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BIOEFFICACY OF *Argemone mexicana* AGAINST AMERICAN BOLLWORM, *Helicoverpa armigera* (HUBNER) (NOCTUIDAE: LEPIDOPTERA)

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ABSTRACT: *Argemone mexicana* is a medicinal plant predominantly present in India. The insecticidal activity of crude extracts and different fractions of the same were investigated against *Helicoverpa armigera*. Pupation was the least (3.33 %) in acetone extract followed by 10 per cent in the chloroform extract. Least pupal weight and maximum malformed adults were observed in chloroform extract. The adult life span was reduced in petroleum ether and water extracts. Fecundity was meager in petroleum ether extract, while its solvent curtailed the hatchability. Among the acetone fractions, the first and fourth fractions resulted in nil pupation. Larval prolongation, reduced pupal weight and malformed moth emergences were also observed.

Keywords: *Argemone mexicana*, *Helicoverpa armigera*, pupation, fecundity

INTRODUCTION

American bollworm, *Helicoverpa armigera,* a polyphagous noctuid, feeds on a large number of plant species. In Tamil Nadu, the pest is wide spread in summer cotton tract often reaching high populations and during 'rabi', the insect is found in serious proportions in cotton as well as on pigeon pea, chickpea, groundnut, sunflower, bhendi etc. causing extensive damage (Duraimurugan and Regupathy, 2005). The repetitive use of synthetic chemicals for several decades to manage this pest resulted in resurgence and outbreak, resistance to insecticides, elimination of existing natural enemies and polluted soil, water, air and food (Mehta *et al.,* 1992; Patel *et al.,* 1992).

Hence, search for viable and sustainable alternatives to synthetic pesticides is given priority. Novel natural substances derived from higher plants are preferred over others due to their environmental safety (Arnason *et al*., 1989). Insecticidal activity of many plants against *Helicoverpa armigera* were reflected in *Ocimum basilicum* (Pandey *et al*., 1983), *Tribulus terrestris* (Gunasekaran and Chelliah, 1985), *Anona squamosa* (Ganeshan *et al.,* 1995), *Melia dubia* (Koul *et al*., 2000), *Cicer judaicum* (Simmonds and Stevenson, 2001), *Trichilia pallida* (Simmonds *et al*., 2001), *Lippia alba* (Tripathi, 2002) and *Tetradium daniellii* (Stevenson *et al*., 2003). Thus, in the present investigation, a less explored potential botanical, *Argemone mexicana* available commonly was screened for its biopesticidal activity against *H. armigera* under laboratory conditions.

MATERIALS AND METHODS

H. armigera larvae were collected from the fields of lady's finger, cotton and chickpea from Kannivadi in Dindigul district. The larvae collected from various hosts were maintained in the laboratory at 22 ± 2°C and 70 – 75 % RH. The larvae were reared on semi-synthetic diet (Shorey and Hale, 1965; Sathiah, 1987) in individual containers to prevent cannibalism and contamination. Leaves of *A. mexicana* were collected from different parts of Thirukazhukundram and Kannivadi, Tamil Nadu.

Extraction of plant material : The leaves of *A. mexicana* collected from Kannivadi, Tamil Nadu were shade dried and powdered. One kg each of powdered leaves was extracted successively using non-polar to polar solvents viz., hexane, petroleum ether, chloroform and acetone. In each solvent the plant material was soaked for 24 h at 30 ± 2°C, filtered and to the residue the same solvent was added.

This procedure was repeated thrice to obtain maximum extractables. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator (Harborne, 1998) at 190 rpm/min (the temperature varies between extracts viz., 40-60°C for petroleum ether, 60-62°C for chloroform and acetone, 66-70°C for hexane). Aqueous extraction was also done. One kg of fresh leaves was macerated in a blender by adding 5 litres of autoclaved distilled water and squeezed with cheese cloth. It was filtered with 595-Bogen sheets twice and later with vacuum filter (15 Hgs vac). The extracts were lyophilized in 100 ml slots at 55° C (26 lbs) for 18 h per slot. Liquid nitrogen was added to this and freezed at –196° C. The crude extracts were measured and used in desired concentrations for bioassay.

Growth inhibition of larvae : The third instar larvae of *H. armigera* were bioassayed using 1) normal diet + extract (10% concentration), 2) normal diet + solvent and 3) normal diet (control). Pupation (%), pupal weight (mg) and malformed moth emergence (%) were recorded. Ten larvae were used per replication and totally three replications were maintained for each treatment.

Adult longevity, fecundity and egg hatchability of *H. armigera* : The adults of the previous (larvae 1st generation) study from the respective treatments, if any, were tested further. Ten per cent solution of hexane, petroleum ether, chloroform, acetone and water extracts of *A. mexicana* were made in the sugar solution with the respective solvents. Few drops of Tween 80 were added to mix the extracts/solvents thoroughly in the sugar solution, fed to the adult moths, and the longevity, fecundity and hatchability were checked. Solvent control (10 %) and 10 % sugar solution (normal control) were also maintained. After confirming the feeding by the adults only they were released into the oviposition chamber for further observations. Five pairs of treated adults were released into the mud pot and maintained. Adult feed was changed daily. Longevity of the moths, eggs laid and hatchability were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4.

Efficacy of primary fractions of *A. mexicana* on *H. armigera* : 40 g of acetone crude extract was dissolved in the respective solvent and fractionated on a silica gel column, using benzene/acetone and the fractions were eluted.

RESULTS AND DISCUSSION

Efficacy of crude extracts of *A. mexicana*: The pupation was drastically affected by acetone extract (3.3 %) followed by chloroform extract (10 %) and petroleum ether extract (20 %), which is in conformity with dichloromethane and methanol extract of *Melia dubia* to *H. armigera* with decreased pupation and increased larval mortality (Koul *et al*., 2000). Similarly, the extract of various parts of *Tagetes patula* and *T. erecta* in combination with NPV proved better in suppressing the pupation of *H. armigera* and caused high mortality (Balasaraswathi, 1990). Petroleum ether leaf extracts of *Gnidia glauca* (Sundararajan and Kumuthakalavalli, 2000), seed extract of *Trichillia havanensis* (Lopez Olguin et al., 1998) and *Melia dubia* (Koul *et al.*, 2000) against larvae of *H. armigera* have been reported. However, the water extract did not have much effect on pupation (80 %) (Table 1). In addition, sluggishness and reduced feeding were observed in acetone extract treated larvae, which led to difficulty in normal metamorphosis, change in colour pigmentation, prolongation and ultimately led to paralysis (Plate 1) as against acetone solvent treated larvae. Significant difference in the pupal weight was not observed between the treatments. However, the pupal weight was comparatively less in the chloroform extract (190 mg) as against untreated (280 mg). Josephrajkumar et al. (2000) observed similar effects in *H. armigera* treated with the methanolic extract of silver fern. In addition to the reduction in pupal weight, incomplete metamorphosis led to larval pupal intermediates, particularly in the hexane and petroleum ether extracts (Plate 3). Similarly, the chloroform extract treated larvae resulted in 100% malformed adults, while the untreated check was able to produce normal adults (Plate 4). Neem leaf extracts against *H. armigera* showed significant reduction in the normal adult emergence (Sharma and Sheiker, 1997).

Efficacy of crude extracts against first-generation adults : Reduced adult longevity and early mortality was the result of moths emerged from petroleum ether extract treatment. The adults could survive only for six days followed by 7.5 days in hexane extract and water extract treatments of *A. mexicana* as against 9 days in untreated control. The overall impact of extracts on adult mortality reflected a quick knock down effect in extracts. The maximum adult mortality was observed in hexane extract (Fig 1). Fecundity was least in petroleum ether extract treated moths (7 eggs), followed by hexane extract (26 eggs) and petroleum ether solvent (81 eggs) compared to the untreated adults (648 eggs). The methanol extracts of neem seed kernel against *H. armigera* also reported significant decrease in the oviposition (Bajpai and Sehgal, 2003).

In the present study, all the extracts were effective on adults which led to sterile egg laying compared to untreated adults which recorded the maximum (91 %) hatchability. This was in agreement with effective inhibitory action on the egg hatchability and deformation of subsequent larvae due to neem seed kernel extract in *H. armigera* (Hassan, 1999).

Efficacy of acetone fractions against *H. armigera* larvae (first generation) : The fourth instar larvae were assayed for their growth using: 1. Normal diet + fractions; 2. Normal diet + solvent and 3. Normal diet (control).

The pupation was completely arrested in fraction 1 and 4. This was followed by severe reduction in pupation (8.7%) in fraction 3, whereas untreated larvae registered 100 % pupation (Table 2). This was in confirmation with Ganeshan *et al*. (1995) wherein exposure of *H. armigera* larvae to Neem and *Annona* resulted in 100% larval mortalities irrespective of the treatments. Methanol fraction of *M. dubia* inhibited larval growth of neonate *H. armigera* larvae in a dose dependant manner, when added to artificial diet in the range of 100 – 500 ppm of the extract. The extract inhibited larval growth by 50 % at 147 ppm (Koul *et al*., 2002).

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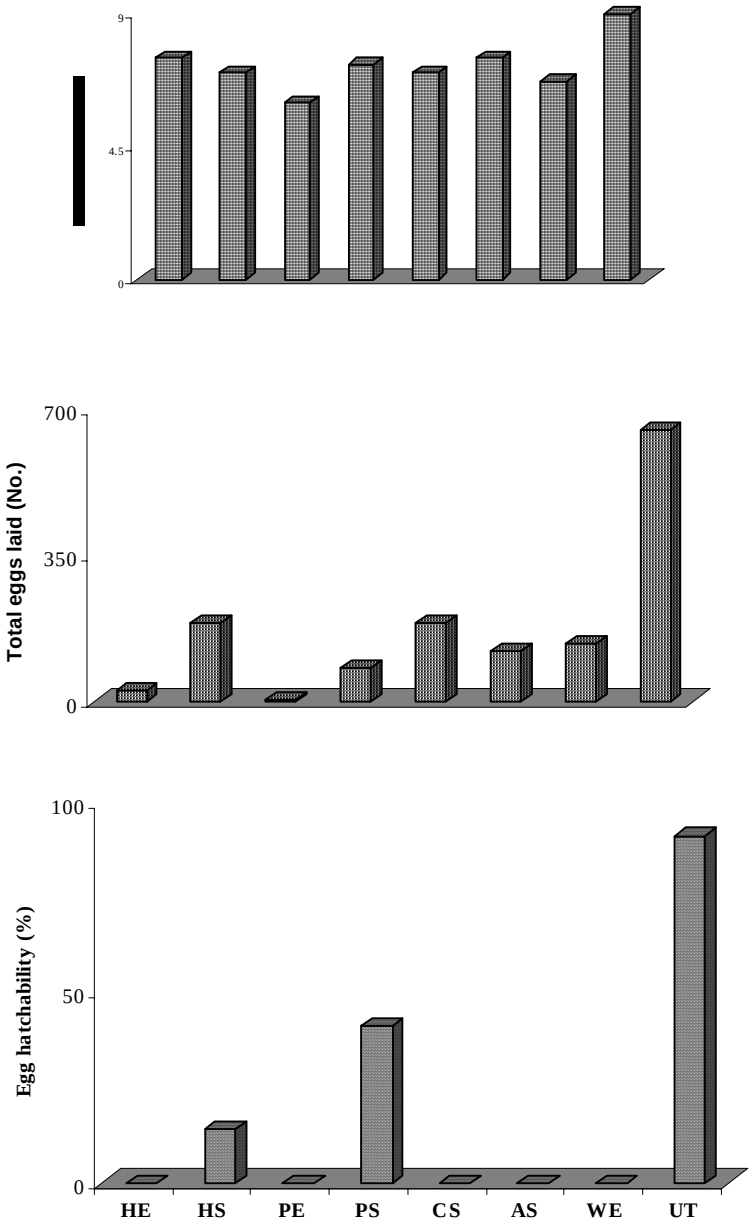
Table 1 Bioefficacy of *Argemone mexicana* crude extracts on *H. armigera* (first generation)

Treatment		Characters*		
		Larval development		Moth emergence %
		Pupation %	Pupal weight (mg)	Malformed/Dead
Hexane	extract	30.0	200	83.3
	solvent	86.6	220	53.4
Petroleum ether	extract	20.0	240	90.0
	solvent	66.6	230	63.3
Chloroform	extract	10.0	190	100.0
	solvent	33.3	220	80.0
Acetone	extract	3.33	200	100.0
	solvent	33.33	250	83.3
Water extract		80.0	210	76.6
Untreated		100.0	280	0.0
CD (P=0.05)		**11.9**	**96.0**	**43.0**

*Each value mean of triplicate

Fig. 1 Efficacy of *Argemone mexicana* crude extracts on *H. armigera*
(adults first generation)



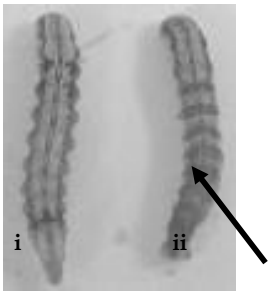
HE-hexane extract; HS-hexane solvent; PE-petroleum ether extract; PS- petroleum ether solvent; CS-chloroform solvent; AS-acetone solvent; WE-water extract; UT-untreated

Table 2 Effect of acetone fractions (primary) of *A. mexicana* on growth of *H. armigera* larvae

Treatment	Characters*		
	Larval development		Moth emergence %
	Pupation %	Pupal weight (mg)	Malformed/Dead
Fraction 1	0.0	-----	-----
Fraction 2	28.6	190	80.5
Fraction 3	8.7	135	66.6
Fraction 4	0.0	-----	-----
Fraction 5	9.0	159	66.6
Fraction 6	9.7	149	22.2
Solvent control	24.0	213	58.3
Untreated	100.0	283	6.6
CD (P = 0.05)	**12.4**	**137.4**	**56.1**

*Each value mean of triplicate; Fraction 1 - (benzene: acetone 90: 10); Fraction 2 - (benzene: acetone 85: 15); Fraction 3 - (benzene: acetone 75: 25); Fraction 4 - (benzene: acetone 65: 35); Fraction 5 (benzene: acetone 40: 60); Fraction 6 (benzene: acetone 25: 75)

Plate 1 Larval prolongation of *H. armigera* due to acetone extract



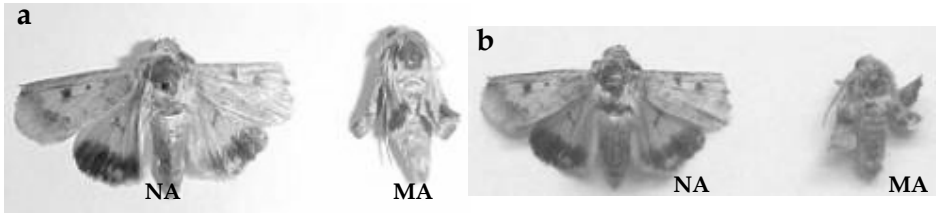
i-normal larva ii- acetone extract treated larva

Plate 2 Impact of disrupted moulting

a – hexane extract; b- chloroform extract
N- normal pupa; RP- reduced/affected pupa

a – hexane extract; b- petroleum ether extract

Plate 4. Deformed adults due to the impact of *A. mexicana* crude extracts



a – hexane extract**;** b- petroleum ether extract; NA- normal adult; MA- malformed adult

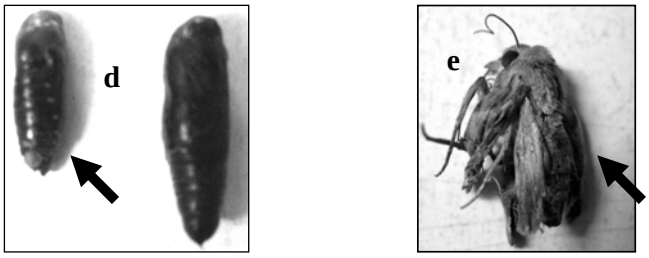
Plate 5. Juvenomimetic effect of *A. mexicana* primary fractions (acetone) on *H. armigera*

i **ii** **i** **ii** **ii** **i** **ii**

i – fraction 2 (arrow);
ii - normal pupa

i – normal pupa; ii – solvent treated;
iii – 3rd fraction –reduced pupal weight (arrow)

i – normal pupa ;
ii -fraction 6 – reduced pupal weight (arrow)



i **ii**
i. fraction 5 (arrow); ii - normal pupa

i
i. malformed adult

a. disrupted moulting; b and c – reduced pupal weight; d- reduced pupal size; e – malformed adult