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Biopesticidal Effect of Leaf Extracts of *Catharanthus roseus* L (G) Don. on the Larvae of Gram Pod Borer - *Helicoverpa armigera* (Hübner)

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ABSTRACT

Biopesticides have gained prominence as potential plant protecting agents. Biological activity of solvent extracts of *Catharanthus roseus* L (G) Don. were evaluated against 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 *C. roseus* 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 to the extracts. All extracts exhibited moderate larvicidal effects. However, highest larval mortality was observed in ethyl acetate fraction (4.1, 4.1, 17.4, 42.2, 55.6 and 84.5) of leaf extract of *C. roseus*, followed by methanol fraction (10.6, 12.7, 26.9, 59.4, 68.3 and 106.7) against the I, II, III, IV, V and VI instar larvae of *H. armigera* respectively. Further, the most active ethyl acetate fraction of *C. roseus* was used to estimate larvicidal activity. The results suggest that ethyl acetate fractions of leaf extract of *C. roseus* holds a potential to be used as bio-pesticide for the control of destructive polyphagous agricultural pest - *H. armigera*.

KEYWORDS: *Helicoverpa armigera; Catharanthus roseus;* Biopesticide; Antifeedant.

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. 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 Hübner (Lepidoptera: Noctuidae) is a polyphagous pest that infests cotton, tomato, bhendi, chickpea, pigeonpea, chilli, maize, sorghum and many other crops, inflicting substantial crop losses every year (Reed and Pawar, 1982; Manjunath *et al.*, 1989; Sharma, 2001; Talekar *et al*., 2006). However, colonization of

new host by *H. armigera* induces selection of adaptive characters and genetic differentiation in population (Rice, 1987; Diehl and Bush, 1989). 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). 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).

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 lees known to promote drug resistance (Liu *et al*., 2000). 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).

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; 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*. 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*.

C. roseus (Madagascar periwinkle) belongs to the family Apocynaceae. Pharmacological studies have revealed that *C. roseus* contains more than 70 different types of alkaloids (indole alklaloids) and chemotherapeutic agents (Verpoorte, 1998). Also, *in vitro* studies have shown that this plant produces large number of alkaloids upon elicitation (Verpoorte et al., 2002). The enormity of work conducted on this medicinal plant is so large that since 1950s more than 2500 publications have come in, ironically, only handful of data is available with regard to its bio-pesticidal potential.

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 . 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.

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 , 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 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 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. The least LD_{50} values ranged from 4.14 to 84.54µg/cm^2 for I to VI instars larvae in the ethyl acetate extracts of leaves of *C. roseus* (Table 1). The mortality rate was observed in the decreasing order of ethyl acetate fraction > methanol fraction > methanol crude > petroleum ether.

The ethyl acetate extracts of *C. roseus* was found to be more active than other fractions tested. Therefore, ethyl acetate extracts 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 significantly different at $P<0.05$, LSD: 6.334.

DISCUSSION

Plants produce a wide spectrum of allelochemicals, however, many of such chemicals have not been explored for

their physiological significance (Norduland and Sauls, 1981). These phytochemicals 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. 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 (Azadirachtin) have been commercialized (Chopra *et al.,* 1994). 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_{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 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, 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 ethylaceteate fraction of *C. roseus* as larvicidal/ antifeedant agent in integrated pest management programs.

References

Abbott WS (1925) A method for computing the effectiveness of an insecticide *J Econ Ent* 18:265-267.

Ahmad M (2007) Insecticide resistance mechanisms and their Management in Helicoverpa armigera (Hübner) - A review J Agric Res 45(4):319-335.

Cabanillas HE and Raulston JR (1995) Impact of Steinernema riobravis (Rhabditida: Steinernematidae) on the control of Helicoverpa zea (Lepidoptera: Noctuidae) in corn J Econ Entomol 88:58-64.

Chopra RN, Badhwar R and Ghosh S (1994) Poisonous Plants of India ICAR ND, India.

Choudhary RK, Veda OP and Mandloi KC (2001) Use of Neem, Azadirechta indica and garlic, Allium sativum in management of bollworms, Helicoverpa armigera in cotton In: Proc 88th Session of Indian Sci Cong Agric Sci, ND. pp 40-42.

Dodia DA, Patel IS and Pathak AR (1995) Antifeedant properties of some indigenous plant extracts against larvae of Helicoverpa armigera Pestol 19:21-22.

Gamble JS (1993). Flora of the Presidency of Madras. Vol I-III. Bishen Singh Mahendra Pal Singh. Dehra Dun-India.

Ge F and Ding Y (1996) The population energy dynamics of predacious natural enemies and their pest control activity in different cotton agro-ecosystems. Acta Entomol Sin 39:266-273.

Jacobson (1982) The potential role of natural product chemistry research in Heliothis management. In: Proc. Internt.

Patacheru, Andrapradesh, India 233-240.

Jacobson M and Crosby DG (1971). Naturally occurring insecticide (Ed Marcel and Dekker) pp 212-219.

Jaglan MS, Khokhar KS, Malik MS and Singh R (1997) Evaluation of Neem (Azadirachta indica A. Juss) extracts against American bollworm, Helicoverpa armigera (Hubner) J Agri Food Chem 45:3262-3268.

Janardhan RS, Chitra KC, Kameswara RP and Subramaniyam RK (1999) Antifeedant and insecticidal properties of certain plant extracts against Helicoverpa armigera J Insect Sci, 5:163-164.

Koul O (1985) Azadirachtin interaction with development of Helicoverpa armigera Fab Indian J Expt Biol 23:160-163.

Koul O, Jain MP and Sharma VK (2000) Growth inhibitory and antifeedant activity of extracts from Melia dubia to Spodoptera litura and Helicoverpa armigera larvae. Indian J Exp Biol 38(1):63-68.

Liu SQ, Shi JJ, Cao H, Jia FB, Liu XQ and Shi GL (2000) Survey of pesticidal component in plant In: Entomology in China in 21st Century, In: Proceedings of Conference of Chinese Entomological Society (Ed: Dianmo) Li Beijing, China: Science & Technique Press. pp 1098-1104.

Manjunath TM, Bhattnagar VS, Pawer CS and Sidhanantham S (1985) Economic importance of Heliothis armigera (Hubner) in India an assessment of their natural enemies and host plants In: Proc. Workshop on Biological control of Heliothis armigera ND, India.

Mathew KW (1985). The Flora of Tamil Nadu Carnatic, The Rapinant Herbarium, St. Josephs College, Tiruchirapalli, India.

Michael AF and Donald CS (1996) Inundative biological control of Helicoverpa zea (Lepidoptera: Noctuidae) with the entomopathogenic nematode Steinernema riobravis (Rhabditida: Steinernematidae) Biol Control 7:38-43.

Murugan K and Babu R (1998) Impact of certain plant products and Bacillus thurengiensis Berliner sub sp. kurstaki on the growth and feeding physiology of Helicoverpa armigera (Hubner) JSIR 57:757-765.

Murugan K, Sivaramakrishnan S, Senthilkumar N, Jayabalan D and Nathan SS (1998) Synergistic interaction of botanicals and Biocides Nuclear polyhedrosis virus on pest control JSIR 57:732-739.

Norduland DA and Sauls GE (1981) Kairomones and their use for the management of entomophagous insects J Gen Ecol 7:1057-1061.

Ramasubramaniam T, Regupathy A. 2004. Pattern of cross resistance in pyrethroid selected populations of Helicoverpa armigera from India. J. Appl. Ent. 128: 583-587.

Ramya S, Rajasekaran C, Sundararajan G, Alaguchamy N and Jayakumararaj R (2008) Antifeedant Activity of Leaf Aqueous Extracts of Selected Medicinal Plants on VI instar larva of Helicoverpa armigera (Hübner) Ethnobotanical Leaflets 12: 938-43.

Reed W and Pawar CS. (1982). Heliothis: a global problem. In: Reed W, Kumble V, editors. Proceedings of International Workshop on Heliothis Management Problem: 9–14. ICRISAT, Patancheru, India.

Roush RT and Tabashnik BE (1990) Pesticide resistance in arthropods. Chapman and Hall, NY.

Sharma HC. 2001. Cotton bollworm/legume pod borer, Helicoverpa armigera (Hübner) (Noctuidae: Lepidoptera): biology and management. Crop protection compendium. ICRISAT, Patancheru, India.

Simmonds MSJ, Blaney WM and Fellows FE (1990) Behavioral and electro-physiological study of antifeedant mechanisms associated with polyhydroxy alkaloids. J Chem Ecol 16:3167-3196.

Sundararajan G and Kumuthakalavalli R (2001) Antifeedant activity of aqueous extract of Gnidia glauca Gilg. and Toddalia asiatica Lam. on the gram pod borer, Helicoverpa armigera (Hubner). J Environ Biol 22(1):11-14.

Talekar NS, Opena RT and Hanson P (2006) Helicoverpa armigera management: a review of AVRDC's research on host plant resistance in tomato Crop Protect 5:461-467.

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

Extract	Larval instars of *Helicoverpa armigera*					
	I	II	III	IV	V	VI
Methanol crude	46.9[c]	52.4 [c]	66.4 [c]	99.4 [c]	138.6 [c]	180.9 [c]
Petroleum ether	160.4[d]	210.6 [d]	290.6 [d]	380.7[d]	420.7 [d]	510.6 [d]
Methanol fraction	10.6[b]	12.7[b]	26.9 [b]	59.4 [b]	68.3[b]	106.7 [b]
Ethyl acetate fraction	4.1 [a]	4.1 [a]	17.4 [a]	42.2 [a]	55.6 [a]	84.5 [a]

Table 2. Larvicidal effect of ethyl acetate fractions of *C. roseus* on the larvae of *H. armigera*.

Larval Instars	ED_{50} ($\mu g/cm^2$)	Fiducial Limits		Slope	Intercepts	χ^2/df
		Upper	Lower			
I	4.14	0.58	0.50	1.959	3.780	1.890/4
II	4.17	0.59	0.51	2.000	3.570	2.490/4
III	17.36	2.75	2.38	1.990	2.530	7.630/4
IV	42.16	7.97	6.70	1.830	2.020	1.120/3
V	55.63	10.30	8.63	1.840	1.780	3.470/3
VI	84.54	9.32	8.40	3.120	-1.020	0.480/2