacillus thurengiensis* cry protein on the growth and feeding physiology of *H. armigera* (Murugan and Babu, 1998) have been less effective.

During the last 50 years, worldwide use of synthetic insecticides to control insect pests has led to both insecticide resistance and environmental persistence (Roush and Tabashnik, 1990). Alternatively, phytochemicals have been used in the management of agricultural pest (Choudhary *et al.*, 2001). Plant derived pesticides are eco-friendly, non-toxic to non target organisms, non persistent in nature, besides they are less known to promote drug resistance. Application of bio-pesticides has been reported to have positive impacts on bollworm population management (Ge and Ding, 1996, Ramya *et al.*, 2008). Therefore, researchers world over are engaged in a mission to hunt for novel phytochemicals that could potentially be used in the management of insect-pests.

Plants are endowed with a potential to produce a wide range of allelochemicals that protect the plants from insect-pests. However, production of phytochemicals has been reported to vary from plant to plant (Ahmad, 2007). Further, parameters like age of the plant, part of the plant (root, stem, leaf, fruit, flower, seed and bark) have been reported to affect the production of such allelochemicals. The phytochemicals produced in response to insect-pest attack, affect feeding and oviposition of insects on the plants (Ramya *et al.*, 2008, 2009).

A number of plants have been shown to have pesticidal and antifeedant activity against *H. armigera*, of which Neem has been subjected to extensive investigation (Koul, 1985, Chopra *et al.*, 1994, Jaglan *et al.*, 1997). Studies have shown that *Acorus calamus*, *Annona squamosa*, *Vitex negundo* are effective in the management of *H. armigera* (Murugan *et al.*, 1998, Janardhan *et al.*, 1999). Sundararajan and Kumuthakalavalli (2001) evaluated antifeedant activity of aqueous extract of *Gnidia glauca* and *Toddalia asiatica* against *H. armigera*. The methanol extract of *Melia dubia* showed growth inhibitory and toxic effect against the larvae of *H. armigera* (Koul *et al.*, 2000), the hexane, methanol, and water extracts of *Cajanus cajan* pods stimulate feeding of fifth instars, with the methanol extract being most stimulatory (Sharma *et al.*, 2001, Green *et al.*, 2002, 2003), the ethanolic extract of seed kernel of *Azadirachta indica* failed to prevent feeding (Jhansi and Singh, 1993), the extracts of *Jatropha curcas* showed insecticidal effects (Solsoloy, 1995) against *H. armigera*.

*Andrographis* is a shrub that is found through out India and other Asian countries. It is sometimes referred to as “Indian echinacea”. *A. paniculata* (Acanthaceae), Kalmegh of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plant body. It grows erect to a height of 30–110 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals. Stem dark green, 0.3–1.0 m in height, 2.0–6.0 mm in diameter, quadrangular with longitudinal furrows and wings on the angles of younger parts, slightly enlarged at nodes; leaves glabrous, up to 8.0 cm long and 2.5 cm broad, lanceolate, pinnate; flowers small, in lax spreading axillary and terminal racemes or panicles; capsules linear-oblong, acute at both ends, 1.9 x 0.3 cm; seeds numerous, sub quadrate, yellowish brown (Mathew, 1985, Gamble, 1993).

Since ancient times, *A. paniculata* is used as a wonder drug in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The plant extracts exhibit antityphoid and antifungal activities. Kalmegh is also reported to possess antihepatotoxic, antibiotic, antimalarial, antihepatitic, antithrombogenic, antiinflammatory, antisnakevenom, and antipyretic properties to mention a few, besides its general use as an immunostimulant agent. *Andrographis* contains, as its primary chemical constituents, diterpenoid lactones (Andrographolides), paniculides, farnesols and flavonoids *Andrographis* is used for prophylactic and symptomatic treatment of upper respiratory infections such as the common cold, as well as for uncomplicated sinusitis, pharyngotonsillitis, pneumonia, and bronchitis (Bobbarala *et al.*, 2009). With this background, in the present study the pesticidal effect of leaf extracts of *A. paniculata* has been evaluated against larvae of *H. armigera*.

## **Materials and Methods**

### ***Collection of Plants***

*A. paniculata* was collected from the wild in Sivagangai District, TN, India. Selection of plants was made on the basis of absence of damage by the insect-pest. Healthy plant materials were collected in poly bags and brought to lab and their botanical

identity was established. Flora of Presidency of Madras (Gamble, 1993) and The Flora of Tamil Nadu Carnatic (Matthew, 1985) were used for authentication of the plants.

### ***Extraction of phytochemicals using different solvents***

Leaves were collected, washed thoroughly in water, air dried in shade and powdered using a pulverizer and stored in plastic containers. The powdered material was weighed and extracted in crude methanol (40–60%) as solvent in the ratio of 1:10 w/v using Soxhlet apparatus at 55°C. The crude methanol extract was filtered through a funnel using glass filter and evaporated using a rotary evaporator. The residue was re-dissolved in methanol and defatted in equal volume of petroleum ether in a separating funnel. The fractions were separated, dried in a rotary evaporator. The methanol fraction was further dissolved in ethyl acetate and insoluble derbies were removed by filtration. Water soluble materials from the ethyl acetate fraction were removed in a separating funnel using double distilled water. The fractions were collected separately and dried. Yields in relation to the initial weight of the powder of the different fractions were determined. One percent stock solutions of all the fractions in methanol were prepared from the residues obtained at each stage of the purification process and the fractions were tested at different concentrations.

### ***Test organism***

The larvae used for the study were collected from the host plants in the fields and brought to lab. They were reared on artificial diet under laboratory conditions. Studies were carried out using I–VI instar larvae of *H. armigera* against the leaf extract of *A. paniculata*. The percentage mortality was calculated after a period of 24 h.

### ***Bioassay studies***

Bioassay studies were carried out with different fractions of *A. paniculata* leaf extracts against the larvae of *H. armigera*. The studies were conducted (24 h) in the laboratory in transparent plastic containers of 4 x 2.5 cm size capped with perforated plastic lids. Fresh leaves of *Gossipium esculentum* (Cotton) were collected from the field and washed in clean water. Excess moisture was removed and the leaves were dipped in one percent test solution, shade dried and served to the larvae of *H. armigera*. Extract free leaves served as the control. For each treatment 10 larvae were singly introduced in separate containers after six hour starvation. Three replicates each of ten larvae were maintained for each treatment. The experiments were conducted at  $27 \pm 1^\circ\text{C}$ , 75% humidity and 14 h dark period. Twenty four hour larval mortality was observed and the percentage mortalities were corrected using Abbott's formula (Abbott, 1925). Ethyl acetate fraction of *A. paniculata* was tested for LD<sub>50</sub> values against the larval stages of *H. armigera*. Mortality was observed after the completion of the larval stages. The fraction which showed high rate of mortality in the least LD<sub>50</sub> values was selected for further studies.

## Results and Discussion

The results of bioassay studies against the larvae of *H. armigera* in the crude extracts, methanol fractions, petroleum ether fractions and ethyl acetate fractions of *A. paniculata* revealed that the LD<sub>50</sub> values for the individual fractions of plant extracts varied significantly from 1.9–120.0 µg/cm<sup>2</sup>. The mortality rate was observed in the decreasing order of methanol crude > methanol fraction > ethyl acetate fraction > petroleum ether. The crude methanol extracts of the leaves of *A. paniculata* was more toxic for the instars I to VI than other fractions. The least LD<sub>50</sub> values ranged from 1.98–127.91 µg/cm<sup>2</sup> for I to VI instars larvae in the extracts of leaves of *A. paniculata* (Table 1).

Table 1 Effect of phytochemical extracts of *A. paniculata* on the larvae of *H. armigera*

Extract	Larval instars of <i>Helicoverpa armigera</i>					
	I	II	III	IV	V	VI
Methanol crude	1.9 <sup>a</sup>	3.2 <sup>a</sup>	8.2 <sup>a</sup>	19.3 <sup>a</sup>	59.0 <sup>a</sup>	127.9 <sup>a</sup>
Petroleum ether	120.0 <sup>d</sup>	190.0 <sup>d</sup>	240.0 <sup>d</sup>	300.0 <sup>d</sup>	370.0 <sup>d</sup>	400.0 <sup>d</sup>
Methanol fraction	3.1 <sup>b</sup>	4.2 <sup>b</sup>	9.4 <sup>b</sup>	20.6 <sup>b</sup>	63.6 <sup>b</sup>	162.6 <sup>b</sup>
Ethyl acetate fraction	4.6 <sup>c</sup>	5.8 <sup>c</sup>	11.0 <sup>c</sup>	22.1 <sup>c</sup>	65.9 <sup>c</sup>	158.7 <sup>c</sup>

*A. paniculata* crude methanol extract was found to inflict a high percentage of mortality on the treated larvae. Larval mortality at the highest dose (1.9 µg/cm<sup>2</sup>) of *A. paniculata* crude methanol extract was 83.3%. Since, crude methanol extracts of *A. paniculata* was more active than other fractions, crude methanol extracts of *A. paniculata* were used to determine the ED<sub>50</sub> values for their effect on the larvae of *H. armigera*. The ED<sub>50</sub> values and its corresponding fiducial limits along with slope and intercept are given in table 2. However, it was observed that the LD<sub>50</sub> values were significantly different at  $P < 0.05$ ; LSD: 6.334. Pupal mortality includes pupae that died before emerging as adults as well as those which died as mal formed pupae subsequent to larval- pupal metamorphosis. While larval mortality was dependent on the dose of the fractions pupal mortality was not influenced by the dose; on the other hand percent pupation and emergence of adults were dose dependent and were inversely related for instance among the *H. armigera* larva as shown in table 2.

Plants produce a wide spectrum of allelo-chemicals; however, many of such chemicals have not been explored for their physiological properties (Norduland and Sauls, 1981). Phytochemicals are known to specifically inhibit growth, morphogenesis, metamorphosis and reproduction (Ahmad, 2007). Currently there is resurgence of interest in plant derived compounds for developing them commercially as eco-friendly insecticides. Tropical plants are more promising for the development of new insecticides (Jacobson and Crosby, 1971). Despite, the fact that hundreds of tropical plants are reported to possess insecticidal property, only few compounds have been commercialized (Chopra *et al.*, 1994).

Table 2 Effect of crude methanol extract of *A. paniculata* on larvae of *H. armigera*

Larval Instars	ED <sub>50</sub> (µg/cm <sup>2</sup> )	Fiducial Limits		Slope	Intercepts	χ <sup>2</sup> /d.f.
		Upper	Lower			
I	1.9	0.500	0.401	1.070	4.680	7.850/4
II	3.2	0.570	0.490	1.450	4.250	4.980/4
III	8.2	1.430	1.190	1.570	3.550	1.210/4
IV	19.3	2.760	2.410	1.880	2.570	0.520/4
V	59.7	8.050	7.100	2.090	1.270	6.440/4
VI	127.9	20.900	17.950	2.310	0.123	2.430/2

For successful exploitation of natural insecticidal compounds, screening for their behavioral and physiological effects in poly-phagous insects with an understanding of structure activity relationship is essential. Unfortunately, many do not provided estimates of critical lethal (LD<sub>50</sub>) or critical effective dose (ED<sub>50</sub>) which prevents feeding or emergence as adults. Nevertheless, such values evaluate the relative efficacy of the extracts and are required for field application. In a study, Simmonds *et al.*, (1990) reported high antifeedancy (low ED<sub>50</sub>) for pure compounds isolated from different plants against the larvae of *H. armigera*. Janarthan *et al.*, (1999) showed that 0.2 and 0.5% petroleum ether extracts of *Parthenium hysterophorus* exhibited 100% feeding difference in *H. armigera*. Similarly, aqueous extracts of *Calotropis procera* and *Datura stromonium* have been shown to display about 90% feeding protection against *H. armigera* (Dodia *et al.*, 1998).

The bioactivity of tested phytochemical extracts varied significantly with solvents used for the extraction and instar stage of the larvae as reported previously Ramya *et al.*, (2008, 2009). Reviewing the prospects of antifeedant for the management of pests, Jermy (1990) and Ahmad (2007) reported that plant extracts/compounds “with combined behavioral and toxic effect are more likely to have successful practical application than the compounds/extracts, which evoke only behavioral effect of antifeedancy”. Briefly, considering the information available in literature on antifeedancy of plant extracts, the present study has shown that there is a wide scope for application of crude methanol extract of *A. paniculata* as larvicidal/ antifeedant agent in pest management programs.

In conclusion, an attempt has been made to evaluate the role of leaf extract of *A. paniculata* as larvicidal/ antifeedant agent in pest management programs. The results of the present study hold a promising possibility of further investigations of efficacy natural plant products of their biopesticidal properties.

**Authors' contributions:** *Dr. R. Jayakumararaj* (Assistant Professor) investigator, contributed in experiment design and corresponding author of manuscript; *Dr. N. Periyathambi* (Associate Professor), contributed in experiment design and preparation of the manuscript; *Dr. G. Sundarajan* (Assistant Professor) co-investigator for the project, performed some part of the experiment and provided the experimental animals; *Dr. SM. Sundarpandian* performed the final editing of the manuscript *Dr. M. Karthikeyan*, contributed in experiment design and provided plant material; *S. Ramya* (Researcher) performed some of the experiment; *K. Gopinath* (Researcher) performed some of the experiment.

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