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ANTI-INSECT EFFECT OF *ARGEMONE MEXICANA* L. SEED SOLVENT EXTRACTS ON *SPODOPTERA LITURA* FAB

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ABSTRACT

Studies were carried out to determine the effect of *Argemone mexicana* L. solvent seed extracts on third instar *Spodoptera litura* Fab. using leaf disc bioassay method. Seven different concentrations (5, 10, 20, 30, 50, 70 and 90 %) along with solvent control and absolute control were tested. The extracts showed diverse anti insect properties such as feeding deterrence, insecticidal and insects growth regulatory activity at different concentrations. Among the different solvent extracts of seed, the acetone extract showed maximum feeding deterrence activity of 95.22 per cent in higher concentration. When concentration increased the feeding deterrence was also found to be increased. In other solvent extracts, feeding deterrence was < 50 per cent. The maximum larval mortality (80%) was noticed in methanol extract. In ethyl acetate extract, maximum of 40 per cent larval malformation and 60 per cent pupal malformation were noticed. The experiment revealed that among various seed solvent extracts of *A. mexicana* tested against *S.litura*, the acetone extract of seed in higher concentration showed maximum feeding deterrence activity, the ethyl acetate extract of seed showed insect growth regulatory activity and methanol extract of seed showed insecticidal activity.

INTRODUCTION

Indiscriminate use of chemical pesticides resulted in many serious problems *viz.*, genetic resistance of pest species, toxic residues, increasing costs of application, environmental pollution and hazards (Ahmed *et al.*, 1981). This has created a world-wide interest in the development of alternative strategies with main focus on traditionally used botanical extracts. They often consist of complex mixtures of compounds which possess varieties of actions and may also act synergistically (Berenbaum, 1985). Hence they are highly effective, safe and ecologically acceptable (Senthilnathan and Kalaivani, 2005). *Spodoptera litura* Fab. (Noctuidie: Lepidoptera) an economically important polyphagous pest reported for its ability to develop resistance against many insecticides was selected as test insect (Sharma and Seth, 2005). In the present investigation seeds of herbaceous medicinal plant Mexican prickly poppy *Argemone mexicana* L.(Papaveraceae) containing toxic alkaloids *viz.*, sanguinarine, dihydro sanguinarine and berberine was investigated. The effective insecticidal action of *A. mexicana* extract against phytophagous insects and mosquitoes has been proved by many authors (Sakthivadivel *et al*., 2012; Kangade and Zambare, 2013; Abou-Elnaga, 2015; Sharma *et al.*, 2016 and Sivaraman *et al*., 2016). However, dose dependent toxicity studies are very scanty and hence, the present investigation was undertaken.

MATERIALS AND METHODS

Tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) egg masses collected from the castor plants grown in and around Annamalainagar (Latitude11°N, Longitude 79°E) were used for initiating the laboratory mass culture. The larvae were reared in Bengal gram flour based semi synthetic diet till pupation. The pupae were surface sterilized with 0.05% sodium hypochlorite and transferred to an oviposition cage. Egg masses laid on *Nerium oleander* Linn. twig were collected daily, sterilized with 0.05 per cent sodium hypochlorite solution and a continuous culture was maintained. The rearing was done at 26 ± 1°C and 75 per cent relative humidity (PDBC, 1998).

Seed extract preparation

Fifty gram of seed powder was weighed and transferred to conical flasks (250 ml capacity) and filled with 100 ml each of acetone, petroleum ether, methanol and ethyl acetate solvents separately. The mixture was soaked for 72 hrs with intermittent shaking. Then, the extract filtered, concentrated in rotary flash vacuum evaporator and stored at - 20°C (Jaglan *et al.*, 1997). This was used as stock solution and various concentrations *viz.*, 5, 10, 20, 30, 50, 70, 90 per cent were prepared.

Anti insect activity bioassay

A no-choice leaf disc assay was carried out using 4 h pre-starved third instar *S. litura* larvae (Bentley *et al*., 1984). Castor leaf discs (3 cm diameter) were cut out and treated with 300 µl of seven concentrations of various solvent esxtracts of seed separately (5, 10, 20, 30, 50, 70, 90 per cent) on both the sides. After shade drying for one minute, leaf discs were placed separately inside a Petri plate (9 cm diameter) lined internally by moist filter paper to avoid early

drying of the leaf discs. Solvent and absolute controls were maintained. After 6h, mortality and leaf area consumed were recorded. The per cent feeding deterrence activity was worked out using the below mentioned formula. The larvae alive were reared using untreated castor leaves till adult emergence and mortality and malformations were recorded (Selvamuthukumaran and Arivudainambi, 2008).

Per cent feeding deterrence =

$$\frac{\text{Leaf disc consumed by the larvae in control} - \text{Leaf disc consumed by the larvae in treated}}{\text{Leaf disc consumed by the larvae in control} + \text{Leaf disc consumed by the larvae in treated}} \times 100$$

RESULTS AND DISCUSSION

The effect of various concentrations of *A. mexicana* seed solvent extracts on third instar *S. litura* by using leaf disc bioassay method was studied. It showed diverse anti insect properties such as feeding deterrence, insecticidal and malformation at different concentrations of various solvent extracts.

Among various concentrations of acetone extract of seed tested (Table 1), lower concentration (5%) showed less feeding deterrence (10.12%). When the concentration increased, the feeding deterrence was also increased. This similar finding was supported by Joshi *et al.* (1984) who reported that the antifeedant activity of neem seed kernel extract was concentration dependent against *S.litura* on tobacco crop. Although at 10 per cent concentration the acetone extract showed 20 per cent larval mortality it was found to be statistically non significant. As for the pupal mortality is concerned it was founded that from 5 to 20 per cent and at 70 and 90 per cent concentrations, 20 per cent mortality was recorded. The medium dose of 30 and 50 per cent concentrations failed to show any mortality. Till 20 per cent concentration of acetone extract of seed 20 per cent larval malformation was recorded and from 30 to 90 per cent concentrations, failure of occurrence of any larval malformation noticed.

In case of pupal malformation, 10 and 30 per cent concentrations recorded malformation and from 50 to 90 per cent concentration, it failed to record any malformation. In acetone extract of seed the adult malformation was noticed in 5 and 50 per cent concentration only. 40 to 60 per cent adult emergence was found in lower to medium doses and in higher dose it was recorded as 80 per cent.

Ethyl acetate extract of seed (Table 2) recorded feeding deterrence in the lowest concentration of 5 per cent as 10.78 per cent. In medium dose of 30 and 50 per cent, it recorded 21.23 and 25.26 per cent feeding deterrence respectively. The feeding deterrence was found to slightly increase along the concentration. Mean while nil larval mortality was noticed in 5to 20 per cent concentrations and 70 and 90 per cent concentration. However in medium dose treatment (30 and 50 % concentration) 20 per cent mortality was noticed. Similarly 20 per cent pupal mortality was recorded only in 70 per cent concentration. As for as larval malformations were concerned lower concentrations of 5 and 10 per cent showed nil malformations and from 20 to 70 per cent concentrations 80 to 60 per cent combined malformations were noticed. In higher concentration (90 %) 40 per cent pupal malformation and 60 per cent of adult malformation were noticed.

The methanol extract of seed when tested at different concentrations showed (Table 3) 12.73 to 25.55 per cent the feeding deterrence in lower concentrations of 5 to 20 per cent. In medium dose concentration of 50 and 70 per cent they showed 45.07 and 48. 83 per cent feeding deterrence. In case of higher concentration, the maximum feeding deterrence recorded was 49.90 per cent. The feeding deterrence of methanol extract of seed moderately increased from lower to higher concentration. As for as insecticidal activity is concerned, the effect was found to increase with increasing dose. Maximum and quick insecticidal action was noticed in the highest concentration tested (90%). It imparted 80 per cent larval mortality which none of the treatment provided. This was followed by 70 per cent concentration imparting 60 per cent and 20 per cent larval and pupal mortality respectively. At further low doses the total mortality was reduced to 60 per cent (50 and 30% concentration).

Table 1 Anti insect effect of acetone extract of *Argemone mexicana* L. seed on third instar *Spodoptera litura* Fab. at various concentration

Concentration	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
5%	10.16 (18.63)	0 (0.0)	20 (26.56)	20 (26.56)	0 (0.0)	20 (26.56)	40 (39.23)
10%	18.91 (25.77)	20 (26.56)	0 (0.0)	20 (26.56)	20 (26.56)	0 (0.0)	40 (39.23)
20%	49.46 (44.71)	0 (0.0)	20 (26.56)	20 (26.56)	0 (0.0)	0 (0.0)	60 (50.77)
30%	60.50 (51.06)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	0 (0.0)	80 (63.44)
50%	77.07 (61.41)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	80 (63.44)
70%	86.90 (68.78)	0 (0.0)	20 (26.56)	0 (0.0)	0 (0.0)	0 (0.0)	80 (63.44)
90%	95.22 (77.34)	0 (0.0)	20 (26.56)	0 (0.0)	0 (0.0)	0 (0.0)	80 (63.44)
Solvent control	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	90 (71.56)
Absolute control	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	0 (0.0)	90 (71.56)
S.Ed	0.242	0.084	0.133	0.126	0.123	0.111	0.118
C.D	0.518	N.S.	0.284	0.269	0.240	0.237	0.252

*Mean of ten replications
Values within parentheses are arc sine transformed

Table 2 Anti insect effect of ethyl acetate extract of *Argemone mexicana* L. seed on third instar *Spodoptera litura* Fab. at various concentration

Concentration	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
5%	10.78 (19.19)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	90 (71.56)
10%	14.38 (22.30)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	90 (71.56)
20%	19.60 (26.28)	0 (0.0)	0 (0.0)	20 (26.56)	40 (39.23)	20 (26.56)	20 (26.56)
30%	21.23 (27.42)	20 (26.56)	0 (0.0)	20 (26.56)	20 (26.56)	20 (26.56)	20 (26.56)
50%	25.26 (30.20)	20 (26.56)	0 (0.0)	20 (26.56)	40 (39.23)	0 (0.0)	20 (26.56)
70%	30.29 (33.20)	0 (0.0)	20 (26.56)	20 (26.56)	0 (0.0)	40 (39.23)	20 (26.56)
90%	35.12 (36.33)	0 (0.0)	0 (0.0)	0 (0.0)	40 (39.23)	60 (50.77)	0 (0.0)
Solvent control	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	90 (71.56)
Absolute control	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	0 (0.0)	90 (71.56)
S.Ed	0.139	0.121	0.084	0.133	0.096	0.111	0.178
C.D	0.297	0.240	0.179	0.284	0.205	0.237	0.380

*Mean of ten replications
Values within parentheses are arc sine transformed

Table 3 Anti insect effect of methanol extract of *Argemone mexicana* L. seed on third instar *Spodoptera litura* Fab. at various concentration

Concentration	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
5%	12.73 (20.88)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	80 (63.44)
10%	13.73 (21.72)	20 (26.56)	20 (26.56)	0 (0.0)	20 (26.56)	20 (26.56)	20 (26.56)
20%	25.55 (30.40)	20 (26.56)	20 (26.56)	20 (26.56)	20 (26.56)	0 (0.0)	20 (26.56)
30%	38.60 (38.41)	40 (39.23)	20 (26.56)	0 (0.0)	20 (26.56)	20 (26.56)	0 (0.0)
50%	45.07 (42.19)	60 (50.77)	0 (0.0)	0 (0.0)	20 (26.56)	20 (26.56)	0 (0.0)
70%	48.83 (44.51)	60 (50.77)	20 (26.56)	0 (0.0)	20 (26.56)	0 (0.0)	0 (0.0)
90%	49.90 (44.94)	80 (63.44)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	0 (0.0)
Solvent control	9.97 (18.44)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	0 (0.0)	90 (71.56)
Absolute control	16.10 (23.66)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	90 (71.56)
S.Ed	0.121	0.109	0.134	0.083	0.136	0.133	0.190
C.D	0.260	0.234	0.285	0.178	0.283	0.284	0.406

*Mean of ten replications
Values within parentheses are arc sine transformed

Still at low doses (20% and 10%) the mortality was 40 per cent which was made to nil at the lowest dose (5%). This clearly revealed the dose dependent nature of the extract.

The petroleum ether of extract of seed (Table 4) failed to significantly reduce the adult emergence. It inhibited only 40 per cent of adult emergence even at higher concentrations (90, 70, 50%). At low dose it imparted 90 per cent (5 and 10 % concentration) and 80 per cent (20 and 30 % concentration) adult emergence. Hence it was found that petroleum ether extract of seed was ineffective.

The experiment reveled that among the various seed solvent extract of *A. mexicana* tested against *S.litura*, numerous anti insect effects were noticed. The acetone extract of seed in the higher concentration showed maximum feeding deterrence activity. In ethyl acetate seed extract the insect growth regulatory activity was noticed and in methanol seed extract insecticidal activity was recorded.

The results clearly indicated that *A. mexicana* seed extract had high anti insect potency at different concentrations. Hence this botanical can be exploited by further purification of the toxic principles, determination of mode of action and product development.

Table 4 Anti insect effect of petroleum ether extract of *Argemone mexicana* L. seed on third instar *Spodoptera litura* Fab. at various concentration

Concentration	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
5%	9.28 (17.76)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	90 (71.56)
10%	12.38 (20.62)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (18.44)	90 (71.56)
20%	16.92 (24.27)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	0 (0.0)	80 (63.44)
30%	20.29 (26.78)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	0 (0.0)	80 (63.44)
50%	25.26 (30.20)	0 (0.0)	0 (0.0)	0 (0.0)	40 (39.23)	0 (0.0)	60 (50.77)
70%	28.15 (32.08)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	40 (39.23)	60 (50.77)
90%	30.13 (33.27)	0 (0.0)	0 (0.0)	0 (0.0)	20 (26.56)	20 (26.56)	60 (50.77)
Solvent control	10.21 (18.63)	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	0 (0.0)	90 (71.56)
Absolute control	0 (0.0)	0 (0.0)	10 (18.44)	0 (0.0)	0 (0.0)	0 (0.0)	90 (71.56)
S.Ed	0.148	-	-	0.086	0.126	0.064	0.164
C.D	0.317	-	-	0.177	0.269	0.136	0.350

*Mean of ten replications
Values within parentheses are arc sine transformed

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