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Effect of *Argemone mexicana* Leaves Extract at Different Solvents on Gut of *Heliothis armigera* (Hub)

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ABSTRACT- Present investigations evaluated the effect of *Argemone mexicana* leaves extract on the gut of *Heliothis armigera* (Hub.) at different solvents. The effect of leaf extract of *A. mexicana* in ethanol and acetone solvent after 24 and 96 hours of treatment with *H. armigera* shows severity of the damage of epithelial lining, epithelial cells showed vacuoles at certain places. The gut lining was also found to be damaged and the lumen became wider after the effect of ethanol extract of *A. mexicana*. In acetone extract of *A. mexicana*, the thickness of the fore gut wall has been increased due to clumping of the tissue and hence the diameter of the foregut was reduced. The lumen therefore became narrower and columnar epithelial cells showed the vacuoles.

Key-words: *Heliothis armigera*, Argemone *mexicana*, Ethanol, acetone, Epithelial lining, Epithelial cells, vacuoles, Gut lining, Gut wall

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INTRODUCTION

Heliothis armigera is a cosmopolitan, polyphagous insect causing serious damage to cultivated crops in India such as cotton, tomato, chickpea, pigeon pea, maize, sorghum etc. It is distributed throughout the India and is reported from Andhra Pradesh, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, New Delhi, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal etc. Chemical pesticides are commonly used to protect crops from the *H. armigera* infestation, however chemical pesticides are very toxic to us and domestic animals and as they have high residual values, they are not suitable to spray on the vegetables and fruits which are to be harvested after short period of spray.

The plant *Argemone mexicana* contains many alkaloids [1,2] and was found to possess larvicidal and growth inhibiting activity against the second instar larvae of *Aedes aegypti*.

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These extracts have also been shown to induce behavioral and morphological modifications in the larvae of *A. aegypti*. Researchers have also reported the larvicidal and chemosterilant activity of phytochemicals derived from *A. mexicana* seeds against *A. aegypti* [3].

Weeds, the so-called nuisance plants, belong to one such group, which has attracted researchers' attention as eco-friendly substitutes to chemical insecticides for the mosquito management [4]. The larvicidal potential of the extracts prepared from the different parts of the Mexican prickly poppy, *Argemone mexicana*, has been reported against the early fourth instars of *A. aegypti* [5].

MATERIALS AND METHODS

The larvae of *Heliothis armigera* were collected from the field of tur (*Cajanus cajan*), tomato (*Lycopersicon esculentum*) and gram (*Cicer arietinum*) etc. near Aurangabad, India. Each larva was reared in different plastic bottles in the Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India in 2010 on artificial diet to avoid cannibalism and food was changed every day (http://www.cicr.gov.in).

Fresh leaves of *A. mexicana* was collected from the field near Aurangabad and were dried in the shade and then into the oven at 55^0C. The dried leaves were powdered in the grinder and powder was stored in the airtight polyethylene

bags. The powder was packed in filter paper and extract was extracted with acetone and ethanol in Soxhlet apparatus in 1:10 ratio i.e. 10 gm powder in 100 ml solvent. After eight hours of continuous extraction the final extract was kept open to evaporate the solvent and obtained extract was stored at 4ºC in a refrigerator until use. The artificial diet prepared to be mixed with each of the extract so as to prepare various concentrations of the extract and was poured in the vials. 10 vials for each concentration of each extract were prepared and the larvae were released one in each vial. There is cannibalism in larvae of *H. armigera* and hence one larva was reared in each vial. Ten larvae one in each vial with food without extract was allowed to grow as control.

Mortality of the larvae was recorded in four days. The mortality data in the extracts showing considerable toxic effect on the larvae was used to calculate the LD_{10} and LD_{50} values.

Calculation of regression equation for LD_{10} and LD_{50} values of *H. armigera* after the treatment of acetone and ethanol extract of *A. mexicana* for 24 and 96 hrs.

After 24 and 96 hours of exposure, the larvae were dissected in 0.6 % saline and the fore gut was fixed in the Bouin's fluid for 24 hours. The tissues were washed and processed as usual and sections of 5 micron thickness were affixed on the slide. The sections were stained by Mallory's triple stain.

RESULTS

Table 1: comparison of LD_{10} and LD_{50} values of leaf extracts of *A. mexicana* to *H. armigera*

Name of plant and Solvent	Time of exposure	Regression equation $Y = \bar{y} + b(x-\bar{x})$	LD_{10} value (ml/Kg)	LD_{50} value (ml/Kg)
Leaf extract of *A. mexicana* in ethanol	24	Y = 4.8633x + 2.7013	2.618	2.969
	96	Y = 2.3775x + 1.9715	1.842	1.878
Leaf extract of *A. mexicana* in acetone	24	Y = 4.4982x + 2.9413	1.488	2.868
	96	Y = 1.7098x + 3.1430	0.4608	1.219

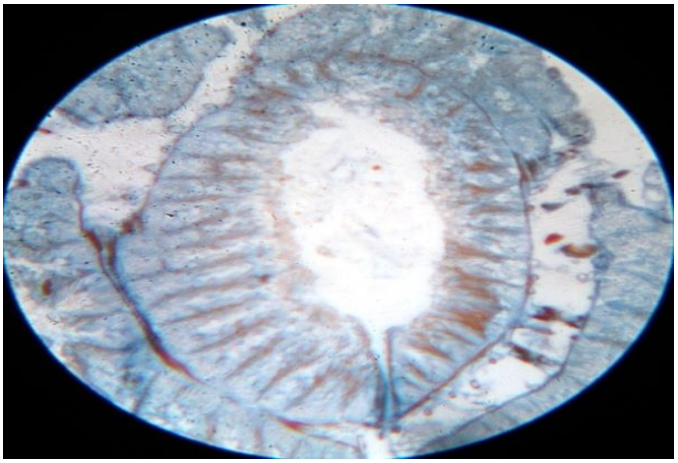

Fig 1: Normal histological structure of the fore gut

Fig. 1 shows the normal histological structure of the fore gut of the larva of *H. armigera.* The fore gut consists of six layers, the innermost lining towards the lumen is cuticular layer outside of which is the single layered columnar epithelium. The columnar epithelial cells are comparatively very tall. The outer side of the epithelium rests on thin basement membrane. The epithelium consists of some goblet cells, which secretes the mucopolysaccharides. Outer side of the basement membrane is surrounded by the circular muscles and then the longitudinal muscle fibers. Outer most layer is the peritoneal membrane. The fore gut on its outer side shows the secretary glands which may be the salivary glands.

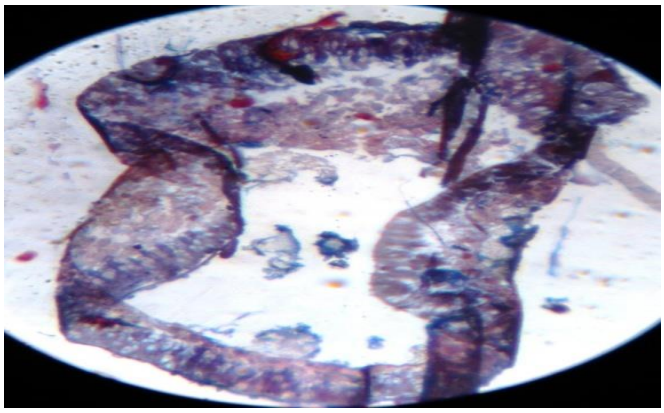

Fig. 2 : Effect of ethanol extract at 24 hrs

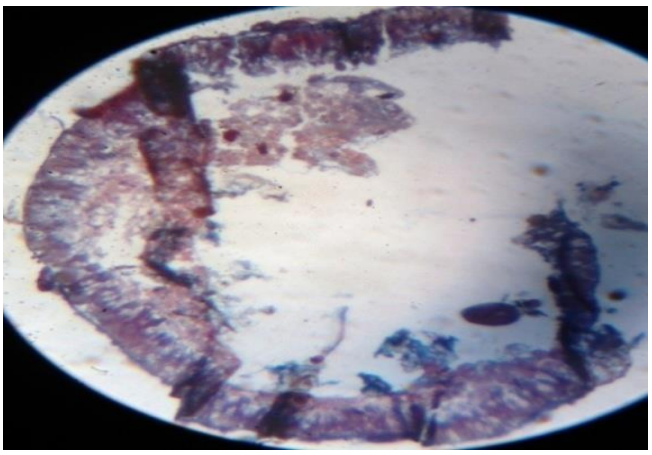

Fig. 3: Effect of ethanol extract at 96 hrs

Fig. 2 and 3 shows the effect of ethanol extract of *A. mexicana* after 24 and 96 hours of treatment. The severity of the damage of epithelial lining is more after 96 hours as compared to that of 24 hours, epithelial cells show vacuoles at certain places. Cuticular lining is also found to be damaged and the lumen became wider.

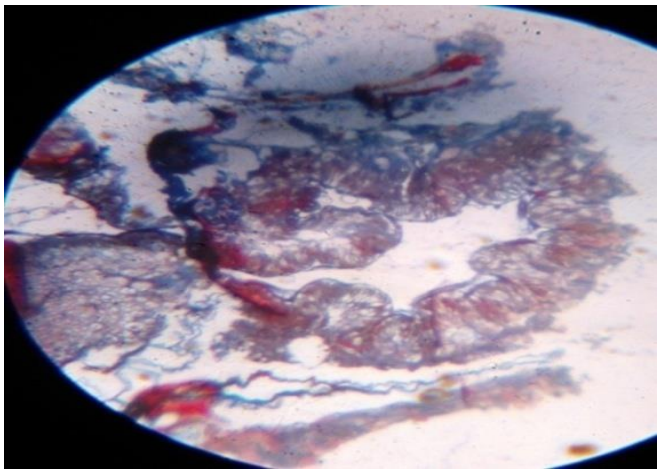

Fig. 4: Effect of acetone extract at 24 hrs

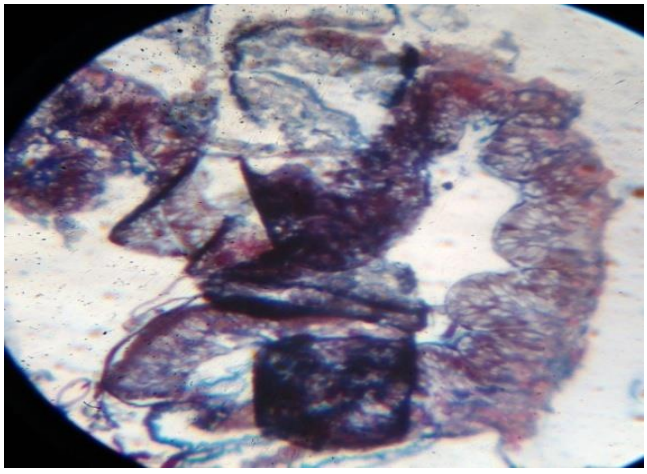

Fig. 5: Effect of acetone extract at 96 hrs

Fig. 4 and 5 shows the effect of acetone extract of *A. mexicana* after 24 and 96 hours of treatment. The thickness of the wall has been increased due to clumping of the tissue and hence the diameter of the foregut was reduced. The lumen therefore became narrower. The columnar epithelial cells show the vacuoles.

DISCUSSION

Effect of *A. mexicana* alone as well as its synergy with other plants revealed less pupation at moderate doses, when applied alone, where as its efficacy was more at lower dose when applied in combination with *Dononae aangustifolia*, *Monactis dubia* and *Castanae dentata*. Similar results were recorded in *H. armigera* with the *Eucalyptus* ground leaf powder (2 %) with significant reduction in population (12 %) and mean larval weight [6]. The insect pests have developed resistance to a variety of insecticides due to the indiscriminate use of chemical pesticides. Insecticides affect the non-target organisms and human beings, directly or indirectly. Plant extracts or pure compounds manifest their effect on insects in several ways, including toxicity, mortality, antifeedancy, growth inhibition, suppression of reproductive behavior and reduction of fecundity and fertility [7].

Ethyl acetate leaf extract of *A. tagala* at 5% showed higher larval mortality of 40.66%. These results support the earlier findings of Jbilou *et al.* [7], who observed potential insecticidal agents for the control of the larvae of *Anticarsia gemmatalis* in acetone and ethanol extracts of *A. pubescens*. *A. albida* plant had acidic metabolites like aristolic acid, aristolochic acid, aristoloctam and aristolone, which exhibited larval mortality against *S. zeamais* [8]. Elumalai *et al.* [9] reported that ethyl acetate leaf extract of *Acorus calamus* at 5.0% exhibited maximum larvicidal activity of 40.24% against *S. litura*. The root extract of *Tageteserecta* proved more toxic to the lesser grain borer and red flour beetle than malathion [10]. *A. mexicana* is a source of medicines as it is a reservoir of chemical agents with therapeutic properties. It provides a good source of anti-infective agents, for example, emetine, quinine and berberine, which still remain to be highly effective in the fight against microbial infections. Various publications have documented the antimicrobial activity of plant extracts [11-13].

CONCLUSIONS

The severity of damage of epithelial lining was more in larvae of 96 hours of exposure as compared to those of 24 hours, epithelial cells showed vacuoles at certain places. The gut lining was also found to be damaged and the lumen became wider after the effect of ethanol extract of *A. mexicana*.

In acetone extract of *A. mexicana* after 24 and 96 hours of treatment, the thickness of the fore gut wall has been increased due to clumping of the tissue and hence the diameter of the foregut was reduced. The lumen therefore became narrower. The columnar epithelial cells showed the vacuoles.

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