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Anti-insect effect of *Senna alata* L. seed solvent extracts on *Spodoptera litura* Fab.

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Abstract
Studies were carried out to determine the effect of *Senna alata* 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 *S.alata* 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.

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

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) [1]. 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) [3]. Hence they are highly effective, safe and ecologically acceptable (Senthilnathan and Kalaivani, 2005) [8]. *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) [9]. In the present investigation seeds *Senna alata* is an important medicinal tree, as well as an ornamental flowering plant in the subfamily Caesalpinioideae. It also known as emperor's candlesticks, candle bush, candelabra bush, Christmas candles, empress candle plant, ringworm shrub, or candletree. *Senna alata.L* containing toxic alkaloids *viz.*, chrysoeriol, kaempferol, quercetin, 5, 7, 4'-trihydroflavanone, kaempferol-3-O-beta-D-glucopyranoside was investigated.

Materials and Methods
Tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) egg masses collected from the castor plants grown in and around Annamalainagar (Latitude11º N, Longitude 79ºE) 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 ºC and 75 per cent relative humidity (PDBC, 1998) [5].

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) [4]. 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) [2]. Castor leaf discs (3 cm diameter) were cut out and treated with 300 µl of seven concentrations of various solvent extracts of seed separately (5, 10, 20, 30, 50, 70, 90 per cent) on both the sides. 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) [7].

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

Results and Discussion

The anti insect effect of promising solvent extracts (All the four solvent extracts of seed) were evaluated at various concentration (5, 10, 20, 30, 50, 70 and 90 %) and are presented in Table 1 – 4. The acetone extract of seed imparted the feeding deterrence activity in concentration dependent manner. The effect was less at the lowest concentration tested and increased with increasing concentration. More than 50 per cent feeding deterrence activity was noticed from 30 per cent concentration. Surprisingly, maximum adult emergence was also noticed in 30 per cent concentration and beyond.

At low doses of 5 and 10 per cent, mere 10.16 per cent and 18.91 per cent feeding deterrence activity were recorded respectively. However, the adult emergence recorded in these two treatments was the least (40%). Such results were supported by the mortality and malformation data. The combined mortality and malformation noticed in 5 and 10 per cent concentration was 60 per cent each whereas in higher concentrations (30% and beyond) it was mere 20 per cent. These confirmatory bioassay results revealed that the main anti insect effect of acetone extract was feeding deterrence activity which increased with increasing concentration. However the extract was also found to impart insecticidal and insect growth regulatory action when applied at low concentrations (5 and 10%) (Table 1).

Table 2 recorded the bioassay results of various concentrations of ethyl acetate extract of seed. It was found that the main anti insect effect of ethyl acetate extract was insect growth regulatory action. At the highest concentration tested (90%), nil adult emergence was recorded. This was facilitated by pupal and adult malformations to the tune of 40 and 60 per cent respectively. When the concentration was reduced (70, 50, 30%), combined insect growth regulatory effect (all malformations) was also found to be reduced (60%). Meanwhile at 20 per cent concentration, the combined insect growth regulatory activity was 80 per cent indicating a slight increase. However, when concentration has been much reduced (5 and 10%) a meager anti insect effect has been recorded (10%). These results revealed that the main anti insect effect was insect growth regulatory action. It was imparted from as low a concentration of 20 per cent to as high a concentration of 90 per cent.

The confirmatory bioassay results of methanol extract of seed revealed that the superior anti insect effect noticed was insecticidal action. The effect was found to increase with increasing concentration.Maximum (80%) and quick (larval mortality) insecticidal action was noticed in the highest concentration tested (90%), which none of the treatment provided. This was followed by 70 per cent concentration imparting 60 and 20 per cent larval and pupal mortalities respectively. At further low doses (50 and 30% concentrations), the total mortality imparted was reduced to 60 per cent. Still at low doses (20 and 10%) the mortality was 40 per cent and was found to be nil at the lowest dose (5%). This clearly revealed the concentration dependent nature of the extract. The maximum feeding deterrence recorded was found to be 50 per cent at 90 per cent concentration. Similarly, maximum insect growth regulatory effects were found to be 40 per cent at the medium concentrations (30 and 50%). Hence, the prime anti insect action was confirmed to be as insecticidal, as noticed earlier in preliminary bioassay (Table 3).

As the petroleum ether extract of seed imparted 56.04 per cent feeding deterrence activity when bioassyed undilutely (preliminary screening), it was again screened at low concentrations in confirmatory bioassay. The extract failed to reduce the adult emergence. It inhibited only 40 per cent of adult emergence even at higher concentrations (90, 70, 50%). At low concentrations (5 and 10% concentration) it resulted 90 per cent and in medium concentrations (20 and 30% concentration) it resulted 80 per cent adult emergence. Hence it was found that petroleum ether extract of seed was ineffective (Table 4).

The results of the confirmatory bio assay again confirmed the supremacy of acetone, ethyl acetate and methanol extract of seed in imparting feeding deterrence, insect growth regulatory and insecticidal actions respectively. These three extracts were selected as potent feeding deterrent solvent extract (Acetone extract of seed), potent insect growth regulatory solvent extract (Ethyl acetate extract of seed) and potent insecticidal solvent extract (Methanol extract of seed) for further fractionation and mode of action studies.

The anti insect effect of promising solvent extracts (All the four solvent extracts of seed) were evaluated at various concentration (5, 10, 20, 30, 50, 70 and 90 %) and the results obtained confirmed their corresponding anti insect activities noticed in the preliminary screening and revealed concentration dependent nature of the extract. Such concentration dependent anti insect effect may be due to presence of increasing amount of active ingredient in increasing concentrations of the extract. Such results corroborated with the preliminary screening results wherein the reason for varied anti insect effect was identified as difference in the amount of active ingredient extracted. Such dose dependent anti insect activity is a common phenomenon in botanicals as reported by Garcia and Rembold (1983) [6]. In azadirachtin.

However in acetone extract of seed, the adult emergence was the least (40%) in the lowest concentration tested (5%) and highest (80%) in highest concentration tested (90%). Such contradictory results were not reported in any other solvent extract tested. The possible reason for such peculiar result may be due to the feeding deterrence effect caused by acetone extract. At low concentrations (5 & 10%), the feeding deterrence was less and the larvae consumed more of treated leaves. This led to toxic and growth regulatory effects and correspondingly more reduction in adult emergence. At high concentrations (30 to 90%), as more feeding deterrence activity was recorded, the larvae consumed less of treated leaves. This led to very minimal toxic or growth regulatory

effects and correspondingly high adult emergence.

In ethyl acetate extract the anti insect effect noticed was again confirmed as insect growth regulatory in nature. It failed to show any effect at low concentrations (5 & 10%). The possible reason may be presence of very less amount of active ingredient in low concentrations. From 20 per cent to 70 per cent, the extract imparted 20 per cent adult emergence. At 90 per cent concentration it resulted in nil adult emergence. Such results supported presence of active ingredient in increasing amounts in increasing concentrations. This was further corroborated by the results obtained in methanol and petroleum ether extract bioassays. In methanol extract treatment bioassay, the insecticidal effect not alone increased also became progressively quicker (larval mortality increased from 20% to 80% along the increasing concentrations).

The petroleum ether extract failed to exhibit any significant anti insect effect even at high concentration indicated the inability of the solvent to extract more

of active ingredient. This was in accordance with the results of preliminary screening. Hence, three seed extracts *viz.*, acetone, ethyl acetate and methanol extracts were selected as potent feeding deterrent solvent extract, potent insect growth regulatory solvent extract and potent insecticidal solvent extract for further fractionation and mode of action studies.

Table 1: Anti insect effects of acetone extract of *Senna alata.* L. seed on third instar *Spodoptera litura* Fab. at various concentrations

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) ^{g}	0 (0.0)	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0) ^{c}	20 (26.56) ^{a}	40 (39.23) ^{a}
10%	18.91 (25.77) ^{f}	20 (26.56)	0 (0.0) ^{b}	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0) ^{b}	40 (39.23) ^{a}
20%	49.46 (44.71) ^{e}	0 (0.0)	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0)	0 (0.0) ^{b}	60 (50.77) ^{b}
30%	60.50 (51.06) ^{d}	0 (0.0)	0 (0.0) ^{b}	0 (0.0) ^{c}	20 (26.56) ^{a}	0 (0.0) ^{b}	80 (63.44) ^{c}
50%	77.07 (61.41) ^{c}	0 (0.0)	0 (0.0) ^{b}	0 (0.0) ^{c}	0 (0.0) ^{c}	20 (26.56) ^{a}	80 (63.44) ^{c}
70%	86.90 (68.78) ^{b}	0 (0.0)	20 (26.56) ^{a}	0 (0.0) ^{c}	0 (0.0) ^{c}	0 (0.0) ^{b}	80 (63.44) ^{c}
90%	95.22 (77.34) ^{a}	0 (0.0)	20 (26.56) ^{a}	0 (0.0) ^{c}	0 (0.0) ^{c}	0 (0.0) ^{b}	80 (63.44) ^{c}
Solvent control	0 (0.0) ^{h}	0 (0.0)	0 (0.0) ^{b}	0 (0.0) ^{c}	10 (18.44) ^{b}	0 (0.0) ^{b}	90 (71.56) ^{d}
Absolute control	0 (0.0) ^{h}	0 (0.0)	0 (0.0) ^{b}	10 (18.44) ^{b}	0 (0.0) ^{c}	0 (0.0) ^{b}	90 (71.56) ^{d}
S.Ed	0.242	0.084	0.133	0.126	0.123	0.111	0.118
C.D (p=0.05)	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

Values with different alphabets with in columns differ significantly

Table 2: Anti insect effects of ethyl acetate extract of *Senna alata.* L. seed on third instar *Spodoptera litura* Fab. at various concentrations

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) ^{g}	0 (0.0) ^{b}	0 (0.0) ^{b}	0 (0.0) ^{c}	10 (18.44) ^{c}	0 (0.0) ^{e}	90 (71.56) ^{c}
10%	14.38 (22.30) ^{f}	0 (0.0) ^{b}	0 (0.0) ^{b}	0 (0.0) ^{c}	0 (0.0) ^{d}	10 (18.44) ^{d}	90 (71.56) ^{c}
20%	19.60 (26.28) ^{e}	0 (0.0) ^{b}	0 (0.0) ^{b}	20 (26.56) ^{a}	40 (39.23) ^{a}	20 (26.56) ^{c}	20 (26.56) ^{b}
30%	21.23 (27.42) ^{d}	20 (26.56) ^{a}	0 (0.0) ^{b}	20 (26.56) ^{a}	20 (26.56) ^{b}	20 (26.56) ^{c}	20 (26.56) ^{b}
50%	25.26 (30.20)^{c}	20 (26.56) ^{a}	0 (0.0) ^{b}	20 (26.56) ^{a}	40 (39.23) ^{a}	0 (0.0) ^{e}	20 (26.56) ^{b}
70%	30.29 (33.20) ^{b}	0 (0.0) ^{b}	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0) ^{d}	40 (39.23)^{b}	20 (26.56) ^{b}
90%	35.12 (36.33) ^{a}	0 (0.0) ^{b}	0 (0.0) ^{b}	0 (0.0) ^{c}	40 (39.23) ^{a}	60 (50.77) ^{a}	0 (0.0)^{a}
Solvent control	0 (0.0) ^{h}	0 (0.0) ^{b}	0 (0.0) ^{b}	0 (0.0) ^{c}	10 (18.44) ^{c}	0 (0.0) ^{e}	90 (71.56) ^{c}
Absolute control	0 (0.0) ^{h}	0 (0.0) ^{b}	0 (0.0) ^{b}	10 (18.44) ^{b}	0 (0.0) ^{d}	0 (0.0) ^{e}	90 (71.56) ^{c}
S.Ed	0.139	0.129	0.084	0.133	0.096	0.111	0.178
C.D (p=0.05)	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

Values with different alphabets with in columns differ significantly

Table 3: Anti insect effects of methanol extract of *Senna alata* L. seed on third instar *Spodoptera litura* Fab. at various concentrations

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) ^{g}	0 (0.0) ^{e}	0 (0.0) ^{b}	0 (0.0) ^{c}	0 (0.0) ^{c}	20 (26.56) ^{a}	80 (63.44) ^{c}
10%	13.73 (21.72) ^{f}	20 (26.56) ^{d}	20 (26.56) ^{a}	0 (0.0) ^{c}	20 (26.56) ^{a}	20 (26.56) ^{a}	20 (26.56) ^{b}
20%	25.55 (30.40) ^{e}	20 (26.56) ^{d}	20 (26.56) ^{a}	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0) ^{b}	20 (26.56) ^{b}
30%	38.60 (38.41) ^{d}	40 (39.23) ^{c}	20 (26.56) ^{a}	0 (0.0) ^{c}	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0) ^{a}
50%	45.07 (42.19) ^{c}	60 (50.77) ^{b}	0 (0.0)^{b}	0 (0.0) ^{c}	20 (26.56) ^{a}	20 (26.56) ^{a}	0 (0.0) ^{a}
70%	48.83 (44.51) ^{b}	60 (50.77) ^{b}	20 (26.56) ^{a}	0 (0.0) ^{c}	20 (26.56) ^{a}	0 (0.0)^{b}	0 (0.0) ^{a}
90%	49.90 (44.94) ^{a}	80 (63.44) ^{a}	0 (0.0) ^{b}	0 (0.0) ^{c}	0 (0.0) ^{c}	20 (26.56) ^{a}	0 (0.0) ^{a}
Solvent control	9.97 (18.44) ^{i}	0 (0.0) ^{e}	0 (0.0) ^{b}	10 (18.44) ^{b}	0 (0.0) ^{c}	0 (0.0) ^{b}	90 (71.56) ^{d}
Absolute control	16.10 (23.66) ^{h}	0 (0.0) ^{e}	0 (0.0) ^{b}	0 (0.0) ^{c}	10 (18.44) ^{b}	0 (0.0) ^{b}	90 (71.56) ^{d}
S.Ed	0.121	0.109	0.134	0.083	0.136	0.133	0.190
C.D (p=0.05)	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
Values with different alphabets with in columns differ significantly

Table 4: Anti insect effects of petroleum ether extract of *Senna alata.* L. seed on third instar *Spodoptera litura* Fab. at various concentrations

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) ^{g}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	10 (18.44) ^{c}	0 (0.0) ^{d}	90 (71.56) ^{c}
10%	12.38 (20.62) ^{f}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	0 (0.0) ^{d}	10 (18.44) ^{c}	90 (71.56) ^{a}
20%	16.92 (24.27) ^{e}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	20 (26.56) ^{b}	0 (0.0) ^{d}	80 (63.44) ^{b}
30%	20.29 (26.78) ^{d}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	20 (26.56) ^{b}	0 (0.0) ^{d}	80 (63.44) ^{b}
50%	25.26 (30.20) ^{c}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	40 (39.23) ^{a}	0 (0.0) ^{d}	60 (50.77) ^{a}
70%	28.15 (32.08) ^{b}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	0 (0.0) ^{d}	40 (39.23) ^{a}	60 (50.77) ^{a}
90%	30.13 (33.27) ^{a}	0 (0.0)	0 (0.0)	0 (0.0) ^{b}	20 (26.56) ^{b}	20 (26.56) ^{b}	60 (50.77) ^{a}
Solvent control	10.21 (18.63) ^{h}	0 (0.0)	0 (0.0)	10 (18.44) ^{a}	0 (0.0) ^{d}	0 (0.0) ^{d}	90 (71.56) ^{c}
Absolute control	0 (0.0) ^{i}	0 (0.0)	10 (18.44)	0 (0.0) ^{b}	0 (0.0) ^{d}	0 (0.0) ^{d}	90 (71.56) ^{c}
S.Ed	0.148	-	0.104	0.086	0.126	0.064	0.164
C.D (p=0.05)	0.317	-	N.S	0.177	0.269	0.136	0.350

*Mean of ten replications
Values within parentheses are arc sine transformed
Values with different alphabets with in columns differ significantly

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