Bioefficacy of Crude and Fractions of *Argemone mexicana* against Tobacco Caterpillar, *Spodoptera Litura* Fab. (Noctuidae: Lepidoptera)

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ABSTRACT

The insecticidal activity of crude extracts and fractions of *Argemone mexicana* L. (Papaveraceae)was investigated against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). The different treatments differed significantly in their efficacy. Pupation was nil in chloroform extract and acetone extract, while water extract treated larvae resulted in least pupal weight and maximum malformed adults. The adult life span was least in acetone solvent followed by hexane and petroleum ether extracts. Most of the treatments resulted in nil fecundity. Among the chloroform fractions, the first fraction arrested the pupation. In addition, disturbed moulting, larval-pupal intermediates and malformed moth emergence/dead pupae were also observed.

Keywords: *Argemone mexicana,* pupation, larval-pupal intermediates, fecundity

INTRODUCTION

The leafworm, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera), a serious but sporadic insect pest causes economic losses of crops from 25.8-100% (Dhir *et al*., 1992) based on crop stage and its infestation level in the field. It has a large host range of more than 120 host plants including crops, vegetables, weeds and ornamental plants (Ramana *et al*., 1988). It feeds gregariously on leaves leaving midrib veins only resulting in great yield loss. In India, 40 species of cultivated crops, wild plants and 11 flowering plants (Ali *et al.,* 1999) are affected by this pest. Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu especially in Coimbatore and Madurai districts (Rao *et al*., 1983). Further, Rao *et al*. (1983) reported that yield losses due to this pest was in the tune of Rs. 281.98 lakhs in tobacco and Rs.275.5 lakhs in chillies in Andhra Pradesh State alone.

It has developed resistance against a variety of insecticides belonging to almost all the insecticide groups used against it (Anonymous, 1999; 2000, Armes *et al*., 1997; Kranthi *et al*., 2002) even against new chemical insecticides like lufenuron (Sudhakaran, 2002). Adverse effects due to synthetic pesticides on pests and their subsequent impact on ecological imbalance (Zadoks and Waibel, 1999) demands ecofriendly alternatives (Parmar, 1993). Botanical is one such alternative and an important component in Integrated Pest Management (IPM) due to its advantages such as availability, least toxicity to beneficials, quick degradation and multiple functions (Isman, 2006). They act as antifeedant, repellent, deterrent, chemosterilants and growth regulator due to the presence of nearly 30,000 secondary metabolites (Bowers and Nishida, 1980; Schoonhoven, 1993; Isman, 2006).

Argemone mexicana (*Family: Papaveraceae*) is an erect prickly annual plant with yellow flower and latex. It is a native of tropical America and now widely naturalized in tropics. The plant is available along riverbanks and in Tamil Nadu; it is predominantly present at Yercaud (1400 m) (Matthew, 1983). The plant contains many alkaloids (Sangwan and Malik, 1998) and is used mostly for the treatment of HIV (YuhChwen *et al*., 2003). A critical literature survey reveals that *Argemone* has not been studied in-depth for its pesticidal character, except against cabbage head caterpillar, *Crocidolomia binotalis* (Facknath and Kawol, 1993) and mosquito, *Aedes aegypti* (Sakthivadivel and Thilagavathy, 2003). Hence, the present study aimed to explore the biopesticidal activity of this plant to combat the devastating pest *S. litura* with the following objectives: to test *A. mexicana* crude extracts against the larval (4th instar) and its adult stages of *H. armigera* and to test *A. mexicana* chloroform fractions against the larval (4th instar) stage of *H. armigera*.

MATERIALS AND METHODS

Collection and Rearing of *Spodoptera litura*

S. litura larvae were collected from infested castor plants from Kannivadi in Dindigul District., Tamil Nadu, India. The larvae collected from castor were maintained in the laboratory at 22 ± 2°C and 70 – 75 % relative humidity (RH). The larvae were reared both on castor and semi-synthetic diet in individual containers to prevent contamination (Santharam, 1985).

Table 1. Bioefficacy of *Argemone mexicana* crude extracts on first generation *S. litura*

Treatment		Characters		
		Larval development		**Moth emergence (%)**
		Pupation %	**Pupal weight (mg)**	**Malformed/Dead**
Hexane	extract	76.6	249	81.9
	solvent	93.3	276	60.0
Petroleum ether	extract	36.6	256	82.2
	solvent	90.0	260	55.5
Chloroform	extract	0.0	—	—
	solvent	66.6	265	70.8
Acetone	extract	0.0	—	—
	solvent	16.6	206	83.3
Water extract		96.6	203	83.3
Untreated		100.0	321	6.6
CD (P=0.05)		**17.02**	**34.86**	**24.08**

Plant Material and Its Extraction

Leaves of *Argemone mexicana* were collected from different parts of Thirukazhukundram and Kannivadi, Tamil Nadu. Collected leaves of *A. mexicana* were shade dried and powdered. One kg of powdered leaves was extracted successively using both non-polar and polar solvents *viz*., petroleum ether, hexane, chloroform and acetone. The powdered leaf material was soaked for 24h at 30 ± 2°C in 2.5 litre of solvent, filtered and to the residue the same solvent was added. The extraction 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).

Bioassay of Host Plants

Growth Inhibition of Larvae

Ten per cent solution of hexane, petroleum ether, chloroform, acetone and water extracts of *A. mexicana* were made in the respective solvents and mixed in the larval diet and fed to the fourth instar larvae only of *S. litura* using 1) normal diet + extract, 2) normal diet + solvent and 3) normal diet (control). Pupation (%), pupal weight (mg) and malformed moth emergence/dead pupae (%) and intermediate forms if any were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4.

Adult Longevity, Fecundity and Egg Hatchability of *S. litura*

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, which was 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. Five pairs of treated adults were released into the mud pot and maintained. 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.

Fractionation of Leaf Extract

Efficacy of primary fractions of *A. mexicana* on *S. litura* 40 g of chloroform crude extract was dissolved in the respective solvent and fractionated on a silica gel column, using hexane/methanol at 9.8:0.2, 9:1, 8:2, 7:3 and 6:4 ratios. Fraction 1 - (hexane: methanol 98: 2); Fraction 2 - (hexane: methanol 90: 10); Fraction 3 - (hexane: methanol 80: 20) Fraction 4 - (hexane: methanol 70: 30); Fraction 5 (hexane: methanol 60: 40). Five fractions named as Fr 1 (dark yellow with slow fractionation), Fr 2 (light yellow with slow fractionation), Fr 3 (reddish brown with moderate fractionation), Fr 4 (brown with high fractionation) and Fr 5 (green with high fractionation) was eluted.

Only the fourth instar larvae of *S. litura* were bioassayed using 1) normal diet + fraction, 2) normal diet + solvent

Table 2 Effect of chloroform fractions (primary) of A. mexicana on the growth of S. litura larvae

Treatments	Characters*		
	Larval development		Moth emergence %
	Pupation %	Pupal weight (mg)	Malformed /Dead
Fraction 1	0	_	_
Fraction 2	13.3	254	83.3
Fraction 3	25.0	253	91.6
Fraction 4	25.0	284	72.2
Fraction 5	21.6	248	88.8
Solvent control	11.6	129	83.3
Untreated	96.6	304	3.33
CD (P=0.05)	9.309	12.556	30.030

*Each value mean of triplicate

and 3) normal diet (control). Pupation (%), pupal weight (mg) and malformed moth emergence/dead pupae (%) and intermediate forms if any were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4.

RESULTS AND DISCUSSION

Efficacy of *A. mexicana* Crude Extracts on *S. litura* Larva

S. litura larvae treated with chloroform and acetone extracts showed no pupation, which was superior over the others. This was followed by acetone solvent and petroleum ether extract as against 100 % pupation in the untreated larvae (Table 1). Deterred feeding and significant larval mortality was reported in *S. litura* treated with methanolic extracts of *Melia dubia* (Opender *et al.,* 2000) and *Adathoda vasica* (Sadek, 2003). In addition to no pupation, phagodepression and difficulty in moulting resulted in pre-pupal malformations (Plate 1). This coincided with significant changes in pupal and pre-pupal stages in *S. litura* fed with different doses of hexane extracts of neem seed kernel (Kaur *et al.*, 2001) and *Tribulus terrestris* (Gunasekaran and Chelliah, 1985). Such potent toxicity leading to high larval mortality exhibited by the fractions of *A. mexicana* could be attributed to the group of toxic bimolecular possessing insecticidal properties particularly, glycosides and alkaloids present in species of the family Papaveraceae (YuChwen *et al.,* 2003). The pupal weight was least in water extract treated ones (203 mg) followed by acetone solvent (206 mg). The hexane extract treated larvae resulted in pupal weight with 249 mg, followed by petroleum ether extract (256 mg) as against 321 mg in untreated control. Similar effects were reported by the extracts of *Ocimum basilicum* against *Helicoverpa armigera* (Pandey *et al.*, 1983), *Melia azedarach* against cabbage diamond-back moth (Dilawari *et al.*, 1994) and *Annona squamosa* against *Helicoverpa* (Ganeshan *et al.*, 1995). Least healthy moth emergence (16.6 %) was recorded in water extract treatment followed by 17.7 % and 18.1 % in petroleum ether and hexane extracts treatment respectively. The control recorded 93.3 % healthy moth emergence. Similarly, furanocoumarin from the dried fruits of *Tetradium daniellii*, exhibited less healthy moth emergence (Tripathi, 2002; Stevenson *et al.*, 2003).

Efficacy of Crude Extracts against First-Generation Adults

The adult longevity was less (0.3 days) in adults emerged from acetone solvent treatment, followed by 1.1 days in hexane and petroleum ether extracts. Chloroform solvent resulted in 2.1 days while 2.5 days survival was observed in hexane solvent and water extract as against 6.0 days in untreated (Fig 1). Petroleum ether extract and its solvent, acetone solvent and water extract treatments recorded no eggs. The fecundity was minimum (17.3 eggs) in hexane extract as against the highest (238) in untreated. Similarly, hexane extract of neem seed kernel induced 86% sterility

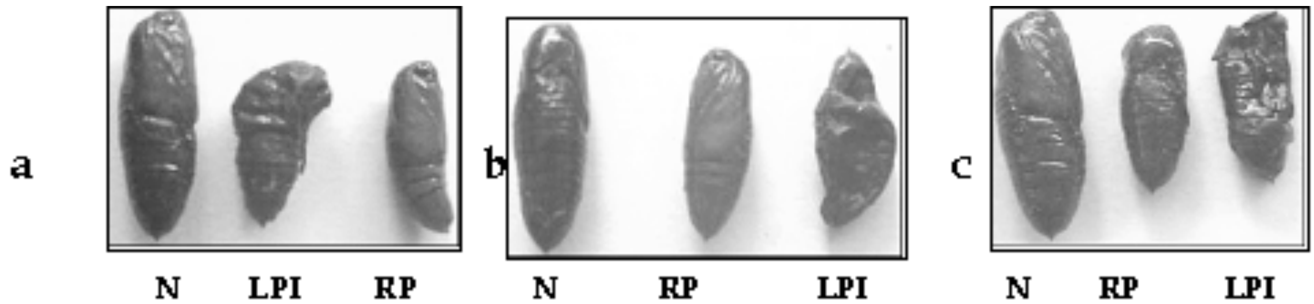


Plate 1. Impact of water (a), hexane (b) and petroleum ether extracts (c) of *A. mexicana* on *S. litura* (N- normal pupa; RP-reduced/affected pupa; LPI-larval-pupal intermediate)

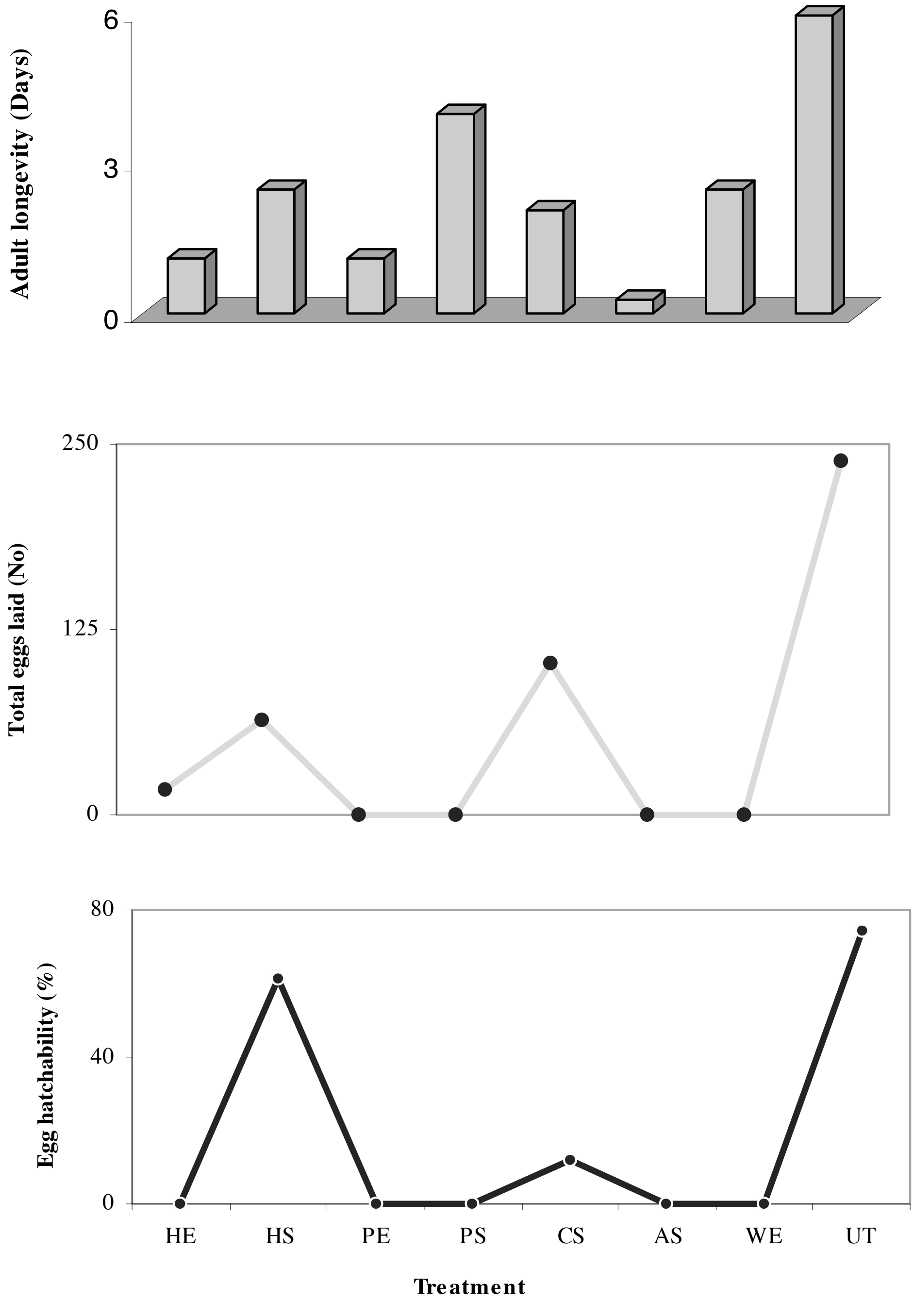
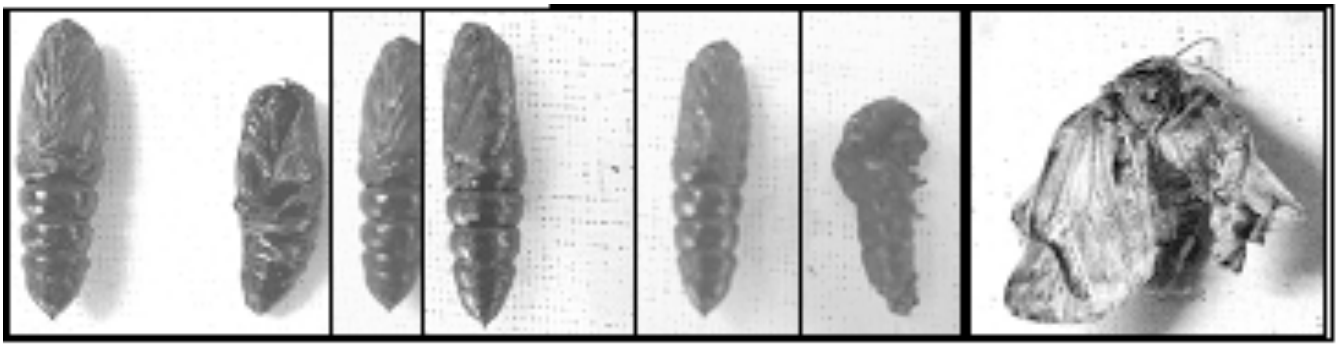


Fig. 1 . Efficacy of *A. mexicana* Hexane Extract (HE), Hexane (HS), Petroleum ether Extract (PE), petroleum ether (PS), Chloroform (CS), Acetone (AS) and Water Extract (WE) on longevity (in days) (a), fecundity (b) and hatchability (c) of *S. litura*



i ii iii iv v vi vii

Plate 2. Juvenomimetic effect of *A. mexicana* primary fractions (chloroform) on *S. litura* larvae– normal pupa; ii – solvent treated; iii – 5th fraction – reduced pupal weight; iv – 3rd fraction with reduced pupal weight; v – 1st fraction; vi – 5th fraction larval-pupal intermediates; vii – 3rd fraction – malformed adult

and further suppressed the reproductive performance (Kaur *et al.,* 2001). Oviposition of the cabbage pest, *Mamestra brassicae* was reduced to half the number of eggs per plant by the neem treatment (Shimizu, 1988). The number of eggs that hatched was not affected by the neem treatment, but development of the larva was strongly inhibited and all larvae in the neem treatment died within two weeks without reaching 2nd instar (Selijasen and Meadow, 2006). Except hexane (61%) and chloroform solvent (12%) treatments the rest of the treatments, resulted in nil hatchability. The impairment of gonotrophic cycle of adults might have prevented the eggs from hatching. Similar trend was also observed in *Earias vitella* (Fab.) (Noctuidae) treated with the leaves of *Azadirachta indica*, *Ocimum basilicum*, *Eucalyptus rostrata, Lantana camara* and *Allium sativum* which significantly reduced the oviposition and hatchability compared to the control (Shukla and Pathak, 1997).

Efficacy of Chloroform Fractions against *S. litura* Larvae

Maximum growth inhibition was observed in larvae treated with fraction 1 and none of the larvae were able to pupate. This was followed by solvent control (11.6 %) and fraction 2 (13.3 %). The control showed 96.6 % pupation. 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).

It was also observed that during development, larvae of *S. litura* lost their body weight rapidly when treated with the fractions and transformed into small sized and shrivelled pupa (Plate 2 - vi). Prolongation in larval developmental periods leading to reduction in pupal weight and malformed larval pupal intermediaries (Plate 2b – iv - vi) are reported to be the physiological effects of the neem (Red Fern *et al.,* 1982; Schmutterer *et al.,* 1983). Similarly, all the fractions of *D. angustifolia* resulted in a drastic reduction in pupal weight and subsequent record of malformed adults (Malarvannan, 2004).

Further, a drastic reduction in pupal weight was recorded in all the treatments. It ranged from 129-284 mg (normal control-304 mg), with 248 mg in fraction 5 (Plate 2 - iii), which subsequently resulted as malformed moths (Table 2; Plate 2 - vii). This may be attributed to the increased energy expenditure in order to detoxify the extracts within the insect body (Schoonhoven and Meerman, 1978; Dowd *et al*., 1983; Al- Sharook *et al*., 1991). Similarly, the postembryonic development and subsequent loss in pupal weight was observed in *S. litura* larvae fed with crude extracts of neem+mahua+jatropha (Ganeshan *et al.,* 1995). Similar effects in *Annona* (Kawazu *et al.,* 1990; Rupprecht *et al.,* 1990; Londershausen *et al.,* 1991; Ohsawa *et al.,* 1991) neem (Schmutterer, 1990) and jatropha and mahua (Grainage and Saleem Ahmad, 1988) have been documented earlier. Maximum malformed moth emergence was recorded in fraction 3 (91.6%) (Plate 2e), followed by fraction 5 (88.8%) as against the untreated ones (3.33%) (Table 2). The results obtained from laboratory studies on feeding of *S. litura* with botanicals are in confirmation with the antifeedant effects of neem seed kernel suspension (Joshi *et al.,* 1984), karanja (Deshmukh and Borle, 1975). Sombatsiri and Tigvattannont (1983) reported that survival rate of the larvae to the adult stage of *S.*

litura was 8.6% when treated with 0.1% neem kernel extract.

The experimental results proved that the biopesticides, particularly plant extracts play a major role in combating the pest. Its wide application as a botanical pesticide could be taken up after exploring its toxicity and field trials.

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