Research Article

Biopesticidal Effect of Ethyl Acetate Leaf Extracts of *Datura metel* L. (Solanaceae) on the larvae of *Helicoverpa armigera* (Hübner)

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ABSTRACT

Plant extracts, especially botanical insecticides, are of interest because of their use in plant protection. In the present study, effects of solvent extracts of *Datura metel* L. were evaluated against larvae of gram pod-borer *Helicoverpa armigera* (Lepidoptera: Noctuidae). Preliminary screening was carried out with aqueous and other solvent extracts of field collected *D. metel* at a concentration of 1,000 ppm. Antifeedant activity of methanol crude, petroleum ether, methanol fraction and ethyl acetate fraction of leaf extracts of *D. metel* were determined to ascertain the active fraction. Larval mortality was observed after 24h of exposure to the plant extracts in different solvents. All the extracts exhibited significant antifeedant and larvicidal effects on the larvae, however, highest larval mortality was observed in ethyl acetate fraction (5.9, 19.3, 31.1, 38.5, 84.8 and 152.6) of leaf extract of *D.metel* against the I, II, III, IV, V and VI instar larvae of *H. armigera* respectively. Further, the most active ethyl acetate fraction of *D. metel* was used to estimate growth inhibition, larvicidal activity and malformation. Results suggest that ethyl acetate fractions of leaf extract of *D.metel* holds a significant potential to be used as bio-pesticide for the control of destructive polyphagous agricultural pest - *H. armigera.*

Keywords: *Helicoverpa armigera, Datura metel,* insecticides, Antifeedant, Larvicidal.

INTRODUCTION

Helicoverpa armigera (Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, Order: Lepidoptera, Family: Noctuidae) is a polyphagous pest that infests cotton, tomato, bhendi, chickpea, pigeonpea, chilli, maize, sorghum and many other crops, inflicting substantial crop loss every year[1,2]. However, colonization of new host by *H.armigera* induces selection of adaptive characters and genetic differentiation in population[3,4].

The ability of this insect pest to thrive on alternate host plants is an adaptive advantage for its survival. Polyphagy, high mobility, high fecundity and facultative diapauses are its key physiological, behavioral and ecological characteristics that facilitate survival even in ramshackle habitats[5]. Polyphagy entails physiological mechanisms in *H.armigera* to confront the varying chemical complexities posed by host plants. Behavioral adaptation and physiological processes are responsible for insect nutrition[6]. An understanding to genetic variation among *H.armigera* populations occurring on host plants has become essential from view point of their susceptibility to different insecticides[7]. Further, *H.armigera* is characterized by high mobility and fecundity that is responsible for its capacity to develop resistance to synthetic insecticides used in its management[8-10]. It has developed resistance against most of the modern classes of synthetic insecticides like DDT[11], pyrethroids[12], carbamates and organophosphate[13].

The versatility of this species due to genetic variability governing the behavior of *Harmigera*[14, 15] making it a menace on several crop species. On the other hand, botanicals have been used in the management of agricultural pest since time immemorial. Plant derived pesticides are eco-friendly, non-toxic to non target organisms, non persistent in nature, besides they are lees known to promote drug resistance[16]. Application of bio-pesticides has been reported to have positive impacts on the management of bollworm population[17]. 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.

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[18-20]. Studies have shown that *Acorus calamus, Annona squamosa, Vitex negundo*[21,22], *Andrographis paniculata*[23], *Gnidia glauca* and *Toddalia asiatica*[24] are effective in the management of *H. armigera*.

Datura metel Linn (Solanaceae) is widely used in phytomedicine to cure diseases. Several studies have documented the scientific basis for the efficacy of plants in phytomedicine[25]. Whole plant particularly the leaves are used as anesthetic, anodyne, anti-asthmatic, antispasmodic, bronchodilator, and hallucinogenic[26]. It is also used in the treatment of burns. The present study seeks to explore the possibilities of exploiting *D.metel* against *H. armigera.*

MATERIALS AND METHODS

Collection of plants

The plants were collected from the wild in Madurai District, Tamilnadu, India. Healthy plant materials were collected in poly bags and brought to lab and their botanical identity was established. The Flora of Presidency of Madras[27] and The Flora of Tamil Nadu Carnatic[28] were used for authentication of the plants.

Extraction of phytochemicals using different solvents

Leaves were 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 ethyl acetate as solvent in the ratio of 1:10 w/v using Soxhlet apparatus. The extract was filtered through a funnel using glass filter and evaporated using a rotary evaporator. The residue was redissolved in the solvent and defatted in equal volume of petroleum ether in a separating funnel. The fractions were separated, dried in a rotary evaporator and insoluble derbies were removed by filtration. Yields in relation to the initial weight of the powder of the different fractions were determined. 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.

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents present in *D.metel* leaf extract such as alkaloid, glycosides, terpenoids, steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

Test for Alkaloids (Meyer's Test)

The extract of *D. metel* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent[29]. Samples were observed for the presence of yellow precipitation.

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and concentrated sulphuric acid are added, and observed for reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer.

Test for Terpenoid and Steroid

Approximately, 4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids and green bluish colour for steroids.

Test for Flavonoids/ flavones

Approximately, 4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

Test for reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Triterpenes

Approximately, 300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 min. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

Test for Phenolic Compounds (Ferric chloride test)

Approximately, 300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

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. The percentage mortality was calculated after a period of 24 h.

Ethics Statement

H. armigera has not been notified under any act or laws and rules thereof of the Government of India as an endangered or threatened species restricting or regulating its collection and observation. No permits were required, for collecting the larvae from the field since *H. armigera* is not an endangered species affecting the biodiversity status.

Bioassay studies

Bioassay studies were carried out with different fractions of the leaf extracts against the larvae of *H. armigera.* The studies were conducted (24h) in the laboratory in transparent plastic containers of 4x2.5 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 6h starvation. Three replicates each of ten larvae were maintained for each treatment. The experiments were conducted at 27±1°C, 75% humidity and 14h dark period, 24h larval mortality was observed and the percentage mortalities were corrected using Abbott's formula[30]. Ethyl acetate fraction was tested for LD_{50} 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_{50} values was selected for further studies.

RESULTS AND DISCUSSION

Phytochemical studies

The results of the phytochemical analysis carried out for *D. metel* are presented in Table 1. The phytochemical tests indicate the presence of alkaloids, terpenoids, steroids, flavonoids, triterpenes, phenolic compounds and tannins. However, glycosides and reducing sugars were absent in *D.metel.*

Table 1: Phytoconstituents present in *Datura metel*

Test	Presence/ Absence
Alkaloids	+
Glycosides	-
Terpenoids and steroid	+
Flavonoids	+
Reducing sugars	-
Triterpenes	+
Phenolic compounds	+
Tannins	+

Estimation of LD_{50} value

The results of bioassay studies, i.e. LD_{50} value of the larvae of *H.armigera* in the crude methanol extracts, methanol fractions, petroleum ether fractions and ethyl acetate factions of *D.metel* is given in Table 2; Figure 1. Based on the LD_{50} values, the most active ethyl acetate fractions were identified and were selected for further study.

Table 2: Effect of phytochemical extracts of *D. metel* on larval instars of *H. armigera*

Extract	Larval instars of *Helicoverpa armigera*					
	I	II	III	IV	V	VI
Methanol crude	57.9 [c]	68.9 [c]	82.4 [c]	120.6 [c]	143.6 [c]	206.4 [c]
Petroleum ether	220.6 [d]	260.8 [d]	290.4 [d]	420.3 [d]	510.4 [d]	590.6 [d]
Methanol fraction	24.9 [b]	38.6 [b]	56.9 [b]	72.6 [b]	108.7 [b]	180.9 [b]
Ethyl acetate fraction	5.9 [a]	19.3 [a]	31.1 [a]	38.5 [a]	84.8 [a]	152.6 [a]

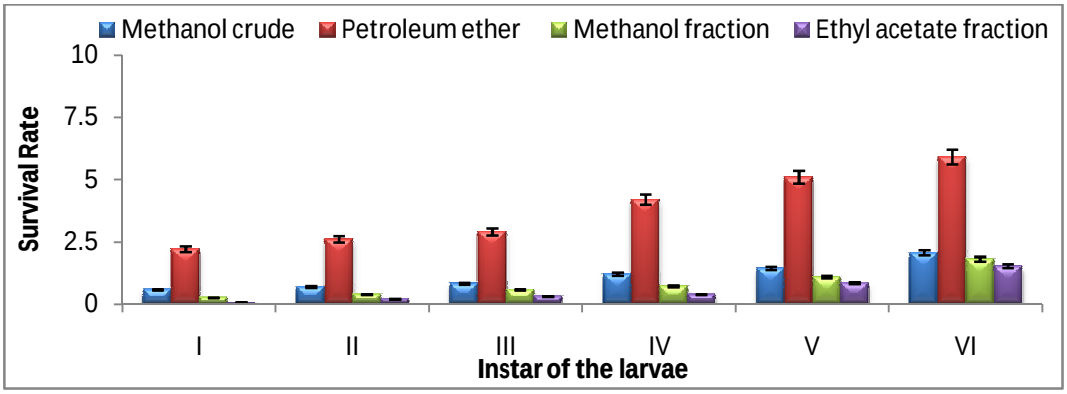

Figure 1: Effect of phytochemical extracts of *D. metel* on larval instars of *H. armigera*

Table 3: Effect of ethyl acetate fraction of *D. metel* on different larval instars of *H. armigera*

Larval Stage	ED_{50} (µg/cm^2)	Fiducial Limits		Slope	Intercepts	χ^2/df
		Upper	Lower			
I	5.99	0.97	0.80	1.790	3.600	3.840/3
II	19.32	7.39	5.34	1.170	3.484	0.605/3
III	31.09	6.47	6.98	0.530	2.700	0.517/3
IV	38.45	4.05	3.07	2.930	0.349	7.260/3
V	108.78	14.58	12.85	2.480	-0.060	1.200/3
VI	152.64	27.96	23.67	2.400	-0.240	1.043/3

Antifeedant activity

The most active ethyl acetate fractions of the selected plant extracts were tested for their antifeedancy against the larvae of *H.armigera.* Feeding inhibition was dose dependent, in addition the dose required for inciting feeding inhibition increased with the age of the larvae. Ethyl acetate fraction of *D.metel* (5.99 to 154.64 µg/cm^2) using the results obtained for the antifeedant activity of the most active fractions, ED_{50} values that is the effective dose at which 50% of the leaves are not feed on by the various stages of the larvae (Table 3).

Growth inhibition of the larvae

Administration of the most active ethyl acetate fractions of the plant extracts showed a significant inhibition of larval growth. Fully grown terminal larva (control) weighed around 890 mg. The weight of the larvae fed on the leaf disc treated with effective fraction showed a significant reduction in weight. The concentration of the dosage required and the number of doses required to effect growth inhibition in the larvae varied for various instars for the various fractions of *D.metel.*

Based on the results obtained for the inhibition of growth (weight of the larva) at various concentrations of the most active fractions, ED_{50} values were calculated along with the statistical analysis (Table 3). Further, administration of the active fractions induced genetic deformities during the larval stage, larval moulting pupation, pupal stage, adult emergence and neonatal stage. Malformed larvae could not successfully complete moulting and consequently the adult could not come out of the pupal case. Incidence of larval malformation increased from about 10% to 27.8 % in the *H. armigera* larva administered 2 µg/cm^2 dose of ethyl acetate faction of *D. metel*.

CONCLUSION

Plants produce a wide spectrum of allelochemicals[31]. Some of the compounds influence the feeding behavior of the insects and inhibit feeding; a few others drastically affect hormonal balance and there by inhibit growth, metamorphosis and reproduction. Currently there is resurgence of interest in plant derived compounds for developing eco friendly insecticides.

Polyphagous insects prefer nutritionally balanced food in terms of both macro and micronutrient constituents, but imbalanced food(s) can also satisfy their nutrient requirements[32]. When fed on different natural diets, *H.armigera* showed significant differences in terms of larval and pupal mass. Adjusting to deficiencies in diet is an energy demanding process and may sometimes prove to be fatal. The rate of insect mortality at a particular larval stage is indicative of failure of the insect's adaptability to a particular diet.

Differing nutrient levels in various host plants have been shown to affect overwintering in *H.armigera* pupae[33]. Late larval stages serve to acquire energy reserves for the maintenance of the adult insect form[34]. Significantly high mortality has been reported in early and late stages of growth and development in *H.armigera.* This has been attributed to their undeveloped mouthparts; neonates are susceptible to death if fed on diets that are difficult to chew[35].

During active development (IV and V instar), *H.armigera* exhibits low mortality on all diets, irrespective of the nutritive value. However, feeding is significantly reduced in the late fifth instar and is almost negligible in the sixth instar or pupation. Consequently, feeding on imbalanced diets is reflected in the survival rate of late larval and pupal stages. Overall, studies indicate that larvae not only try to complete their life cycle on nutritionally poor diets but also extend their larval period to gain nutrition for better survival. Results indicate that ethyl acetate fractions of *D. metel* have strong antifeedant, growth inhibitory, insecticidal activity.

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