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Larvicidal potential of *Argemone mexicana* L. Plant extracts against *Spodoptera litura* fab.

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Abstract
Larvicidal activity of ethanol and aqueous leaf extracts of *Argemone mexicana* were studied against third instars larvae of *Spodoptera litura* at different concentration (5, 10, 15, 20 and 25 mg/ml.). The plant leaves were dried, powdered and extracted in soxhlet apparatus in ethanol and aqueous solvent for 24 hrs. The third instars larvae of *S. litura* were exposed to various concentration and percent mortality were recorded after 96hrs.
The larvicidal activity of leaf extract of *A. mexicana* were (LD_{10}= 7.65 mg/ml, LD_{50}= 20.54 mg/ml) in ethanol and (LD10= 10.17 mg/ml, LD 50= 31.55 mg/ml) in aqueous respectively. Results revealed that the mortality was increased with increasing in concentration of the plant extracts.
The ethanol solvent extract of *A. mexicana* showed higher larvicidal property against third instars larvae of *S. litura*. Statistical variance, 95% confidence limits and regression equations are presented.

Keywords: *Spodoptera litura*, *Argemone mexicana* and mortality

Introduction
The tobacco caterpillar, *Spodoptera litura* Fab. is one of the serious and dominant polyphagous pest of cotton, soya bean and other major crops which caused much damage and affect agricultural productivity (Balaraju *et al*., 2011) [5]. It is also one of the most economically important insect pests of 51 countries including India, Japan, China, and other countries of Southeast Asia infesting 112 species of plants belonging to 44 families including groundnut, cotton, chilly, tobacco, castor and soyabean (Chari and Patel, 1983) [9]. In India *Spodoptera litura* feeds on 74 species of cultivated crops and some wild plants (Rao *et al.*, 2008) [26]. The average crop losses worldwide due to pests and diseases are 60% of potential production.
Chemical pesticides have been used for many years in controlling pests as they give high mortality rate in less time. The huge amount of synthetic pesticides is applied in the fields of cotton and other economically important crops to protect them from insect attack. However, the indiscriminate use of synthetic pesticides resulted in many problems such as resistance of pest to pesticides, resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affect human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment. With a greater awareness of hazards associated with the use of synthetic pesticides there has been an increase need to explore suitable alternative method of pest control. Farmers use different plant material to protect their crops from pest infestation. Natural products in their crude form or plant extract provide unlimited opportunities as biopesticide.
In recent years research efforts are reported on development of insecticides of plant origin. Botanical insecticides are ecofriendly and environmentally safer alternative method for crop protection (Mansour *et al*., 2011; Kabili *et al*., 2012; Abbad and Basheli, 2013) [22, 16, 1]. Plant derivatives are highly toxic to many insect species and more than 2000 plant species are known to possess some insecticidal properties. Some of the plants from Meliaceae, Rutaceae, Asteriaceae, Labiatae, Convolvulaceae and Pedaliaceae are promising sources of insecticide based property (Schutterer, 1990; Isman, 1995; Sujatha *et al.*, 2010) [30, 15, 32]. Thangarasu *et al.*, (2015) [33], evaluated the role of different extracts of *Abrus precatorius* for their ovicidal activity, oviposition deterrent activity, antifeedant activity and larvicidal activities against various life stages of selected agricultural field pest *S. litura*. This paper reports the results of research on the effect of *Argemone mexicana* L. plant extracts against the tobacco cutworn, *Spodoptera litura.*

The plant, *Argemone mexicana* Linn. belongs to the family Papaveraceae and It is a common plant found everywhere by road-sides and fields in India. *A. mexicana* is considered as an important medicinal plant in India and long been used for dropsy, jaundice, ophthalmia, scabies and cutaneous infections (Chopra *et al.*, 1956; Ambasta, 1986; Sharma *et al.*, 2012) [10, 2, 35]. Various plant parts of this species (the roots, branches, stems, leaves, flowers, seed extracts, and oils possess insecticidal, repellent, nematicidal and bactericidal effects, and they were tested against a wide range of pests of agricultural importance (Prakash and Rao, 1997) [24]. Crude *A. mexicana* extracts were demonstrated to have ovicidal and larvicidal activity against lepidopteran, dipteran, coleopteran, and hemipteran pests (Cepero 1994; Zambare *et al*., 2012; Kangade and Zambare 2013) [8, 12, 17]. These extracts have a wide range of sub lethal effects including growth delay (Rao and Chitra 2000) [25], reduced fecundity and fertility, molting disorders, morphogenetic defects (Malarvannan *et al*. 2008) [19], and repellency (Majeed and Abidunnisa 2011) [18].

Therefore, the present study was undertaken to evaluate the larvicidal potential of ethanol and aqueous leaf extracts of *Argemone mexicana* against the tobacco cut worm, *Spodoptera litura.*

Materials and Methods

Plant Collection and Extraction

Leaves of *Argemone mexicana* were collected from local area and were properly identified from taxonomist. The leaves were shade dried and were powdered in domestic grinder and stored in air tight container in refrigerator till further use. From the stock 100 g of powdered was extracted with 1000 ml of ethanol and aqueous using Soxhlet apparatus for 24 hrs separately.

Insect Culture

The eggs of *S. litura* (NBAII-MP-NOC-02: *S.litura*) were purchased from National Bureau of Agriculture Insect Resources, Bangalore and were surface sterilized with 0.02% Sodium Hypochlorite solution, dried and allowed to hatch. After hatching, the larvae were reared on normal diet with castor leaf, *Ricinus communis.* Third instars larvae were used for further study to minimize handling effects.

Insecticidal bioassay

Third instars larvae of *S. litura* were used for the insecticidal assay. Fresh Castor leaf was taken in each acrylic plastic jar and was exposed to several doses of ethanol and aqueous extracts of *A. mexicana*. The dose was prepared by mixing the extract with respective solvent and was sprayed on castor leaves. One jar of control containing only fresh castor leaf sprayed with respective solvent was maintained. The treated castor leaves were allowed to evaporate the solvent. 10 newly emerged III instars larvae were released in each experimental and control acrylic plastic jar containing fresh castor leaves. Three replications for each solvent were conducted. The percent mortality was calculated after 96 h and the observed data was subjected to probit analysis (Finney, 1947; Busvine, 1971) [13, 7].

Results

The toxic effect of leaf extract of *A. mexicana* was evaluated against *S. litura*. The numbers of dead *S. litura* were counted after 24, 48, 72 and 96 h at (5, 10, 15, 20, and 25 mg/ml) doses of ethanol and aqueous extract. The total percent mortality was observed after 96 h, and then the corrected mortality was calculated using Abbott's formula and the results are presented. The results showed that, the mortality increases with increasing in concentrations (Figure and Tables).

The results of probit analysis for the estimation of LD_{10}, LD_{50}, variance, 95% confidence limits and regression equation at 96h for the mortality of third instar larvae of *S. litura* are presented in Table-2.

The insecticidal bioassay in ethanol solvent extracts of *Argemone mexicana*, LD_{10} = 7.65 mg/ml and LD_{50} = 20.54mg/ml and in aqueous extract of *Argemone mexicana*, LD_{10} = 10.17 mg/ml and LD_{50} = 31.55mg/ml. Among the various estimate of regression based probit analysis, the $\chi 2$ values for the regression coefficients showed homogeneity to the data.

Table 1: Mortality rate of *Spodoptera litura* treated with leaf extracts of *Argemone Mexicana*

S. No.	Dose in mg/ml	No. of insects used	Mortality after 96 hrs. (Ethanol)	Mortality after 96 hrs. (Aqueous)
1.	Control	10	-	-
2.	5	10	-	-
3.	10	10	20	10
4.	15	10	30	20
5.	20	10	50	30
6.	25	10	60	40

Table 2: LD_{10}, LD_{50} values with variance, 95% confidence limits and probit analysis parameters for larvae of *Spodoptera litura* after 96h of exposure.

Solvent	LD_{10}	LD_{50}	Variance	95% CL		Regression equations	$\chi 2$
				Lower	Upper		
Ethanol	7.65	20.54	0.003658	1.1942	1.4312	Y=2.9876x+1.0779	0.043
Aqueous	10.17	31.55	0.002168	1.4077	1.590	Y=2.6048x+1.101	0.538

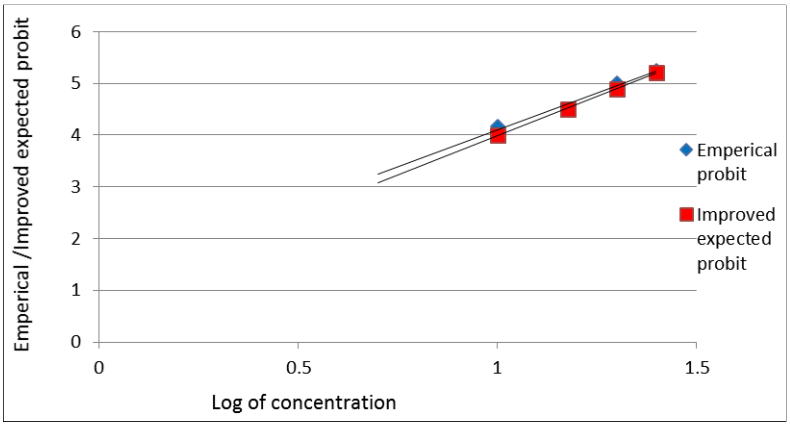

Fig 1: Regression and provisional lines for *Spodoptera litura* exposed to ethanol leaf extract of *Argemone mexicana* after 96 h

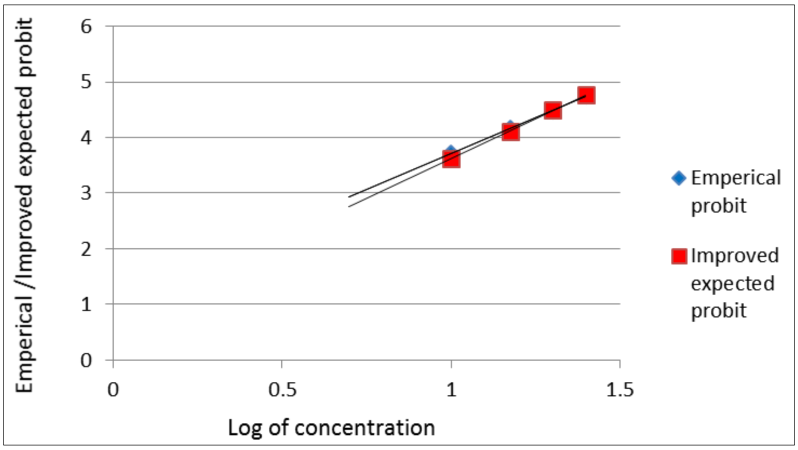

Fig 2: Regression and provisional lines for *Spodoptera litura* exposed to aqueous leaf extract of *Argemone mexicana* after 96 h

Discussion

Spodoptera litura is one of severe agricultural pest. In insect-plant interactions, insects often have unique adaptation to their host plants in locating and selecting the plants by the use of chemical, visual and mechanical cues (Schoonhoven *et al*., 1998) [29]. According to Mustaparta., (2002) [23], unsuitable plants are avoided by detection of other chemical cues; such chemical substances may have repellent or toxic properties against insects. Based on this principle, botanical pesticides are invented and utilized for control of insect pests. Crude extracts from the leaf, stem, root and seeds of various plant species have been reported to possess antifeedant, insecticidal, and/or growth inhibitory properties (Ekesi., 2000) [11]. Hummel and Isman, (2001) [14] reported that synergistic effects of complex mixtures (crude extracts) of phytochemicals are also thought to be important in plant defenses against insect herbivores.

In the present investigation, the toxicity of ethanol and aqueous leaf extract of *Argemone mexicana* was tested against III instar larvae of *S. litura*. In our study mortality increased with increase in concentration at all the doses up to 96 hrs of exposure. Similar to the present investigation, several studies documented the insecticidal activity of *Argemone mexicana* on different pests.

Malarvannan *et al.* (2008, 2008a) [19, 20] found that toxic as well as growth inhibitory properties of leaf crude extracts of *A. maxicana* against two lepidoptenan species, *S. litura* and *H. armigera*. The chloroform fraction and acetone fraction of this plant extracts showed reduced pupal weight, deformed moths and reduced adults longevity of *S. litura* and *H. armigera*, respectively. Bosch, (2007) [6] studied the effect of crude extract from leaves of of *A. mexicana* having antifeedant effect on the larvae of *Crocidolomia binotalis* Zeller (Lepidoptera: Crambidae) and *S. litura*. The type of organic solvent that is used to extract the metabolites is very crucial; the same concentrations (≤2 mL/kg) of *A. mexicana* leaf extracts caused different mortality rates in *Corcyra cephalonica* 4th instar larvae when they were extracted in different solvents: the methanolic 10 to 20% extracts, the acetonic 10 to 70% extracts, the ethanolic 20 to 90% extracts, and the chloroformic 10 to 100% extracts was experimented by Kangade and Zambare., (2013) [17].

Zeinab and Abou, (2015) [36] showed the toxic effect of chloroform and methanol leaves and seeds crude extracts of *A. Mexicana* against medically important vectors *Cx. pipiens* and *Ae. Aegypti*. Majeed and Abidunnisa., (2011) [18] reported that ultra-low concentrations (0.5 to 0.80 g/cm2) of aqueous crude extracts from leaves of *A. mexicana* caused 80 to 96% repellency of adults of *T. castaneum* and the rice weevil *Sitophilus oryzae* (L.) at 1 h after application. A field application of a 50% concentration of extracts from leaves of *A. mexicana* caused 90% mortality in adults of the stink bug *Oebalus insularis* at 48 h after application (Cepero, 1994) [8].

Under laboratory conditions when *C. cephalonica* larvae were fed with rice grains coated with diverse types of organic extracts from *A. mexicana* at concentrations of 1.5 and 2.0 mL/kg, the pupal mortality reached up to 40% (Kangade and Zambare 2013) [17]. Sharma *et al.*, (2016) [31] evaluated the effect of *Argemone mexicana* leaves extract of different solvents on gut of *Heliothis armigera* (Hub.) and after 24 and 96 hours of treatment *Heliothis armigera* showed severity of the damage of epithelial lining and epithelial cells with vacuoles at certain places. Ashwini *et al*., (2017) [4] found that the toxicity bioassay of *A. mexicana* extracts caused greater mortality on third instars larvae (LD50 = 5.33 mg-1) than *C. inerme* (LD50 = 7.26 mg-1).

In many countries, plant derived products are being used by the farmers from ancient times and it triggered the scientists to search for ecofriendly insecticides from plant kingdom.

Several hundred plants have been reported as insect repellents, antifeedants, attractants, insecticides, ovicides & oviposition deterrents (Arnason *et al*., 1992; Ewete *et al.*, 1996) [3, 12].

Vetal and Pardeshi, (2019) [34] reported the insecticidal potential of hexane and ethanol seed extracts of *Annona squamosa* against III instar larva of *Spodoptera litura* and found LD_{10} (5.91mg/mL and 11.72 mg/mL) and LD_{50} (13.98 mg/mL and 22.48 mg/mL) respectively.

The genus *Argemone* is very rich in alkaloids, which have both pharmaceutical and pest control properties. This genus is used to treat various human ailments because of its antimicrobial, antiparasitic, antimalarial, cytotoxic, and neurological properties (Rubio-Pina and Vazquez-Flota 2013) [27]. Aqueous and organic extracts of *Argemone mexicana* was effective for checking crop damage due to the presence of sanguinarine, berberine and palmatine, among other compounds, which have antifeedant and repellent effects on a wide range of pests (Malikova *et al*., 2006; Schmeller *et al*. 1997) [21, 28].

The finding of the present investigation revealed that, the leaves extract of *Argemone mexicana* possesses remarkable insecticidal activity against *Spodoptera litura*. The LD_{10}= 7.65mg/ml, LD_{50}=20.54 mg/ml in ethanol and LD_{10}=10.17 mg/ml, LD_{50}=31.55 mg/ml in aqueous is reported. The study needs further investigation to find out active ingredients responsible for insecticidal properties of *A. mexicana* and to reach any final recommendations.

Conclusion

The result of this study has confirmed that the *Argenome mexicana* have explored the potential biopesticide and plant protecting activity against cut worm, *Spodoptera litura*.

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