



LARVICIDAL EFFECT OF *CATHARANTHUS ROSEUS* L (G) DON. LEAF EXTRACTS AGAINST THE LARVAE OF *HELICOVERPA ARMIGERA* (HUBNER)

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ABSTRACT

Use of biopesticides has gained prominence as potential plant protecting agents. Biological activity of aqueous extracts of *Catharanthus roseus* were evaluated against larvae of gram pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae). Screening for larvicidal activity with the aqueous leaf extracts at a concentration of 1000 ppm revealed that the larvicidal activity invariably increased with the stage of larval development. Larval mortality was observed after 24 hrs of exposure to the extracts. All extracts exhibited moderate larvicidal effects (2.60, 3.94, 7.75, 30.83, 38.53 and 63.34) towards I, II, III, IV, V and VI instar of *H. armigera* larvae. The results suggest that aqueous leaf extract of *C. roseus* holds a potential to be used as bio-pesticide for the management of *H. armigera*.

Keywords: *Helicoverpa armigera*, *Catharanthus roseus*, Biopesticide, Larvicidal.

INTRODUCTION

India is basically an agro-based country; more than 80% 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. *Helicoverpa armigera* belongs to the family Noctuidae and act as a serious pest in most of the agro ecosystem. It inhabits most areas between the latitudes of 40 degrees North, and 40 degrees South (Fitt, 1991). It is a serious pest in India and many parts of the world, including Africa, Australia, China, India and Pakistan (Reed and Pawar, 1982; Sharma, 2001; Liu *et al.*, 2004; Talekar *et al.*, 2006). The species is highly polyphagous; eggs and or larvae have been recorded on more than 60 plant species belonging to 47 families. *H. armigera* is recorded as a pest of virtually all field and horticultural crops, being a major pest of maize, sorghum, tomato, lucerne, tobacco, cotton and cowpea. McGahan *et al.* (1991) have reported that the direct damage to flowering and fruiting structures by larvae, and extensive insecticide spraying result in low yields and high control costs. Increased resistance to insecticides in *H. armigera* has led to a renewed interest in developing alternatives to insecticidal control, such as behavioral control and the development of resistant genotypes (Wilson *et al.*, 1998).

The ability of populations of *H. armigera* to persist in agricultural areas and seemingly to adapt to changes in agricultural practices taking place in its environment, is one

of the major factors contributing to the pest status of this moth (Fitt, 1989). Intra-specific variation in host selection behavior, among as well as within populations, is an important element in the dynamics and persistence of insect populations (Via, 1987). Determining the extent and nature of this variation in *H. armigera* is essential for the design and implementation of effective pest management strategies, which are based on our understanding of the host selection behavior of this moth. 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.

Since, *H. armigera* can survive on alternate host it is characterized by high mobility and fecundity. Different plants vary in their suitability as a breeding ground for *Heliothine* larvae, and therefore they also vary in their attractiveness to females as an oviposition site (Fitt, 1991). This is because the fitness and survivorship of the offspring may depend on the environment in which they develop (Liu *et al.*, 2004). Some females will deposit small batches of eggs on many different plants, (especially when host distribution is patchy), to maximize her chances of having successful offspring (Fitt, 1991).

Interestingly, differences in egg load influenced the choosiness of female moths. The larger the egg load, the more likely the female would oviposit on less favorable and

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less suitable hosts. *H. armigera* display a hierarchy of plants that they prefer (Jallow and Zalucki, 1998), and it is likely that a trade-off occurs between host availability and suitability (Fitt, 1991). Upon hatching, the young larvae are relatively restricted in their movement, and therefore unable to exercise choice of the feeding site (Fitt, 1991); the host plant is chosen solely by the adult.

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). Further, it has been reported to develop resistance to synthetic insecticides used in its management (Ramasubramaniam and Regupathy, 2004). On the other hand, botanicals have been used in the management of agricultural pest since time immemorial (Ramya and Jayakumararaj, 2009).

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 (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).

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; Jaglan *et al.*, 1997; Koul *et al.*, 2000). 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*.

Recently, it has been reported that switching of the host plant by *H. armigera* larvae significantly affects feeding behavior. Further, leaf phyllosphere bacterial composition determines resistance to the host plant, indicating that the feeding habit is significantly influenced by the phyllosphere microbial composition of the crop plant. Thus both the host plant, as well as the plant's geographical location significantly affect the gut bacterial population of the insect and determine host preference (Gayatri Priya *et al.*, 2012).

Catharanthus roseus L (G) Don. (Madagascar periwinkle) belongs to the family Apocynaceae. Pharmacological studies have revealed that *C. roseus*

contains more than 70 different types of alkaloids. Furthermore, *in vitro* studies have shown that this plant produces large number of alkaloids upon elicitation (Verpoorte *et al.*, 2002). With this background, in the present study the pesticidal effect of leaf extracts of *C. roseus* has been evaluated against the larvae of *H. armigera*.

MATERIALS AND METHODS

Collection of plants

C. roseus was collected from the wild in Vellore 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. The Flora of Presidency of Madras (Gamble, 1993) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) 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 *C. roseus*. The percentage mortality was calculated after a period of 24 h.

Ethics Statement

Helicoverpa 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 *C. roseus* 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 14h 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 *C. roseus* was tested for LD₅₀ 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 LD₅₀ values was selected for further studies.

RESULTS

The results of bioassay studies against the larvae of *H. armigera* in the aqueous, crude extracts, methanol fractions, petroleum ether fractions and ethyl acetate fractions of *C. roseus* revealed that the LD₅₀ values for the individual fractions of plant extracts varied significantly with the solvent system used for extraction of the phytochemicals from the selected plant source. The least LD₅₀ values ranged from 2.6 to 63.34 $\mu\text{g}/\text{cm}^2$ for I to VI instars larvae in the aqueous extracts of leaves of *C. roseus* (Table 1). The mortality rate was observed in the decreasing order of aqueous > ethyl acetate fraction > methanol fraction > methanol crude > petroleum ether.

The aqueous leaf extract of *C. roseus* was found to be more active than other fractions tested. Therefore, aqueous leaf extract of *C. roseus* were used to determine the ED₅₀ values for their effect on the larvae of *H. armigera*. The ED₅₀ values and its corresponding fiducial limits along with slope and intercept are given in Table 2. However, it was

observed that the LD₅₀ values were significant at $P < 0.05$.

DISCUSSION

Plants produce a wide spectrum of phytochemicals that specifically inhibit growth, morphogenesis, metamorphosis and reproduction (Ahmad, 2007). Currently there is resurgence of interest in plant derived compounds for developing them commercially as ecofriendly insecticides. Jacobson and Crosby (1971) pointed out the use of plants as promising source for the development of new insecticides. Despite, the fact that hundreds of tropical plants are reported to possess insecticidal property, only few compounds have been commercialized. For successful exploitation of natural insecticidal compounds, screening for their behavioral and physiological effects in polyphagous insects with an understanding of structure activity relationship is essential. Unfortunately, many do not provided estimates of critical lethal (LD₅₀) or critical effective dose (ED₅₀) 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 ED₅₀) 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.*, 1995).

The bioactivity of tested phytochemical extracts varied significantly with solvents used for the extraction and instar stage of the larvae. Reviewing the prospects of antifeedant for the management of pests, 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 ethylacetate fraction of *C. roseus* as larvicidal/ antifeedant agent in integrated pest management programs.

Table 1. Effect of phytochemical extracts of *C. roseus* on *H. armigera* larvae.

Extract	Larval instars of <i>Helicoverpa armigera</i>					
	I	II	III	IV	V	VI
Aqueous	2.60	3.94	7.75	30.83	38.53	63.34
Methanol crude	46.9	52.4	66.4	99.4	138.6	180.9
Petroleum ether	160.4	210.6	290.6	380.7	420.7	510.6
Methanol fraction	10.6	12.7	26.9	59.4	68.3	106.7
Ethyl acetate fraction	4.1	4.1	17.4	42.2	55.6	84.5

Table 2. Larvicidal effect of aqueous of *C. roseus* on the larvae of *H. armigera*.

Larval Instars	ED ₅₀ (µg/cm ²)	Fiducial Limits		Slope	Intercepts	χ^2/df
		Upper	Lower			
I	2.60	0.36	0.32	2.040	4.150	6.670/4
II	3.94	0.43	0.36	1.470	4.570	7.140/4
III	7.75	1.06	0.94	2.020	3.190	7.180/4
IV	30.83	4.69	4.08	1.890	2.170	4.240/3
V	38.53	26.42	15.68	2.030	1.760	17.020/3
VI	63.34	5.47	5.03	3.740	1.740	1.450/2

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