



ANTI-INSECT PROPERTIES OF VARIOUS SOLVENT EXTRACTS OF DIFFERENT PLANT PARTS OF *CASCABELA PERUVIANA* PERS. AGAINST *SPODOPTERA LITURA* FAB.

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Abstract

The present study was carried out to find out the Anti-insect properties of various part of *Cascabela peruviana* (Pers.) on *Spodoptera litura* Fab. Various solvent extracts viz., acetone, ethyl acetate, petroleum ether and methanol were used for this study. Among the solvents tested, acetone imparted maximum antifeedant action of 97.21 per cent followed by methanol. Between the plant 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 emergence by imparting 40 and 60 per cent larval and adult malformations respectively. Among the solvent extracts, petroleum ether exhibited minimum anti insect effects in all the plant parts tested.

Key words: various solvent extracts, *Cascabela peruviana* Pers., *Spodoptera litura* Fab.

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). 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). 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). 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; Siqueira *et al.*, 2000; Cork *et al.*, 2003; Aktar *et al.*, 2009).

Hence, a world-wide interest in the development of alternative strategies, including the search for new eco friendly insecticides and use of traditional botanical insecticides has emerged strongly (Dayane *et al.*, 2009). Plant crude extracts often consist of complex mixtures of compounds viz., alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides etc. acting synergistically were known to possess multifarious anti insect properties such as repellence, feeding deterrence, growth inhibition and insecticidal actions (Berenbaum, 1985; Singh and Saratchandra, 2005; Isman, 2006). They gain more importance since they are comparatively effective, safe and ecologically acceptable (Senthilnathan *et al.*, 2005).

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 (Sinha and Biswas, 2008).

Among many promising botanicals of herbaceous

plant One such promising plant called *Cascabela peruviana* (*Thevetia peruviana*) an evergreen shrub, belonging to Apocynaceae family, is a very poisonous shrub in nature and the kernels being the most toxic. *C. peruviana* seed kernels are very rich in cardioactive glycosides, triosides *i.e.* the aglycone of these glycosides consists of three sugar units (Ellenhorn and Barceloux, 1988). Thevetin is a mixture of two triosides namely Thevetin A and Thevetin B (cereberoside). Seed kernel also contains neriifolin, acetylneriifolin, thevefolin, theveneriin and peruvoside which are monoside in nature. Fatty oils constitute more than 62% of the seed kernel. Seed also contains small quantity of theveside, viridoside and perusitin. Apigenin-5-methyl ether has been isolated from seed shells (Oji and Okafor, 2000). Hence, the present investigation was conducted to study the anti-insect properties of various solvent extracts of different plant parts of *C. peruviana* against third instar larvae of *S. litura*.

Materials and methods

Mass culturing of *Spodoptera litura* Fab.

Tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae:Lepidoptera) egg masses were collected from the castor plants grown in and around Annamalainagar (Latitude:11°N, Longitude:79°E). The emerging larvae were maintained in castor leaves up to second instar. Then, they were reared in Bengal gramflour based semi synthetic diet till pupation. The pupae were collected, cleaned, surface sterilized with 0.05% sodium hypochlorite, sexed and transferred into an oviposition cage. Egg masses laid were collected daily, sterilized with 0.05 per cent sodium hypochlorite solution and a continuous culture was maintained. The rearing is done at 26°C and 75 per cent relative humidity (PDBC, 1998).

Collection and extraction of plant materials

Cascabela peruviana whole plants were collected from Annamalainagar and shade dried. Leaves, flowers, seeds, stem and roots were separated, powdered by using wiley mill. Cold solvent extraction method described by Jaglan *et al.* followed for extracting active principle from leaf powder. Powdered plant material was packed as 100 g packets by using Whatman No.40 filter paper and placed inside stoppered round bottom flasks (Capacity: 2 lit.) at the rate of five packets per flask. The flasks were then filled with one liter of respective solvents separately and kept for 72 h at room temperature. Then the extract was filtered and concentrated under reduced pressure by a rotary flash vacuum evaporator. The semisolid extract thus obtained was stored in deep freezer at (Selvamuthukumaran and Arivudainambi, 2010).

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 undiluted solvent extract 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. Each Petri plate was provided with one 4 h pre starved third instar larvae and each treatment was replicated ten times. Solvent and absolute controls were also maintained. Treated leaf discs were collected from the containers after six hours. Then, the leaf area fed was measured graphically an per cent feeding deterrence was computed. The larvae alive were reared using untreated castor leaves till adult emergence and mortality and malformations were recorded (Selvamuthukumaran and Arivudainambi, 2008).

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 *Cascabela peruviana* stem, leaf, root, flower and seed were extracted using solvents such as acetone, ethyl acetate, petroleum ether and methanol. 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 (97.21%) followed by leaf (53.12%) and root (40.29%). 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 *Cascabela peruviana* 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 19.12 per cent in flower to a maximum of 44.34 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 (78.63%) 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. Such similar inhibition in adult emergence was noticed in root extract also. The other extracts of plant parts viz., stem and flower exhibited 60 and 80 per cent adult emergence respectively. These results revealed superior insecticidal action of methanol extract of seed (Table 3).

Table 4 providing the effect of petroleum ether extract of various plant parts of *Cascabela peruviana* 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%, 25.12%, 28.13% and 31.33%

Table 1: Anti insect effects of *Cascabela peruviana* 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	97.21 (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
Stem	31.12 (33.89) ^d	0 (0.0)	0 (0.0)	0 (0.0) ^b	20 (26.56) ^b	0 (0.0) ^c	80 (63.44) ^b
Root	40.29 (39.41) ^c	0 (0.0)	0 (0.0)	0 (0.0) ^b	0 (0.0) ^d	20 (26.56) ^a	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 *Cascabela peruviana* 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	44.34 (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
Stem	30.16 (33.34) ^d	0 (0.0)	0 (0.0)	20 (26.56) ^b	0 (0.0) ^c	0 (0.0) ^d	80 (63.44) ^b
Root	38.19 (38.17) ^b	0 (0.0)	0 (0.0)	0 (0.0) ^d	0 (0.0) ^c	20 (26.56) ^b	80 (63.44) ^b
Flower	19.12 (26.64) ^e	0 (0.0)	0 (0.0)	0 (0.0) ^d	10 (18.44) ^b	0 (0.0) ^d	90 (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 3: Anti insect effects of *Cascabela peruviana* methanol 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	78.63 (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
Stem	33.16 (35.18) ^c	0 (0.0) ^c	0 (0.0) ^b	20 (26.56) ^a	0 (0.0) ^c	20 (26.56) ^a	60 (50.77) ^c
Root	40.12 (39.29) ^b	0 (0.0) ^c	20 (26.56) ^a	20 (26.56) ^a	0 (0.0) ^c	20 (26.56) ^a	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 4: Anti insect effects of *Cascabela peruviana* 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	56.12 (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	31.34 (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
Stem	28.33 (32.01) ^c	0 (0.0)	0 (0.0)	0 (0.0) ^c	20 (26.56) ^a	0 (0.0) ^b	80 (63.44) ^b
Root	25.23 (30.07) ^d	0 (0.0)	0 (0.0)	0 (0.0) ^c	20 (26.56) ^a	0 (0.0) ^b	80 (63.44) ^b
Flower	16.24 (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 (p=0.05)	0.243	-	-	0.214	0.296	0.209	0.309

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

in flower, root, stem 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 petroleum ether extract was found as less superior solvent extract. The 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 petroleum ether 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.

This finding was in accordance with the report of Larvicidal efficacy of methanol leaf extract of *Cascabela peruviana* was tested against the aquatic stages of *Aedes aegypti*. The mortality was noticed against I, II, III, IV instar larvae and pupae of *A. aegypti* after 24 hrs. of treatment with different concentrations (500-700ppm). In this study IV instar larvae of *A. aegypti* showed least

susceptibility than pupae and larval stages. The mortality rate was increased with concentrations and the larvae also undergo malanization slowly (Sathish *et al.*, 2015). Leaf extracts of *Cascabela peruviana* was used to study its larvicidal properties against the larvae of malaria (*Anopheles stephensi*) and dengue (*Aedes aegypti*) vectors. Mean LC50 values of the petroleum ether, chloroform, acetone and methanol extracts of leaves of *Cascabela peruviana* were determined as 0.045, >0.05, 0.026, 0.041 and 0.038, >0.05, 0.021, 0.036%, against the larvae of *A. stephensi* and *A. aegypti* mosquitoes respectively after 24 hours. Delayed impact after 3 days with chloroform extract indicated that the larvicidal action is probably due to the insect growth inhibition (Yadav *et al.*, 2013).

The leaf of *Cascabela peruviana* were extracted in aqueous and treated against the adults of *Holotrichia serrata* (Fab.). The tested aqueous leaf extract effectively produced 50% mortality of *Holotrichia serrata* (Fab.) and their toxicity was 0.025% after 48 hours bioassay (Theurkar *et al.*, 2014). Hence, the present investigation was conducted to study the anti-

insect properties of various solvent extracts of different plant parts of *Cascabela peruviana* against third instar larvae of *S. litura*. The present study revealed presence of multifarious toxic properties of *Cascabela peruviana*, which product development after in depth mode of action and toxicological studies.

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