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**Research Article**

# Larvicidal effect of *Catharanthus roseus* L (G) Don. aqueous leaf extracts on the larvae of *Helicoverpa armigera* (Hübner)

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**Abstract**

Use of biopesticides has gained prominence as potential plant protecting agents. Biological activity of aqueous extracts of *Catharanthus roseus* L (G) Don. 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 1,000 ppm revealed that the larvicidal activity invariably increased with the stage of larval development. Larval mortality was observed after 24h 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 the 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 and Larvicidal

## Introduction

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. 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 phyto-chemicals 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 the host plant by *H. armigera* larvae significantly affects feeding behavior. Further, the

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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 affects 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* contain more than 70 different types of alkaloids. Furthermore, *in vitro* studies have shown that this plant produces a 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* were collected from the wild in Madurai District, TN, India. Selection of plants was made on the basis of absence of damage by the insect-pest. A healthy plant materials were collected in poly bags and brought to the 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**

The leaves were collected, washed thoroughly in water, air dried in the 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 a 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 the 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.

**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 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 six hour 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. 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 $LD_{50}$ values against the larval stages of *H. armigera.* Mortality was observed after the completion of the larval stages. The fraction which showed a high rate of mortality in the least $LD_{50}$ 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 factions of *C. roseus* revealed that the $LD_{50}$ 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 $LD_{50}$ values ranged from 2.6 to 63.34 µg/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 $ED_{50}$ values for their effect on the larvae of *H. armigera*. The $ED_{50}$ values and its corresponding fiducial limits along with slope and intercept are given in Table - 2. However, it was observed that the $LD_{50}$ 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 a 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 a promising source for the

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

| Extract | Larval instars of *Helicoverpa armigera* | | | | | |
|---|---|---|---|---|---|---|
| | 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 | $\chi^2$/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 |

development of new insecticides. Despite, the fact that hundreds of tropical plants are reported to possess insecticidal property, only a 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 provide estimates of critical lethal ($LD_{50}$) or critical effective dose ($ED_{50}$) 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 $ED_{50}$) 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 histerophorus* 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 testing 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, Jermy (1990) and Ahmad (2007) reported that plant extracts/compounds "with combined behavioral and toxic effect are more likely to a 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 ethylaceteate fraction of *C. roseus* as larvicidal/ antifeedant agent in integrated pest management programs.

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