Evaluation of anti insect properties of *Senna alata* L against *Spodoptera litura* Fab[Lepidoptera: Noctuidae]

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Abstract

The effect of acetone, ethyl acetate, hexane and methanol extract of various plant parts of. *Senna alata* L against *Spodoptera litura* Fab. revealed presence of various anti insect properties such as feeding deterrence, insecticidal and insect growth regulatory activities. Among the solvents tested acetone imparted maximum antifeedant action of 98.01 per cent followed by methanol. Between the plants parts tested the seed extract had shown higher feeding deterrence followed by leaf extract. Various solvent extracts of other plant parts failed to exhibit significant feeding deterrence (> 60%). Supreme insecticidal action was noticed only in methanol extract of seed (80% larval mortality) and it caused complete death of all the treated insects (Nil adult emergence). Insect growth regulatory activity alone was noticed as the supreme anti insect action in ethyl acetate solvent extract. It caused nil adult emergences by imparting 40 and 60 per cent larval and adult malformations respectively. Among the solvent extracts, Hexane exhibited minimum anti insect effects in all the plant parts tested.

Keywords: Anti insect properties, *Senna alata, Spodoptera litura*

INTRODUCTION

The damage caused by the insect pests is one of the major concerns for the farmers across the world. An estimated one third of global agricultural production is destroyed annually by over 20,000 species of insect pests in field and storage (Mariapackiam and Ignacimuthu, 2008)[1]. The annual average loss due to insect pests has been estimated as around 15.7 per cent in India, a monetary loss of about US $36 billion (Dhaliwal *et al.*, 2015) [7]. Although varieties of pest management tools are available to tackle such problem, farmers are mainly depending on insecticides for their crop protection endeavors (Dhaliwal and Koul, 2010) [6]. This over reliance coupled with improper use has resulted in serious problems such as development of genetic resistance of pest species, leading to vicious spray cycle. This has resulted in many harmful effects *viz.*, toxic residues, environmental pollution, health hazards and reduction in non target organisms (Ahmed *et al.*, 1981[1] ; Siqueira *et al*., 2000[17]; Cork *et al*., 2003[4]; Aktar *et al.*, 2009[2]).

Although there is a rich source of plants that could be harnessed for their anti insect properties,

commercialization of botanicals has not gained ground. The market share of botanicals along with other bio pesticides remains at a mere 2 per cent level. One such a plant candle bush *Senna alata* L. (Fabaceae) a shrub, possessed anti insect properties, Further, the effective insecticidal action of *S.alata* extract against *Callosobruchus chinensis* L. and mosquitoes has been proved . However, the information regarding the anti insect properties effects have been proved in few insect. Hence, the present study is aimed at screening of various solvent extracts of *S. alata* plant parts for their anti insect properties against *S. litura* Fab. (Noctuidae: Lepidoptera)

MATERIALS AND METHODS

Preparation of plant extracts of *S. alata*

Collection of various plant parts and shade drying

S. alata whole plants were collected from Annamalainagar. The collected plants were shade dried for 10 days. Various plant parts like leaves, flowers and seeds were separated and powdered in a Wiley mill. They were then stored separately in air tight containers (15 cm × 10 cm) and used for further extraction.

Preparation of extract

Room temperature solvent extraction method using acetone (Boiling point: 56.5°C), ethyl acetate (Boiling point: 77°C), methanol (Boiling point: 65°C) and hexane (Boiling point: 58°C) separately as solvents was done for extracting the active principle from the plant parts as described by Jaglan *et al.* (1997) [9]. The powdered plant materials of *S. alata* were extracted by using various solvent like acetone, methonal, ethyl acetate and hexane in conical flask (500 ml) for a period of three days with intermittent shaking and filtered through What man no 40 filter paper. The crude extracts thus obtained were stored under room.

Preliminary screening of solvent extracts of various plant parts of *S.alata* for their anti insect properties

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 dia.) were cut out and treated on both the sides with 300 µl of undiluted solvent extracts of acetone, ethyl acetate, methanol and hexane separately. After shade drying for a minute, leaf discs were placed separately inside a Petri plate (9 cm dia.) lined internally by moist filter paper to avoid early drying of leaf disc. Each Petri plate was provided with one 4 h pre starved third instar larvae and each treatment was replicated ten times. Respective solvent and absolute controls were also maintained. Treated leaf discs were collected after six hours. Then, the leaf area fed was measured graphically and per cent leaf area protection over absolute control was computed and

feeding deterrence activity was worked out as indicated below. The larvae alive were reared using untreated castor leaves till adult emergence and mortality and malformations were recorded periodically (Selvamuthukumaran, 2008) [16].

$$\text{Percent feeding deterrence activity} = \frac{\text{Leaf disc consumed by the larvae in control} - \text{Leaf disc consumed by the larvae in treated}}{\text{Leaf disc consumed by the larvae in control} + \text{Leaf disc consumed by the larvae in treated}} \times 100$$

RESULTS AND DISCUSSION

Various plant parts of *S .alata viz*., seed, leaf and flower were extracted using solvents such as acetone, methanol , ethyl acetate and hexane. These extracts were tested undilutedly against third instar *S. litura.* The results obtained were tabulated in Tables 1- 4. The acetone extract was found mainly to impart feeding deterrence activity. It failed to show any significant mortality. Similarly the larval malformation induced was also not more than 20 per cent except in acetone extract of seed and leaf. The maximum feeding deterrence activity was noticed in seed (98.01%) followed by leaf (53.12%). The increased feeding deterrence noticed in seed resulted in delayed larval mortality, larval and pupal malformations. Hence in this treatment alone, a meager 20 per cent adult emergence was noticed. Although the leaf extract imparted more than 50 per cent feeding deterrence activity, it recorded 80 per cent adult emergence. Similarly the remaining plant parts failed to show any considerable reduction in adult emergence. These treatments resulted in 80 or 90 per cent adult emergence. The results revealed that acetone extract of seed alone possessed maximum feeding deterrence activity under preliminary bioassay (Table 1). The effects of undiluted ethyl acetate extract of various plant parts of *S.alata* were tabulated in Table 2. The results revealed that none of the plant parts tested exhibited more than 50 per cent feeding deterrence activity. It ranged from a minimum of 20.12 per cent in flower to a maximum of 45.74 per cent in seed extract. Conspicuous absence of insecticidal activity was noticed. Meanwhile, marked insect growth regulatory activity was noticed in seed extract. It imparted 40 per cent larval malformation and 60 per cent adult malformation resulting in nil adult emergence. However the extract of other plant parts recorded 80 or 90 per cent adult emergence. Hence it was found that ethyl acetate extract of seed possessed insect growth regulatory activity.

Superior insecticidal activity (80%) was noticed in methanol extract of seed . The seed extract also imparted 20 per cent pupal malformation resulting in nil adult emergence. It also imparted nearly 80 per cent (79.93%) feeding deterrence activity. This was followed by methanol extract of leaf which imparted 20 per cent mortality in both larva and pupa. It also imparted 20 per cent pupal malformation and 60 per cent adult emergence inhibition. These results revealed superior insecticidal action of methanol extract of seed (Table 3). Table 4 providing the effect of hexane extract of various plant parts of *S.alata* against third instar *S. litura* revealed the extract's inability to induce any considerable anti insect effect. This was

evident from the fact that the minimum adult emergence recorded itself was 60 per cent (seed and leaf extract).

Further there was nil mortality recorded in any of the treatment. Similarly the malformations recorded were also very less to the tune of 10 to 40 per cent. However the seed extract alone imparted a slightly better feeding deterrence activity (56.04%) compared with extracts of other plant parts (16.14%, and 31.33% in flower, and leaf respectively). On the whole, as none of the extracts of plant parts imparted more than 40 per cent adult emergence inhibition even under undiluted condition the Hexane extract was found as less superior solvent extract. The preliminary bioassay results revealed that the acetone extract, ethyl acetate extract and methanol extract of seed alone showed promising feeding deterrence, insect growth regulatory and insecticidal action respectively. Further, it was evident that the extracts of other plant parts failed to exhibit any considerable anti insect effect. Hence these three extracts along with Hexane extract of seed, imparting 56.04 per cent feeding deterrence were selected as promising solvent extracts for further confirmation of their anti insect activity at reduced concentrations.

Screening carried out using undiluted solvent extracts (acetone, methanol, ethyl acetate and hexane) of various plant parts of *S.alata* against third instar *S. litura* revealed the presence of any one of the anti insect action at more than 50 per cent level in all the four solvent extracts of the seed tested. Such supreme anti insect effect of *S. alata* seeds were supported by the findings of Pandey *et al*. (1981) [13], Sakthivadivel and Thilagavathy (2003) [15], Priya and Rao (2012) [14], Guerrero *et al.* (2015) [18] and Sivaraman *et al.* (2016) [15]. The reason for such results may be due to the presence of more amounts of alkaloids in the seeds. This was corroborated by the reports of Das and Khanna (1997) [15] that identified the presence of 0.44 per cent to 0.50 per cent of alkaloids in seed oil on a V/V basis. This may be the reason for better performance of hexane extract of seed (>50 % feeding deterrence) and failure of hexane extracts of leaf and flower to record any significant anti insect action. Comparatively, it was found that the seed extract irrespective of the solvent used possessed significant anti insect properties.

However, the anti insect activities exhibited by various solvent extracts of seed were found to be highly variable. The acetone and hexane extracts imparted strong and weak feeding deterrence activities respectively; ethyl acetate extract exhibited insect growth regulatory and methanol extract imparted insecticidal action. The possible reason for such variability may be due to the ability of the solvent to dissolve the active ingredient in the extract. The reports of Marek *et al*. (2003) [11] and Waksmundzka-Hajnos and Petruczynik (2008) [18] revealed the presence of benzo isoquinoline alkaloids as their water soluble salts and bases. Kostalova *et al*. (1982) [10] reported utilization of methanol as the best solvent for initial extraction protoberberine alkaloids. These reports supported the present findings wherein the polar solvents *viz.*, methanol and acetone imparted quick anti insect actions like very strong insecticidal and

feeding deterrence activities respectively whereas less polar solvents *viz.*, ethyl acetate imparted delayed anti insect effect like insect growth regulatory action and hexane exhibited a weak feeding deterrence action. The possible reason may be that the most polar solvents (methanol and acetone; polarity index: 5.1) extracted more amounts of water soluble salts of the active alkaloids easily leading to corresponding quick anti insect actions visualized and *vice versa* for less polar and non polar solvents [ethyl acetate (less polar; polarity index: 4.4) and hexane (non polar; polarity index: 0.1)]. Further, this same polarity concept may be responsible for the non significant effect of Hexane extract, the most non polar solvent used in the present study.

Table 1. Anti insect effects of *Senna alata* L. acetone extract on third instar *Spodoptera litura* Fab.

Plant parts	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
Seed	98.01 (81.87) [a]	20 (26.56)	0 (0.0)	20 (26.56) [a]	40 (39.23) [a]	0 (0.0) [c]	20 (26.56) [a]
Leaf	53.12 (46.78) [b]	0 (0.0)	0 (0.0)	20 (26.56) [a]	0 (0.0) [d]	0 (0.0) [c]	80 (63.44) [b]
Flower	21.21 (27.42) [e]	0 (0.0)	0 (0.0)	0 (0.0) [b]	0 (0.0) [d]	10 (18.44) [b]	90 (71.56) [c]
Solvent control	0 (0.0) [f]	0 (0.0)	0 (0.0)	0 (0.0) [b]	0 (0.0) [d]	10 (18.44) [b]	90 (71.56) [c]
Absolute control	0 (0.0) [f]	0 (0.0)	0 (0.0)	0 (0.0) [b]	10 (18.44) [c]	0 (0.0) [c]	90 (71.56) [c]
S.Ed	0.699	0.108	–	0.134	0.121	0.119	0.105
CD (p=0.05)	1.541	N.S.	–	0.296	0.266	N.S	0.231

*Mean of ten replications

Values within parentheses are arc sine transformed

Values with different alphabets with in columns differ significantly

Table 2. Anti insect effects of *Senna alata* L. methanol extracton third instar *Spodoptera litura* Fab.

Plant parts	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
Seed	79.93 (63.44) [a]	80 (63.44) [a]	0 (0.0) [b]	0 (0.0) [c]	20 (26.56) [a]	0 (0.0) [b]	0 (0.0) [a]
Leaf	40.12 (39.29) [b]	20 (26.56) [b]	20 (26.56) [a]	0 (0.0) [c]	20 (26.56) [a]	0 (0.0) [b]	40 (39.23) [b]
Flower	23.17 (28.79) [d]	0 (0.0) [c]	20 (26.56) [a]	0 (0.0) [c]	0 (0.0) [c]	0 (0.0) [b]	80 (63.44) [d]
Solvent control	0 (0.0) [e]	0 (0.0) [c]	0 (0.0) [b]	0 (0.0) [c]	10 (18.44) [b]	0 (0.0) [b]	90 (71.56) [e]
Absolute control	0 (0.0) [e]	0 (0.0) [c]	0 (0.0) [b]	10 (18.44) [b]	0 (0.0) [c]	0 (0.0) [b]	90 (71.56) [e]
S.Ed	0.099	0.123	0.134	0.122	0.116	0.104	0.168
CD (p=0.05)	0.217	0.271	0.296	0.282	0.279	0.272	0.371

* Mean of ten replications

Values within parentheses are arc sine transformed

Values with different alphabets with in columns differ significantly

Table 3. Anti insect effects of *Senna alata* L. ethyl acetate extract on third instar *Spodoptera litura* Fab.

Plant parts	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
Seed	45.74 (42.53) [a]	0 (0.0)	0 (0.0)	40 (39.23) [a]	0 (0.0) [c]	60 (50.77) [a]	0 (0.0) [a]
Leaf	33.12 (35.12) [c]	0 (0.0)	0 (0.0)	0 (0.0) [d]	20 (26.56) [a]	0 (0.0) [d]	80 (63.44) [b]
Flower	20.12	0	0	0	10	0	90

	(26.64) [e]	(0.0)	(0.0)	(0.0) [d]	(18.44) [b]	(0.0) [d]	(71.56) [c]
Solvent control	0 (0.0) [f]	0 (0.0)	0 (0.0)	0 (0.0) [d]	0 (0.0) [c]	10 (18.44) [c]	90 (71.56) [c]
Absolute control	0 (0.0) [f]	0 (0.0)	0 (0.0)	10 (18.44) [c]	0 (0.0) [c]	0 (0.0) [d]	90 (71.56) [c]
S.Ed	0.093	-	-	0.117	0.095	0.101	0.164
CD (p=0.05)	0.205	-	-	0.221	0.209	0.224	0.362

*Mean of ten replications

Values within parentheses are arc sine transformed

Values with different alphabets with in columns differ significantly

Table 4. Anti insect effects of *Senna alata* L. hexane extract on third instar *Spodoptera litura* Fab.

Plant parts	Per cent feeding deterrence activity*	Per cent mortality*		Per cent malformation*			Per cent adult emergence*
		Larva	Pupa	Larva	Pupa	Adult	
Seed	56.04 (48.45) [a]	0 (0.0)	0 (0.0)	20 (26.56) [a]	20 (26.56) [a]	0 (0.0) [b]	60 (50.77) [a]
Leaf	31.33 (34.02) [b]	0 (0.0)	0 (0.0)	0 (0.0) [c]	20 (26.56) [a]	20 (26.56) [a]	60 (50.77) [a]
Flower	16.14 (23.66) [e]	0 (0.0)	0 (0.0)	10 (18.44) [b]	0 (0.0) [c]	0 (0.0) [b]	90 (71.56) [c]
Solvent control	0 (0.0) [f]	0 (0.0)	0 (0.0)	0 (0.0) [c]	10 (18.44) [b]	0 (0.0) [b]	90 (71.56) [c]
Absolute control	0 (0.0) [f]	0 (0.0)	0 (0.0)	0 (0.0) [c]	10 (18.44) [b]	0 (0.0) [b]	90 (71.56) [c]
S.Ed	0.110	-	-	0.087	0.134	0.095	0.140
CD	0.243	-	-	0.214	0.296	0.209	0.309

(p=0.05)

*Mean of ten replications

Values within parentheses are arc sine transformed

Values with different alphabets with in columns differ significantly

References

1. Ahmed, S.M., H. Chander and J. Pereira. 1981. Insecticidal potential and biological activity of India indigenous plants against *Musca domestica*. **International Pest Control, 23**: 170-175.

2. Aktar, M.W., D. Sengupta and A. Chowdhury. 2009. Impact of pesticide use in Indian agriculture-Their benefits and hazards. **Interdisciplinary Toxicology, 2**(1): 1-12.

3. Bentley, M.D., D.E. Leonard, W.F. Stoddard and L.H. Zalkow. 1984. Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm *Choristonewa fumiferana* (Lepidoptera: Tortricidae). **Annals of the Entomological Society America, 7**: 393-397.

4. Cork, A., N.Q. Kamal, S.N. Alam, J.C.S. Choudhury and N.S. Talekar. 2003. Pheromone and their applications to insect pest control. **Bangladesh Journal of Entomology, 13**: 1-13.

5. Das, M. and S.K. Khanna. 1997. Clinicoepidemiological, toxicological, and safety evaluation studies on oil. **Critical Reviews in Toxicology**, **27**(3): 273–297.

6. Dhaliwal, G.S. and O. Koul. 2010. Quest for pest management: from green revolution to gene revolution. **Kalyani Publishers,** New Delhi.

7. Dhaliwal, G.S., J. Vikas and M. Bharathi. 2015. Crop losses due to insect pests: Global and Indian Scenario. **Indian Journal of Entomology**, **77:** 165-168

8. Guerrero, R.R., M.A. Rodríguez Pérez and M.N. Campos. 2015. Toxicity of Mexican native plant extracts against larvae of *Aedes aegypti* (Diptera:Culicidae). ***Asian Pacific Journal of Tropical Biomedicine,*** **5**(4): 287-291.

9. Jaglan, M.S., K.S. Khokhar, M.S. Malik and J.S. Taya. 1997. Standardization of method for extraction of bioactive components from different plants for insecticidal property. **Indian Journal of Agricultural Research, 31**(3): 167 - 173.

10. Kostalova, D., B. Brazdovicova and H.Y. Jin. 1982. Alkaloids from the above-ground parts of *Berberis coreana* palib. **Farmaceuticky Obzor,** 213–216.

11. Marek, R., P. Seckarova, D. Hulova, J. Marek, J. Dostal and V. Sklenar. 2003. Palmatine and berberine isolation artifacts. **The Journal of Natural Products**, **66**: 481–486.

12. Mariapackiam, S. and S. Ignacimuthu. 2008. Larvicidal and histopathological effects of the oil formulation on *Spodoptera litura* Fab. In: Recent trends in Insect Pest Management. (Ignacimuthu, S. and S. Jeyaraj. eds.). Elite Publishing House Pvt. Ltd., New Delhi, 1, 128,3(10).

13. Pandey, G.P., R.B. Doharey and B.K. Varma. 1981. Efficacy of some vegetable oils for protecting green gram against the attack of *Callosobruchus maculates* Fab. **Indian Journal of Agricultural Science, 51**: 910-912.

14. Priya, C.L. and K.V.B. Rao. 2012. Ethanobotanical and current ethanopharmacological aspects of *Senna alata* L Linn: An overview. **International Journal of Pharmaceutical Science and Research, 3**(7): 2143-2148.

15. Sakthivadivel, M. and D. Thilagavathy. 2003. Larvicidal and chemosterilant activity of the acetone fraction of Hexaneextract from *Senna alata L* L. seed. **Bioresource Technology, 89**(2): 213-216.

16. Selvamuthukumaran, T. 2008. Pesticidal studies on certain chemical fractions of *Cleistanthus collinus* (Roxb.) Benth. leaves. **Ph.D., Thesis,** Annamalai University, Annamalainagar, 233p.

17. Siqueira, H., A. Alvaro, A. Guedes, N.C. Raul and M.C. PicanÇo. 2000. Insecticide resistance in population of *Tuta absoluta* (Lepidoptera: Geleiidae). **Agricultural and Forest Entomology, 2**: 147-153.

18. Waksmundzka-Hajnos, M. and A. Petruczynik, 2008. TLC of isoquinoline alkaloids. *In:* Waksmundzka-Hajnos, M. Sherma, J., Kowalska, T. (eds.), Thin layer chromatography in phytochemistry, CRC Press, Taylor and Francis, Group, p. 641-684.