

Chemical composition and growth inhibitory activities of *Solanum pseudocapsicum* against *Spodoptera litura* and *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

The aim of this research works was to determine the chemical composition and insect growth inhibitory activity of ethyl acetate crude extract of *Solanum pseudocapsicum* fruits against the *Spodoptera litura* and *Helicoverpa armigera*. Ethyl acetate extracts of *S. pseudocapsicum* fruits were fractionated using Silica-gel Column Chromatography and analysed by GC-MS analysis. Total of 47 compounds were identified in the ethyl acetate extracts of *S. pseudocapsicum*. The major compounds which is responsible for the insect growth inhibitory activity on tested larvae of *S. litura* and *H. armigera* were found in n-Propyl acetate, 1,2,3-Propanetriol, monoacetate, Tetradecanoic acid, 1,2,3-Propanetriol, 1-acetate, Triacetin, 9-Hexadecenoic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester. The maximum larval mortality was observed in Tetradecanoic acid (98.80 ± 0.88 and 96.70 ± 3.29), 9-Hexadecenoic acid (98.40 ± 4.33 and 95.70 ± 3.26) and n-Hexadecanoic acid (98.30 ± 4.33 and 95.20 ± 3.26) also exhibited contact toxicity against *S. litura* and *H. armigera*, respectively. The results indicated that this compounds of Tetradecanoic acid, 9-Hexadecenoic acid and n-Hexadecanoic acid showed potential in to serve as an alternate botanical pesticide in the management of *S. litura* and *H. armigera*.

Keywords: Antifeedant, Insecticidal, growth inhibition activities, *Spodoptera litura*, *Helicoverpa armigera*, *Solanum pseudocapsicum*

Introduction

Synthetic insecticides are used widely for the control of various insect pests because they can be applied whenever and wherever needed, economical and most important thing is the reliability of control methods. Hence the production and consumption of pesticides has greatly increased in recent years. Now Indian pesticide industry is the fourth largest in the world and 75% output of total market. At present India is the largest manufacturer of chemical pesticides in Asia and twelfth ranks in globally for use pesticides with an annual production of 90,000 tones (Mathur, 1999; Chitra *et al.* 2006)^[33, 13]. Chemical pesticides are generally persistent in nature. The World Health Organization (WHO) estimated to have 2,00,000 people are killed worldwide (CAPE, 2009)^[11] and Due to a higher dose and repeated frequency of application, every year one million people suffer from pesticide poisoning, cardiopulmonary, neurological and skin disorders, fetal deformities, miscarriages, lowering the sperm count of applicators (Bami, 1997; Abhilash and Singh, 2009)^[6, 2]. Many neurotoxic insecticides are damaging the environment and pose a hazard to public health via food residues, ground water contamination and accidental exposure (Isman *et al.* 1990; Ezhil Vendan, 2016)^[21, 18]. Frequent exposure of *Spodoptera litura* to synthetic pesticides resulted in the development of resistance, as a consequence of which an unexpected population outbreak was observed in the major cotton and groundnut growing regions of Andhra Pradesh and Tamil Nadu (Johny and Muraliragan, 2000)^[28]. Indiscriminate use chemical pesticides have resulted in pollinators and other wildlife, upsetting the balance of nature

through disruption of natural enemies and outbreaks of secondary pests (Jeyasankar and Jesudasan, 2005; Dubey *et al.* 2011)^[22, 15].

Furthermore, *S. litura* has been reported to develop a high resistance against some variety of chemical pesticides, including organophosphate, carbamate, pyrethroids and few newer synthetic insecticides (spinosad, indoxacarb, abamectin, emamectin fipronil) with field control failure observed very frequently (Ahmad, 2008^[3]; Saleem *et al.* 2008)^[41]. 50% of all insecticides used in India and China are to control against *Helicoverpa armigera* respectively (Lammers and Macleod, 2007)^[31] but the continuous and indiscriminate use of synthetic insecticides over the years was explode to developed resistance of certain molecules belonging to different classes of insecticides in various parts of the world in *H. armigera* (Chaturvedi, 2007; Yang *et al.* 2013)^[12, 43]. Dreadful facts of chemical pesticides demand for Eco friendly and environmentally safer alternate methods for crop protection. Chemical control is an effective strategy used extensively in daily life activity against insects. At the present investigation known about a variety of new insect control agents have been developed, or are being developed, which may fit a variety of insect pest management needs (Hirashima, 2008)^[20].

Plants synthesize innumerable chemical substances with certain biological activities, which are used in industry for medicinal and agricultural purpose in ancient time. Repellency activity in plant material has been demoralized for thousands of years before by man by hanging bruised plants in houses against to control of insects. Plant secondary compounds have been systematically exhibited in an effort to discover new

sources of botanical insecticides. The secondary metabolites like *alkaloids, terpenoids, flavonoids, phenols, glycosides, sitosterols, and tannis*. These phytochemicals are known to crop protection against insect pests (Ahmad, 2007) [4]. Botanical pesticides are experiencing a revival due to their ecotoxicological properties (Cosimi *et al.* 2009) [14] and its play a vital role in ecological systems (Garcia *et al.* 2007) [19]. They may provide possible alternative of currently used insect control agents because they constitute a rich source of bioactive chemicals in plants (Qin *et al.* 2010) [37]. However these phytopesticides are naturally slow acting substance (Leatemia and Isman, 2004) [32], mostly safer to non target organisms and integrated crop management approaches (Khater, 2011) [30]. Even though plant based pesticides are structurally more complex, selective and biodegradable compared with common chemical pesticides (Rattan, 2010) [39]. Furthermore antifeedant and insecticidal activities of *Phyllanthus niruri, Hibiscus ovalifolius, Achyranthes aspera, Abutilon indicum, Senna auriculata* and *Solanum trilobatum* were tested against *Henosepilachna vigintioctopunctata* (Jeyasankar *et al.* 2014) [24]. Repellent and larvicidal activity of *Corymbia citridora, Cymbopogon citrates, syzygium aromaticum, Gaultheria procumbens* and *Cymbopogon nardus* soils were tested against stored insect pests (Jeyasankar *et al.* 2016) [23]. Botanical pesticide of azadirachtin induces no accumulations in the soil, no phytotoxicity and accumulation is seen in plants, and no adverse effects occur in groundwater (Mehlhorn, 2011) [34]. Previous studies revealed that *S. pseudocapsicum* seed extracts showed significant antifeedant, insecticidal and growth inhibition activities tested against *Spodoptera litura, Helicoverpa armigera* and black cutworm, *Agrotis ipsilon* (Jeyasankar *et al.* 2012a,b) [26, 27]. Further, the present investigation was carried out to evaluate the chemical composition and growth inhibitory activities of isolated fractions of *Solanum pseudocapsicum* against economically important lepidopteron pests.

Materials and Methods

Collection of plant materials

The fruits parts of *Solanum pseudocapsicum* were collected from Pullian, Namakkal District, Tamil Nadu, India. The voucher specimen of *S. pseudocapsicum* (IPH No. 23) was prepared and deposited in PG and Research Department of Zoology, Government Arts College, Coimbatore, Tamil Nadu, India.

Soxhlet extraction method

The plant materials were thoroughly washed with tap water and shade dried under room temperature (27.0± 2°C and 75 ± 5% RH). After complete drying the plant materials were powdered using electric blender and sieved through a kitchen strainer. 1000g of plant powder was extracted by Soxhlet extraction methods with ethyl acetate solvents and filtered through Whatman's No. 1 filter paper. The solvent from the crude extract were evaporated to air dried at room temperature. The crude extracts were collected in clean borosil vials and stored in the refrigerator at 4°C for subsequent bioassay against *S. litura* and *H. armigera*.

Field collections and rearing of *Helicoverpa armigera* and *Spodoptera litura*

Different larval stages of *H. armigera* and egg masses and larvae of *S. litura* were collected from bhendi and caster fields at Anaipatty near Arignar Anna Government Arts College Musiri, Tiruchirappalli district, Tamil Nadu, India. The collected *H. armigera* larvae were reared in fresh bhendi vegetables on individually in a plastic container (for avoiding cannibalistic activity) and eggs and larvae of *S. litura* were reared on leaves of castor (*Ricinus communis*) till they attain the pupal stage under laboratory conditions (27 ± 2°C and 75 ± 5% RH). Sterilized soil will be provided for pupation. After pupation, the pupae were collected from soil and placed inside the oviposition samplers' (46×40.5×40.5). After adult emergence, cotton soaked with 10% sugar solution with a few drops of multivitamins was kept inside oviposition cage for adult feeding. After hatching newly emerged larvae will be providing bhendi for feeding of *H. armigera* and castor leaf for *S. litura*. Generally, healthy and uniform sized fourth instar larvae, pupae, meat and eggs were used for the experiments and the cultures were maintained throughout the study period.

Antifeedant assays

Antifeedant activity was studied using leaf disc no choice method (Isman *et al.* 1990) [21]. Required concentration of fractions was prepared by dissolving in acetone and mixing with dechlorinated water. Polysorbate 20 (Tween 20) at 0.05% was used as an emulsifier (Subramonithangam and Kathiresan, 1988) [42]. Fresh cotton leaf (for *H. armigera*) and castor leaf (for *S. litura*) discs of 3 cm diameter were punched using a cork borer and dipped in 250, 500, 750 and 1000 ppm for fractions separately and air dried for 5 minutes. After air drying, treated leaf discs were kept inside the Petri dishes (15mm × 90 mm diameter) separately containing wet filter paper to avoid drying of the leaf disc and single 2hrs post starved fourth instar larva of *H. armigera* and *S. litura* was introduced on each treated leaf disc. Neemazal was considered as constant. Ten replications were maintained for each treatment. A progressive consumption of leaf area by the larva in 24 hrs period was recorded in control and treatments using a leaf area meter (systronics 211). Leaf area consumed in plant extract and fraction treatments was corrected from the control. The percentage of antifeedant index was calculated using the formula of (Ben Jannet *et al.* 2000) [8].

$$AFI = \frac{C - T}{C + T} \times 100$$

Where AFI = Antifeedant Index; C = Area protected in control leaf disc; T = Area protected in treated leaf disc.

Larvicidal assays

For the evaluation of larvicidal activity of the fractions against the selected pest, the fractions was tested on a wide range of concentration, from that a narrow range of concentration was derived. Thus, 250, 500, 750 and 1000 ppm concentrations for fractions were tested against the freshly moulted (0-6h) fourth instar larvae of *H. armigera* and *S. litura*. The branches

bearing cotton leaves were tied with wet cotton plug to avoid early drying and placed in a plastic trough (29cm × 8cm). In each concentration 10 pre-starved (2hrs) fourth instar larvae were introduced individually and covered with muslin cloth. Neemazal was considered as constant. Five replicates were maintained for each concentration, each replicates comprised of 25 numbers of larvae. After 24h of the exposure period, the number of dead larvae was recorded from each replicates at all the concentrations and the percentage of larval mortality was calculated using Abbott's formula (Abbott 1925) [1]. The larvae with no symptom of a movement or shake while touching with soft camel brush were considered as dead.

$$\text{Abbotts Percent Corrected Mortality} = \frac{\% \text{ MT} - \% \text{ MC}}{100 - \% \text{ MC}} \times 100$$

Where, % MT = % Larvae mortality in treatment and % MC = % Larvae mortality in control. The LC₅₀ and LC₉₅ values were calculated by probit analysis using software SPSS16.00.

Growth Regulation Activity

Growth regulation activity of fractions was studied at four different concentrations (as mentioned in antifeedant activity) against fourth instar larvae of *S. litura* and *H. armigera*. Ten larvae were introduced in a Petri-plate containing tomato leaves treated with different concentrations of fractions. Neemazal was considered as constant. After 24 hrs of 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.

2.7 Gas Chromatography and Mass Spectroscopy Analysis

Gas chromatography-mass spectrometer analysis was performed on an Elmer Clarus 500 Software GC equipped with capillary column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane). The oven temperature was programmed from 200°C to 150°C at the rate of 4 oC min⁻¹ and held at this temperature for 5 min. The inlet and interface temperature were 250 to 280 °C. The carrier gas was helium at a flow rate of 1.0 mL min⁻¹ (constant flow). The sample (1.0µL) was injected with split 20:1. Electron impact mass spectrometry was carried out at 70eV. Ions source and quadrupole temperature were maintained at 230 to 150 °C. The Mass range was 40-600amu and compounds were identified using the library-NIST 2005.

Statistic Analysis

Data analysis was carried out using Microsoft Excel 2007. One -Way ANOVA was performed for all the experimental data from that Least Significant Difference was calculated and the significant differences were marked with different alphabet. LC₅₀, LC₉₀ was carried out using SPSS 16.00.

Results

Antifeedant activity of different fractions isolated from ethyl acetate extracts of *S. pseudocapsicum* tested against fourth instar larvae of *S. litura* and *H. armigera* and the results

pertaining to different concentrations are presented in the Table: 1 Different concentration showed varying range of antifeedant activity. It was noted that the antifeedant activity of fraction 7 was found to be statistically significant than the other fractions tested against the fourth instar larvae of *S. litura* and *H. armigera*. Initially, at 1000ppm concentration of fraction 7 showed 78.99±1.64 and 70.23±3.11 area of leaf protection from the larvae feeding followed by 67.88±4.34 and 61.35±8.48 (fraction 3), 59.99±3.33 and 59.92±3.56 (fraction 6), 34.73±2.31 and 31.35±1.26 (fraction 5), 28.81±3.19 and 22.23±2.02 (fraction 4), 27.88±2.31 and 20.44±3.15 (fraction 8), 21.66±1.33 and 20.11±2.14 (fraction 2), 19.17±3.53 and 18.61±2.14 (fraction 1), respectively (Table 1).

Insect growth inhibition activity of different fractions isolated from ethyl acetate extracts from *S. pseudocapsicum* against fourth instar larvae of *S. litura* and *H. armigera* with the results pertaining to the above experiments are presented in Table 2. Initially, Fraction 7 showed (25.30±1.71 and 27.00±1.58%) larval, (25.40±2.43 and 22.80±1.59%) pupal and (25.80±1.99 and 26.20±3.16 %) of adult deformities against *S. litura* and *H. armigera* at 1000ppm concentration. In addition to 23.50±3.02 and 24.20±3.04% of successful adult emergence was noticed in 7th fraction isolated from ethyl acetate fruits extracts of *S. pseudocapsicum* exerted at the same concentration respectively (Table:2).

Besides, fraction 3 caused (25.30±3.04 and 25.80±0.95%) larval, (24.70±2.06 and 22.20±2.22%) pupal and (20.60±1.11 and 21.50±2.87%) of adult deformities at 1000ppm concentration. Even though, 29.40±3.23 and 30.50±3.78% of successful adult emergence was recorded in same fraction, respectively. Fraction 6 caused (22.40±4.21 and 25.20±1.52%) larval, (25.80±5.18 and 26.40±4.00%) pupal and (25.30±2.81 and 21.10±3.03%) of adult deformities at 1000ppm concentration. Even though, 28.50±3.37 and 27.30±2.66% of successful adult emergence was recorded in same fraction, respectively. Fraction 5 caused (20.20±4.81 and 17.40±2.19%) larval, (17.80±3.27 and 18.00±3.67%) pupal and (18.60±2.60 and 18.00±2.44%) of adult deformities at 1000ppm concentration. Even though, 43.40±4.22 and 47.60±3.78% of successful adult emergence was recorded in same fraction, respectively.

Fraction 2 caused (19.60±2.36 and 17.40±2.21%) larval, (17.40±1.84 and 13.60±0.83%) pupal and (12.60±1.92 and 9.40±1.88%) of adult deformities at 1000ppm concentration. Even though, 50.40±5.98 and 59.60±5.15% of successful adult emergence was recorded in same fraction, respectively. Fraction 4 caused (8.25±3.27 and 6.24±2.89%) larval, (5.30±4.80 and 3.62±2.17%) pupal and (9.50±3.34 and 7.00±3.58 %) of adult deformities at 1000ppm concentration. Even though, 76.85±2.44 and 82.13±2.87% of successful adult emergence was recorded in same fraction, respectively. Fraction 1 caused (8.96±2.44 and 4.00±1.58%) larval, (5.44±1.12 and 4.32±0.64%) pupal and (8.8±1.67 and 5.38±1.25%) of adult deformities at 1000ppm concentration. Even though, 76.80±2.77 and 85.80±4.08% of successful adult emergence was recorded in same fraction, respectively. Fraction 8 caused (7.40±1.66 and 4.50±1.26%) larval, (6.40±1.58 and 5.00±1.11%) pupal and (2.20±2.36 and 5.50±1.79%) of adult deformities at 1000ppm concentration. Even though, 81.00±5.61 and 86.10±4.61% of successful

adult emergence was recorded in same fraction, respectively. The previous experiment the seven fractions obtained from the ethyl acetate extracts of *S. pseudocapsicum* was tested for their larvicidal activity against the fourth instar larvae of *S. litura* and *H. armigra* on perusal of the (Table:3) revealed that Fraction 7 showed the LC₅₀(LCL-UCL) value of 502.36 (343.17-735.11) and 588.83(509.52-688.71) and LC₉₀(LCL-UCL) was calculated to be 1200.22 (902.56-2006.14) and 1412.03 (1213.85-1717.24), ($\chi^2= 6.79$ and 4.906) against *S. litura* and *H. armigra* respectively. Accordingly, Fraction 3 showed the LC₅₀ (LCL-UCL) value of 693.466 (609.17-804.13) and 733.94 (640.75-861.14) and LC₉₀ (LCL-UCL) was calculated to be 2199.56 (1776.02-1858.83) and 1576.19 (1350.25-1930.66) ($\chi^2= 2.705$ and 4.406) against *S. litura* and *H. armigra* respectively. Likewise, for Fraction 6 showed the LC₅₀ (LCL-UCL) value of 719.68 (630.22-838.14) and 762.72 (660.24-908.04) and LC₉₀ (LCL-UCL) was calculated to be 1523.33(1305.77-1874.60) and 1672.34(1414.86-2087.60), ($\chi^2=0.511$ and 4.883) against *S. litura* and *H. armigra* respectively. Likewise, for Fraction 5 showed the LC₅₀ (LCL-UCL) value of 1168.23 (970.40-1523.37) and 1297.23(1048.35-1796.59) and LC₉₀ (LCL-UCL) was calculated to be 2300.23 (1845.81-1796.59) and 2576.22(2003.71-3782.36), ($\chi^2= 0.068$ and 4.344) against *S. litura* and *H. armigra* respectively. Likewise, for Fraction 4 showed the LC₅₀ (LCL-UCL) value of 1427.23 (1142.22-2069.67) and 1644.32(1253.21-2629.06) and LC₉₀ (LCL-UCL) was calculated to be 2765.32(2115.04-4016.16) and 3175.03(2344.47-5392.40), ($\chi^2=3.770$ and 1.283) against *S. litura* and *H. armigra* respectively. Likewise, for Fraction 2 showed the LC₅₀ (LCL-UCL) value of 1496.03 (1200.41-2111.14) and 1597.12(1261.94-2338.55) and LC₉₀ (LCL-

UCL) was calculated to be 2709.33(2098.19-4026.98) and 2858.21(2178.70-4409.89), ($\chi^2=3.720$ and 4.70) against *S. litura* and *H. armigra* respectively. Likewise, for Fraction 1 showed the LC₅₀ (LCL-UCL) value of 1798.03 (1351.40-2996.00) and 1743.22(1336.44-2761.27) and LC₉₀ (LCL-UCL) was calculated to be 3325.00(2402.07-5866.06) and 3136.21(2315.61-527.74), ($\chi^2=2.547$ and 4.687) against *S. litura* and *H. armigra* respectively. Likewise, for Fraction 8 showed the LC₅₀ (LCL-UCL) value of 1814.23 (1371.32-2977.69) and 1963.03(1436.82-3542.59) and LC₉₀ (LCL-UCL) was calculated to be 3270.33(2378.68-5672.44) and 3581.00(2517.73-6856.12), ($\chi^2=4.712$ and 2.920) against *S. litura* and *H. armigra* respectively.

Ethyl acetate extracts of *S. pseudocapsicum* fruits parts were obtained from hydrodistillation and investigated by GC and GC-MS. A total of 47 compounds (table 4) of the ethyl acetate extracts of *S. pseudocapsicum* fruits parts were identified, respectively. The principal compounds in *S. pseudocapsicum* fruits parts were n-Propyl acetate, 1,2,3-Propanetriol, monoacetate, Tetradecanoic acid, 1,2,3-Propanetriol, 1-acetate, Triacetin, 9-Hexadecenoic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (table 5). The maximum larval mortality was observed in Tetradecanoic acid (98.80±0.88 and 96.70±3.29), 9-Hexadecenoic acid (98.40±4.33 and 95.70±3.26) and n-Hexadecanoic acid (98.30±4.33 and 95.20±3.26) also exhibited contact toxicity against *S. litura* and *H. armigera* respectively. The results indicated that this compounds of Tetradecanoic acid, 9-Hexadecenoic acid and n-Hexadecanoic acid show potential in to serve as an alternate botanical pesticide in the management of *S. litura* and *H. armigera* (table 6).

Table 1: Antifeedant activity different fractions of *Solonom pseudocapsicum* tested against fourth instars larvae of *S.litura* and *H.armigera*

Fractions tested	<i>S. litura</i>				<i>H. armigera</i>			
	Concentrations tested (ppm)							
	125	250	500	1000	125	250	500	1000
Fraction 1	7.22±2.27 ^a	9.89±4.32 ^a	12.22±2.23 ^a	19.17±3.53 ^a	6.41±3.41 ^a	8.16±4.88 ^a	11.99±3.33 ^a	18.61±2.14 ^a
Fraction 2	9.80±3.27 ^{ab}	12.22±2.27 ^b	17.27±2.33 ^b	21.66±1.33 ^b	8.17±2.54 ^{ab}	13.12±5.58 ^b	17.22±3.42 ^{bc}	20.11±2.14 ^{ab}
Fraction 3	13.11±2.72 ^b	21.11±3.33 ^c	40.22±3.34 ^f	67.88±4.34 ^f	19.91±2.88 ^c	29.14±6.63 ^d	43.63±4.87 ^e	61.35±8.48 ^d
Fraction 4	9.63±2.04 ^{ab}	17.92±2.91 ^b	21.27±1.51 ^c	28.81±3.19 ^c	8.94±4.48 ^{ab}	13.32±3.53 ^b	15.59±4.31 ^b	20.44±3.15 ^{ab}
Fraction 5	10.12±3.22 ^b	14.12±4.23 ^{bc}	23.21±3.24 ^{cd}	34.73±2.31 ^d	11.11±3.45 ^c	18.33±4.28 ^c	22.81±3.12 ^d	31.35±1.26 ^{cd}
Fraction 6	15.22±2.77 ^b	28.21±3.55 ^d	33.75±2.70 ^{cd}	59.99±3.33 ^e	16.12±3.88 ^b	23.37±4.72 ^c	39.16±5.22 ^c	59.92±3.56 ^c
Fraction 7	21.22±2.79 ^a	34.73±2.17 ^f	56.99±4.23 ^e	78.99±1.64 ^e	20.89±2.74 ^d	31.90±2.49 ^{df}	50.89±3.33 ^f	70.23±3.11 ^f
Fraction 8	10.00±3.45 ^b	13.63±3.26 ^b	18.10±5.02 ^b	27.88±2.31 ^c	9.23±3.27 ^{ab}	17.39±5.31 ^{bc}	20.25±3.13 ^{cd}	22.23±2.02 ^b
Neemazal	78.88±2.11	100±00.00	100±00.00	100±00.00	75.34±2.99	100±00.00	100±00.00	100±00.00

Values are mean ±Standard deviation of five replications; Values in parentheses are angular transformed; ANOVA followed by Duncan Multiples Range Test (DMRT) was performed; Superscripts alphabet in the values are significantly different at p<0.05% Control group was fed with host plant without the treatment of chemicals

Table 2: Insect growth inhibitory activity of different fractions of *Solonom pseudocapsicum* against *S. litura* and *H. armigera* at 1000 ppm Concentration

Fractions tested	<i>S. litura</i>				<i>H. armigera</i>			
	Larvae	Pupae	Adult	Successful adult emergence	Larvae	Pupae	Adult	Successful adult emergence
Fraction 1	8.96±2.44	5.44±1.12	8.8±1.67	76.80±2.77	4.00±1.58	4.32±0.64	5.38±1.25	85.80±4.08
Fraction 2	19.60±2.36	17.40±1.84	12.60±1.92	50.40±5.98	17.40±2.21	13.60±0.83	9.40±1.88	59.60±5.15
Fraction 3	26.30±3.04	21.70±2.06	22.60±1.11	29.40±3.23	26.80±0.95	24.20±2.22	23.50±2.87	25.50±3.78
Fraction 4	8.25±3.27	5.30±4.80	9.50±3.34	76.85±2.44	6.24±2.89	3.62±2.17	7.00±3.58	82.13±2.87

Fraction 5	20.20±4.81	17.80±3.27	18.60±2.60	43.40±4.22	17.40±2.19	18.00±3.67	18.00±2.44	47.60±3.78
Fraction 6	22.40±4.21	25.80±5.18	25.30±2.81	28.50±3.37	25.20±1.52	26.40±4.00	21.10±3.03	27.30±2.66
Fraction 7	25.30±1.71	25.40±2.43	25.80±1.99	23.50±3.02	27.00±1.58	22.80±1.59	26.20±3.16	24.20±3.04
Fraction 8	7.40±1.66	6.40±1.58	2.20±2.36	81.00±5.61	4.50±1.26	5.00±1.11	5.50±1.79	86.10±4.61
Neemazal	50.20±1.33	20.20±1.24	29.60±0.30	00.00±0.00	51.60±2.51	31.40±4.59	16.00±1.45	00.00±0.00
Control	1.50±0.43	0.40±0.54	2.00±0.70	97.10±1.00	0.60±0.51	0±50.69	0.40±0.45	98.50±0.44

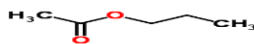
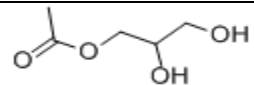

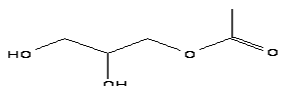
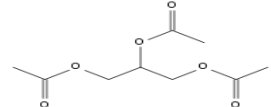
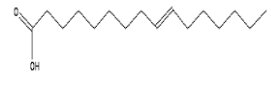
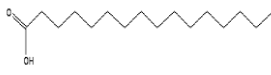
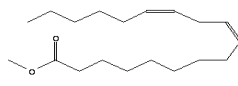
Values are mean ±Standard deviation of five replications

Table 4: List of compounds identified from *S. pseudocapsicum* using GC-MS analysis.

S. No.	Name of the Compounds	Molecular Formula	Molecular Weight	Retention Time	Peak Area	% Peak area
1	Propanoic acid, ethyl ester	C ₅ H ₁₀ O ₂	102	4.80	6531158	0.1515
2	n-Propyl acetate	C ₅ H ₁₀ O ₂	102	4.90	63493828	1.473
3	3,5-Dimethyl-5-hexen-3-ol	C ₈ H ₁₆ O	128	7.57	1052588	0.0244
4	p-Xylene	C ₈ H ₁₀	106	13.84	577839	0.0134
5	Pyrimidine, 4-hydroxy-	C ₄ H ₄ N ₂ O	96	15.74	362589	0.0084
6	Hexanoic acid	C ₆ H ₁₂ O ₂	116	19.62	1096527	0.0254
7	Phenol	C ₆ H ₆ O	94	20.07	605486	0.0140
8	Glycerin	C ₃ H ₈ O ₃	92	20.59	16735126	0.3883
9	1-Hepten-3-ol, 3-methyl	C ₈ H ₁₆ O	128	20.98	4304152	0.0999
10	1,1-Ethanediol, diacetate	C ₆ H ₁₀ O ₄	146	22.03	3017018	0.0700
12	1,2,3-Propanetriol, monoacetate	C ₅ H ₁₀ O ₄	134	22.59	68387504	1.586
13	Benzenepentanoic acid, 4-methyl- α -oxo-	C ₁₂ H ₁₄ O ₃	206	23.95	3922464	0.0910
14	Benzenecarboxylic acid	C ₇ H ₆ O ₂	122	24.60	6406623	0.1486
15	Dianhydromannitol	C ₆ H ₁₀ O ₄	146	24.88	2705639	0.0628
16	1,2,3-Propanetriol, 1-acetate	C ₅ H ₁₀ O ₄	134	25.26	172108240	3.993
17	5-Acetoxyethyl-2-furaldehyde	C ₈ H ₈ O ₄	168	26.49	4461149	0.1035
18	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	26.72	7258137	0.1684
19	Triacetin	C ₉ H ₁₄ O ₆	218	26.82	31844884	0.7388
20	3-Acetyl-cyclopentanone	C ₈ H ₁₂ O ₂	140	27.05	1169476	0.0271
21	Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	C ₁₃ H ₁₆	172	27.31	399419	0.0093
22	Naphthalene, 1,6-dimethyl-	C ₁₂ H ₁₂	156	28.52	1734789	0.0402
23	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	C ₁₃ H ₂₂ O	194	28.86	798020	0.0185
24	1,4-Diacetyl-3-acetoxyethyl-2,5-methylene-l-rhamnitol	C ₁₄ H ₂₂ O ₈	318	29.61	6723022	0.1560
25	3(2H)-Furanone, dihydro-2-methyl-	C ₅ H ₈ O ₂	100	30.44	1252576	0.0291
26	1,3,4-Oxadiazole, 2-(acetyloxy)-2,5-dihydro-2,5,5-trimethyl-	C ₇ H ₁₂ N ₂ O ₃	172	30.62	3624327	0.0841
27	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	31.28	6665647	0.1546
28	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	C ₁₃ H ₁₈ O	190	31.54	465640	0.0108
29	1,3-Propanediol, 2,2-dimethyl-, diacetate	C ₉ H ₁₆ O ₄	188	31.64	537289	0.0125
30	3-Hydroxy-4-methoxybenzoic acid	C ₈ H ₈ O ₄	168	32.37	6419206	0.1489
31	2-Heptadecenal	C ₁₇ H ₃₂ O	252	33.46	5684738	0.1319
32	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	34.81	112279992	2.604
33	Benzoic acid, 4-amino-, ethyl ester	C ₉ H ₁₁ NO ₂	165	35.84	1206668	0.0280
34	2-Pentadecanone, 6,10,14-trimethyl-	C ₁₈ H ₃₆ O	268	35.99	2903305	0.0674
35	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242	37.05	5755714	0.1335
36	2-Nonadecanone	C ₁₉ H ₃₈ O	282	37.63	721578	0.0167
37	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	C ₂₀ H ₃₂	272	37.86	3882320	0.0901
38	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	C ₁₈ H ₃₀ O	262	38.00	1139650	0.0264
39	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	38.22	7578437	0.1758
40	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	39.42	78250360	1.8154
41	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	40.38	2138582784	49.615
42	Estra-1,3,5(10)-trien-17 α -ol	C ₁₈ H ₂₄ O	256	40.86	17330716	0.4021
43	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	42.08	46558292	1.080
44	15-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	42.18	20666826	0.4795
45	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	43.74	209456432	4.859
46	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264	44.09	141477280	3.282
47	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264	44.20	689662912	16.000

48	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264	44.33	402402624	9.335
49	Propanoic acid, ethyl ester	C ₅ H ₁₀ O ₂	102	4.80	6531158	0.1515
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59	1,2,3-Propanetriol, monoacetate	C ₅ H ₁₀ O ₄	134	22.59	68387504	1.586
60	Benzenepentanoic acid, 4-methyl- δ -oxo-	C ₁₂ H ₁₄ O ₃	206	23.95	3922464	0.0910
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64	5-Acetoxymethyl-2-furaldehyde	C ₈ H ₈ O ₄	168	26.49	4461149	0.1035
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66	Triacetin	C ₉ H ₁₄ O ₆	218	26.82	31844884	0.7388
67	3-Acetyl-cyclopentanone	C ₈ H ₁₂ O ₂	140	27.05	1169476	0.0271
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70	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	C ₁₃ H ₂₂ O	194	28.86	798020	0.0185
71	1,4-Diacetyl-3-acetoxymethyl-2,5-methylene- β -D-glucopyranoside	C ₁₄ H ₂₂ O ₈	318	29.61	6723022	0.1560
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83	2-Nonadecanone	C ₁₉ H ₃₈ O	282	37.63	721578	0.0167
84	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	C ₂₀ H ₃₂	272	37.86	3882320	0.0901
85	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	C ₁₈ H ₃₀ O	262	38.00	1139650	0.0264
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88	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	40.38	2138582784	49.615
89	Estra-1,3,5(10)-trien-17 α -ol	C ₁₈ H ₂₄ O	256	40.86	17330716	0.4021
90	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294	42.08	46558292	1.080
91	15-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	42.18	20666826	0.4795
92	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	43.74	209456432	4.859
93	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264	44.09	141477280	3.282
94	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264	44.20	689662912	16.000
95	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264	44.33	402402624	9.335

Table 5: Larval mortality of major compounds of *S. pseudocapsicum* against larvae of *S. litura* and *H. armigera*.

S. No.	Compounds tested	n	Corrected % Mortality of <i>s.litura</i>	Corrected % Mortality of <i>H.armigera</i>	Significance ($P<0.05$) ^b <i>s.litura</i>	Significance ($P<0.05$) ^b <i>H.armigera</i>	Structure of the compounds
1	n-Propyl acetate	25	47.5	45.5	*	*	
2	1,2,3-Propanetriol, monoacetate	25	46.5	43.6	*	*	
3	Tetradecanoic acid	25	98.8	96.7	***	***	
4	1,2,3-Propanetriol, 1-acetate	25	62.4	53.8	*	*	
5	Triacetin	25	56.8	52.2	**	**	
6	9-Hexadecenoic acid	25	98.4	95.7	***	***	
7	n-Hexadecanoic acid	25	98.3	95.2	***	***	
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	25	58.6	50.6	**	**	

n=Number of larvae introduced; ^aCorrected % mortality (Abbott, 1925) ^[2]; ^bTWO way ANOVA with replication was performed; *** =Highly Significant; ** =Moderate Significant; * = Significant

Discussion

Antifeedant activity of botanicals against insect pests has been studied in many countries. Quantification of the antifeedant effect of botanicals is of great importance in the field of insect pest management. From an ecological point of view, antifeedants are very important since they never kill the target insects directly and allow them to be available for their natural enemies and thus help in the maintenance of natural balance. The monophagous or oligophagous insects die due to the application of antifeedants on their food plants, due to starvation. The rate of feeding significantly varied depending on the concentration of the plant extracts. This indicates that the active principles present in the particular solvent extracts inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence (antifeedant). In the present study, fraction IV isolated from the ethyl acetate extract of *S.pseudocapsicum* showed statistically significant antifeedant, larvicidal, Growth regulation activity than the other fractions against fourth instar larvae of *S. litura* and *H. armigera*. Antifeedant activity of twelve fractions isolated from hexane extract of *Atlantia monophylla* against *H. armigera*. Among them, ninth fraction of hexane extract showed promising antifeedant, larvicidal, pupicidal activities and disrupted adult emergence in *H. armigera* at 1000ppm concentration

respectively (Baskar *et al.* 2009) ^[9]. Whereas strong antifeedant activity presented in sixth fraction against fourth instar larvae of *S. litura* (91.58%) at 1000ppm concentration compared with other fractions and positive control (Azadirachtin) showed 92.18%.

Similarly Jeyasankar *et al.* (2014b) ^[25] reported that feeding deterrent activity of *Barleria buxifolia* were tested against *S. litura* and *H. armigera*. Maximum percentage of antifeedant activity was recorded in ethyl acetate extract against *S. litura* (78.5%) and *H. armigera* (75.4%) at 5% concentration compared to other solvent extracts and control. Methanol extract, its fraction and isolated compound furocoumarin and quinolone alkaloid from *Ruta chalepensis* exhibited maximum larvicidal activity with the LC₅₀ values of 2.42, 0.89, 1.59 and 1.21 mg/ml for larvicidal activity against *S. littoralis* (Emam *et al.* 2009) ^[17]. Leaf methanol extract of *Jatropha gossypifolia* showed significant larval mortality with LC₅₀ value of 19.75 mg/ml concentration against *S. litura* (Bhagat *et al.* 2012) ^[10]. Toxic effects of leaf extract of *Acacia arabica* have also been reported against *S. litura* (Rajguru and Sharma, 2012) ^[38]. Further, the fractions from the chloroform extract of *Caesalpinia bonduc* reduced the emergence of *S. litura* (Baskar *et al.* 2012) ^[8]. This was in agreement with the earlier findings of Muthu *et al.* (2012) ^[36] who found that chloroform extract of *Clerodendrum phlomidis* completely prevented the

adult emergence of *E. vittella*. Jeyasankar and Chinnamani (2017) [28] also observed the antifeedant, larvicidal and ovicidal activities of fractions isolated from ethyl acetate crude extracts of *Barleria buxifolia* leaves were tested against fourth instar larvae of *S. litura* and *H. armigera*. Similarly, Anshul *et al.* (2013) [5] reported that the isomeric flavonoid from methanol extract of *Artemisia annua* exhibited promising growth inhibitory activities against *H. armigera*.

Present results agreed with earlier works, Sahayaraj *et al.* 2008 [40] stated that, the compounds 2-(5,6-dimethyl pyrazinyl) methyl; O-Terphenyl-13C and 3,3A, 4,9B-Tetrahydro-2H-Furo(3,2-C) (1) Benzopyran were identified from *Pedaliium murex* showed more significant feeding deterrent and larvicidal activity against *S. litura*. Moon *et al.* 2010 [35] observed that Kaempferol-3-O- α -L-rhamnopyranosyl-(1 -2)- β -D-xylopyranoside - C₂₆H₂₈O₁₄ isolated from the seeds of *Drabanemorosa*. Duraipandiyar *et al.* 2014 [16] reported that the isolated compounds, 5,7-dihydroxy- 4',6-dimethoxy-flavone from *Clerodendrum phlomidis* showed significantly greater antifeedant, larvicidal, oviposition deterrent and ovicidal activities against *H. armigera*. In conclusion, isolated fractions and identified compounds from the *S. pseudocapsicum* may be used for controlling the economically important pests.

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