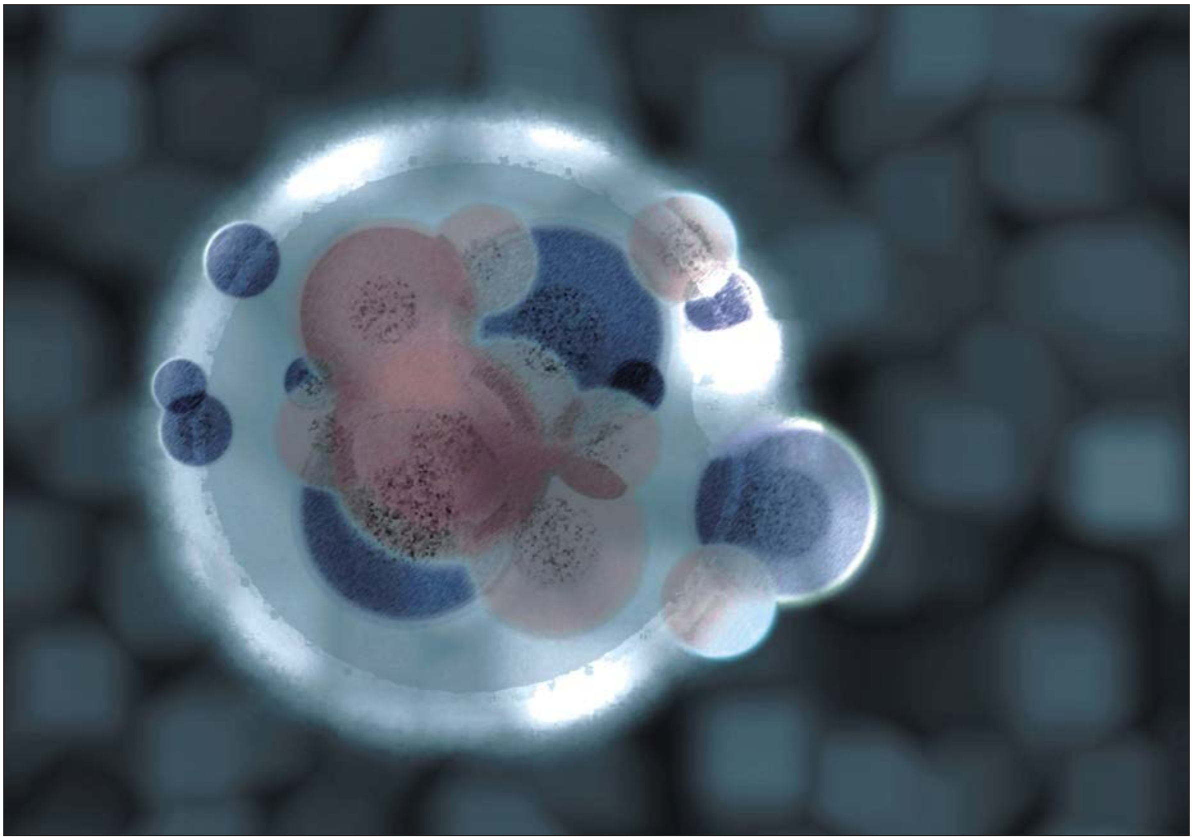

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Bio-efficacy of *Solanum pseudocapsicum* L. (Solanaceae) against Black Cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae)

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Abstract: Bio-efficacy of hexane, dichloromethane and ethyl acetate leaves and seeds extracts of *Solanum pseudocapsicum* were studied against black cutworm *Agrotis ipsilon* to find out their antifeedant, insecticidal and growth regulations activities. Significant antifeedant, insecticidal and growth regulatory activities were noticed on ethyl acetate extracts in seeds. Percentage of deformed larvae, pupae and adults were showed high on seeds extracts. Preliminary phytochemical analysis showed the presence of triterpenoids flovonoids, alkaloids and quinine in both leaves and seeds extracts. This plant can be explored as biopesticidal plant to serve as an alternate control of the management of economically important pest.

Key words: Bioefficacy, *Solanum pseudocapsicum, Agrotis ipsilon,* antifeedant, insecticidal

INTRODUCTION

Applications of chemical pesticides minimize the threat from pest manifestation by rapid knock down effect, *Albeit* with little consideration to the quality (nutritional contents) of the crop and agro-residues (Katyal and Satake, 1996; Kannaiyan, 2002). Due to higher dose and repeated frequency of application, every year one million people suffer from pesticide poisoning (Bami, 1997). Many neurotoxic insecticides are damaging the environment and or pose a threat to public health via food residues, ground water contamination, or accidental exposure (Isman *et al*., 1990).

Plant derived pesticides offer a more natural, 'environmentally friendly' approach to pest control than synthetic insecticides (Leatemia and Isman, 2004). Screening plant extracts for their deleterious effects on insects is one of the approaches used for the search of novel botanical insecticides (Arnason *et al*., 1985; Isman, 1995). Plants synthesize innumerable chemical substances with certain biological activities, which are used in industry for medicinal and agricultural purpose. Chemical examination of natural products for plant protection for the past fifty years has been yielding botanical pesticides based on plant compounds like, azadirachtin, pyrethrins, rotenone, nicotine and physostigmine. Extensive toxicological and biological data are available for such natural products (Koul, 1999; Rahman *et al*., 2003; Raja *et al*., 2005; Iloba and Ekrakene, 2006; Jeyasankar and Jesudasan, 2005; War *et al*., 2011; Jeyasankar *et al*., 2012).

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a polyphagous pest, such as beans, broccoli, cabbage, carrot, spinach, egg plant, lettuce, potato, tomato, turnip, as well as many other plants (Rings *et al*., 1975; Boughton *et al*., 2001). This insect is nearly cosmopolitan and occurs in the US, Europe, Canada, Japan, New Zealand, South Africa, South America, India and the Pacific. *A. ipsilon* has developed resistance in recent years to some of the conventional insecticides. Several attempts to combat the pest species on different crops using synthetic chemical pesticides culminated in problems like insecticide resistance, pest resurgence, outbreaks of secondary pests and environmental pollution (Capinera, 2001). In the present study to evaluate the bioefficacy of plant extracts of *Solanum pseudocapsicum* against black cutworm, *Agrotis ipsilon*.

MATERIALS AND METHODS

Collection and extraction of plant materials: The leaves and seeds of *Solanum pseudocapsicum* (Solanaceae) were collected from the areas in and around Stone house Hill, Ooty, The Nilgiris on January 2010. Plant specimen was identified by Dr. Franklin Jose, Department of Botany, Government Arts College, Ooty, Tamil Nadu, India.

The plant materials were thoroughly washed with tap water and shade dried under room temperature (25.0±2°C) at Department of Wildlife Biology, Government Arts College, Ooty, Tamil Nadu, India. After complete

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drying the plant materials were powdered using electric blender and sieved through kitchen strainer. The 1000 g of plant powder was extracted with hexane, diethyl ether and ethyl acetate, sequentially with increasing polarity of solvents and filtered through Whatman's No. 1 filter paper. The solvents from the crude extract were evaporated to air dryness at room temperature. The crude extracts were collected in clean borosil vials and stored in the refrigerator at 4°C for subsequent bioassay against *A. ipsilon*.

Rearing of black cutworm, *Agrotis ipsilon*: The larvae were collected from cabbage field at Kodappamund, Ooty. Larvae were reared in laboratory condition at the Department of Zoology (Wildlife biology), Government Arts College, Ooty. These laboratory-reared larvae were used for bioassays and the cultures were maintained throughout the study period.

Antifeedant activity: Antifeedant activity of crude extracts was studied using leaf disc no choice method (Isman *et al*., 1990). The stock concentration of crude extracts (5%) was prepared by dissolving in acetone and mixing with dechlorinated water. Polysorbate 20 (Tween 20) at 0.05% was used as emulsifier (Saxena and Yadav, 1983; Thangam and Kathiresan, 1988). Fresh potato leaf discs of 3 cm diameter were punched using cork borer and dipped with 0.625, 1.25, 2.50 and 5.0% concentrations of crude extracts, individually. Leaf discs treated with acetone and without solvent (water) were considered as control. After air-drying, each leaf disc was placed in petri dish (1.5×9 cm) containing wet filter paper to avoid early drying of the leaf disc and a single 2 h pre-starved fourth instar larva of *A. ipsilon* was introduced. For each concentration five replicates were maintained. Progressive consumption of leaf area by the larva after 24 h feeding was recorded in control and treated discs using graph sheet method. Leaf area consumed in plant extract treatment was corrected from the control. The percentage of antifeedant index was calculated using the formula of Ben Jannet *et al*. (2000):

$$\text{Antifeedant index} = \frac{C-T}{C+T} \times 100$$

where, C and T represent the amount of leaf eaten by the larva on control and treated discs respectively.

Insecticidal activity: Fresh potato leaves were treated with different concentrations (as mentioned in antifeedant activity) of crude extracts. Potato leaves treated with acetone and without solvent were considered as control. Petioles of the potato leaves were tied with wet cotton plug (to avoid early drying) and placed in round plastic trough (29 cm×8 cm). In each concentration 10 pre-starved (2 h) IV instar larvae of *A. ipsilon* were introduced individually and covered with muslin cloth. Five replicates were maintained for all concentrations and the number of dead larvae was recorded after 24 h up to pupation. Percentage of larval mortality was calculated and corrected by Abbott's formula (Abbott, 1925):

$$\text{Abbott's corrected mortality (\%)} = \frac{\text{\% Mortality in treated-\% Mortality in control}}{\text{100-\% mortality in control}} \times 100$$

Growth regulation activity: Growth regulation activities of crude extracts were studied at four different concentrations against IV instar larvae of *A. ipsilon*. Ten larvae were introduced in a petri plate having potato leaves treated with different concentrations of crude extracts. Water or acetone treated leaves were considered as control. After 24 h feeding, the larvae were transferred to normal leaves for studying the developmental period. For each concentration five replicates were maintained. During the developmental period, deformed larvae, pupae, adults and successful adults emerged were recorded. In addition, weight gain by the treated and control larvae were also recorded.

RESULTS

Crude extracts prepared from *Solanum pseudocapsicum* using solvents of hexane, diethyl ether, ethyl acetate and their bioactivities were tested at different concentrations against larvae of *Agrotis ipsilon*. The bioactivity data collected were subjected to one-way analysis of variance (ANOVA). Significant difference between the mean was separated using Least Significant Difference (LSD) test.

Antifeedant activity of crude extracts: Antifeedant activity of the crude extracts of the leaves and seeds of *S. pseudocapsicum* were studied at different concentrations and the results are presented in Table 1.

Table 1: Antifeedant activity of crude extracts of *S. pseudocapsicum* (leaves and seeds) against fourth instar larvae of *A. ipsilon*

	Concentrations (%)			
Solvent extracts	0.625	1.25	2.50	5
Leaves				
Hexane	12.15±6.42ᵃ	26.52±4.12ᵇ	35.31±3.44ᵇ	39.43±6.87ᵇ
Diethyl ether	25.74±4.45ᵇ	26.35±3.45ᵇ	39.94±4.86ᵇ	45.84±6.38ᵇ
Ethyl acetate	32.54±2.41ᶜ	35.28±4.12ᶜ	51.93±5.80ᶜ	68.31±8.63ᶜ
Solvent control	4.20±3.61ᵃ	4.20±3.61ᵃ	4.20±3.61ᵃ	4.20±3.61ᵃ
Seeds				
Hexane	13.25±6.40ᵃ	28.56±5.13ᵇ	37.33±3.40ᵇ	40.46±6.57ᵇ
Diethyl ether	24.7±4.53ᵇ	29.34±2.43ᵇ	42.90±3.81ᵇ	55.82±7.33ᶜ
Ethyl acetate	32.02±7.41ᶜ	45.23±4.84ᶜ	58.94±5.22ᶜ	81.30±8.62ᵈ
Solvent control	5.22±4.62ᵃ	5.22±4.62ᵃ	5.22±4.62ᵃ	5.22±4.62ᵃ

Values are mean of five replications, within the row similar alphabets are statistically non significant at p>0.05 by LSD

Table 2: Insecticidal activity of crude extracts of *S. pseudocapsicum* (leaves and seeds) against fourth instar larvae of *A. ipsilon*

	Concentrations (%)			
Solvent extracts	0.625	1.25	2.5	5
Leaves				
Hexane	4.12±3.12[a]	3.87±3.02[a]	4.36±4.41[a]	7.40±5.82[a]
Diethyl ether	2.65±3.43[a]	6.43±5.44[a]	12.93±6.8[ab]	19.84±6.35[b]
Ethyl acetate	12.60±7.44[b]	16.20±4.17[b]	19.17±3.81[c]	38.50±7.23[c]
Solvent control	1.20±3.12[a]	1.20±3.12[a]	1.20±3.12[a]	1.20±3.12[a]
Seeds				
Hexane	8.24±5.48[a]	12.68±7.14[b]	14.63±6.45[b]	18.43±5.87[b]
Diethyl ether	8.95±6.40[a]	13.46±9.47[b]	19.49±4.8[bc]	23.87±6.32[b]
Ethyl acetate	10.60±9.42[ab]	24.23±4.16[c]	45.40±7.83[cd]	60.12±6.62[c]
Solvent control	1.20±3.12[a]	1.20±3.12[a]	1.20±3.12[a]	1.20±3.12[a]

Values are mean of five replications, within the row similar alphabets are statistically non significant at p>0.05 by LSD

Table 3: Percentage of deformed stages of *A. ipsilon* due to the treatment of ethyl acetate extract (5%) of *S. pseudocapsicum*

Treatment	Larvae	Pupae	Adult	Successful adult emergence
Leaves	10.0[a]	7.5[a]	9.2[a]	73.3[b]
Seeds	27.2[b]	21.8[ab]	22.5[b]	28.5[a]
Control	1.0[a]	0.5[a]	1.0[a]	97.5[c]

Values are mean of five replications, within the row similar alphabets are statistically non significant at p<0.05 by LSD

Antifeedant activity of solvent extracts was assessed based on antifeedant index. Higher antifeedant index normally indicates decreased rate of feeding. In the present study irrespective of concentration and solvents used for extraction the antifeedant activity varied significantly. The antifeedant activity of the extract of leaves and seeds were tested at different concentrations. Data pertaining to the above experiment clearly revealed that maximum antifeedant activity was recorded in ethyl acetate extract of seeds (81.30%) and followed by ethyl acetate extract of leaves extract (68.31) at 5% concentration compared to control. One-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test showed statistical significance (p<0.05) compared to control.

Insecticidal activity of crude extracts: Insecticidal activity of crude extracts of leaves and seeds of *S. pseudocapsicum* was studied at different concentrations and the results are presented in Table 2. Insecticidal activity of solvent extracts was calculated based on larval mortality after treatment. High larval mortality normally indicates potential insecticidal activity of plant extracts. In the present study irrespective of concentration and solvents used for extraction the insecticidal activity varied significantly. Data pertaining to the insecticidal activity clearly revealed that maximum insecticidal activity was recorded in ethyl acetate extract of seeds (60.12%) whereas in leaves extract from ethyl acetate showed 38.5% at 5% concentrations compared to control. One-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test showed statistical significance (p<0.05) compared to control.

Percentage of deformities of crude extracts: Percentage of deformities due to the treatment of crude extracts from leaves and seeds of *S. pseudocapsicum* at 5% concentration is presented in Table 3. Maximum larval, pupal and adult deformities were observed in seeds extract compared to leaves extract and control. Percentage of successful adult emergence was found maximum in leaves extract (73.3%).

Preliminary phytochemical analysis: Ethyl acetate extract of leaves and seeds were subjected to preliminary phytochemical analysis for the confirmation of major group of compounds. Both extracts showed positive results for confirmation of triterpenoids, flovonoids and alkaloids. Phenols only present in leaves extract and quinine found in seeds extract.

DISCUSSION

Plants are a rich source of organic chemicals on earth. Already 10,000 secondary metabolites have been chemically identified. In nature many plants have unpalatable substances like high content of phenols, alkaloids, flavonoids, terpenes, quinone, coumarin etc, which play a defensive role against insect pests. These substances possess wide range of biological activities including antifeedant, oviposition deterrent, insecticidal, ovicidal and Insect Growth Regulators (IGRs). Identifying sources with useful biological activity is only the starting point in the long process of development of a botanical pest management product. Success of botanical in the field depends on number of factors such as, ongoing availability of the natural resources, adequate biomass to justify extraction, the feasibility of extraction near the harvest site and the stability of the extract in storage after preparation (Isman *et al*., 1997).

Antifeedant activity: Antifeedant is defined as a chemical that inhibits feeding without killing the insect directly, while the insect remains near the treated foliage and dies through starvation (Yasui *et al*., 1998). Most potent insect antifeedants are quinoline, indole alkaloids, sesquiterpene lactones, diterpenoids, and triterpenoids (Schoonhoven, 1982). The present study, ethyl acetate extract of *S. pseudocapsicum* (seeds) was promising in reducing feeding rate of *A. ipsilon.* The rate of feeding significantly varied depending on the concentration of the plant extracts. This indicates that the active principles present in the seeds inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence. Several authors have reported that plant extracts possess similar type of antifeedant activity against lepidopteran pests (Jeyarajan *et al*., 1990; Sahayaraj, 1998; Morimoto *et al*., 2002; Jeyasankar and Jesudasan, 2005; Abdullah and Subramanian, 2008; Jeyasankar *et al*., 2010, 2011, 2012; Pavunraj *et al*., 2012).

Antifeedant chemicals play a major role in the unsuitability of non-host plants as food for insects. Isolation and structure elucidation of these active chemicals is important not only for understanding the ecological aspects of insect pest's relationship, but also for their potential in insect pest's control (Yasui *et al*., 1998). In the present study, preliminary phytochemical analysis revealed that triterpenoids, flovonoids, alkaloids and quinines present in the ethyl acetate extracts (seeds) indicates that higher percentage of antifeedant activity. These findings are in agreement with the earlier reports of Morimoto *et al*. (1999). They have reported that quinone, remirol and cyperquinone isolated from the plants of the family Cyperaceae had strong antifeedant activity against *S. litura*.

Insecticidal activity: Screening plant extracts for deleterious effects on insects is one of the approaches used in the search for novel botanical insecticides (Isman *et al*., 2001). Secondary plant compounds act as insecticides by poisoning *per se* or by production of toxic molecules after ingestion. These compounds also deter or possibly repel an insect from feeding (Lajide *et al*., 1993; Jeyasankar *et al*., 2011; Khatter, 2011). In the present study ethyl acetate extract from seeds of *S. pseudocapsicum* exhibited significant insecticidal activity at 5% concentration. It is possible that the insecticidal property present in the selected plant compound may arrest the various metabolic activities of the larvae during the development and ultimately the larvae failed to moult and finally died.

In the present study preliminary phytochemical analysis revealed that alkaloid and quinones present in the ethyl acetate extract indicate that higher percentage of insecticidal activity observed in seeds extract of *S. pseudocapsicum*. Similar works have already reported insecticidal activity of many plants and their compounds against different groups of insects (Rajam, 1991; Bohnenstengel *et al*., 1999; Isman, 2000; Leatemia and Isman, 2004; Jeyasankar *et al*., 2010; Khatter, 2011).

Insect growth regulation: Insect growth regulation properties of plant extracts are very interesting and unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active principles enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying (Koul and Isman, 1991). In the present study, deformed development of larvae, pupae and adults were noted. Among the extract tested, percentage of deformed larvae, pupae and adults were observed maximum on seeds extract of *S. pseudocapsicum*.

The morphological deformities are due to toxic effects of plant crude extracts or active compounds on growth and developmental processes of *A. ipsilon*. Since morphogenetic hormones regulate these processes, it can be suggested that these active chemicals interfere with juvenile hormones of the insects. These results are consistent with the earlier reports on various lepidopteran species (Caasi-Lit and Morallo-Rejesus, 1990; Koul and Isman, 1991; Sahayaraj, 1998; Jeyasankar and Jesudasa, 2005; Jeyasankar *et al*., 2011, 2012).

CONCLUSION

In conclusion, ethyl acetate extract of seeds of *S. pseudocapsicum* showed higher insecticidal and growth inhibition activities against *A. ipsilon* and it is first time report on *A. ipsilon*. Hence, it may be suggested that the extract of *S. pseudocapsicum* can be used for controlling the insect pest, *A. ipsilon* and need to be explore the active chemicals of *S. pseudocapsicum*.

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