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Antifeedant, larvicidal and growth regulatory activities of fractions isolated from ethyl acetate extract of *Pseudocalymma alliaceum* against *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hübner (Lepidotera: Noctuidae)

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Abstract

Antifeedant, larvicidal and growth regulatory activities of fractions isolated from ethyl acetate crude extracts of *Pseudocalymma alliaceum* leaves were tested against fourth instar larvae of *Spodoptera litura* and *Helicoverpa armigera.* All the fractions showed biological activity in a dose dependent manner. The maximum antifeedant activity was recorded in ninth fraction of *P. alliaceum* against *S. litura* (88.23%) and *H. armigera* (86.31%) at 1000ppm concentration. Whereas significant larval mortality was observed in ninth fraction of *P. alliaceum* on *S. litura* (91.04%) and *H. armigera* (89.14%) at the same concentration. In addition to 9th fraction showed maximum larval, pupal and adult deformities followed by 6th and 2nd fractions on both insect pests. Ninth fraction caused 20.84% of successful adult emergence with 79.16% of larval, pupal and adult deformities at 1000ppm concentration respectively. These results indicate that *P. alliaceum* has the potential to serve as an alternate botanical pesticide in the management of lepidopteron pests.

Keywords: Antifeedant, Insecticidal, Growth regulatory activities, *Spodoptera litura*, *Helicoverpa armigera*, *Pseudocalymma alliaceum.*

Introduction

In India, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is one of economically important insect and it damages many economically important crops including cotton, pigeonpea, chickpea, tomato, okra, and black gram (Gupta *et al*., 2005; Reena *et al.*, 2006; Sahayaraj and Sathyamoorthi, 2010). It causes economic loss of crops from 25.8 to 100% based on crop stage and its infestation level in the field. It has large host range of more than 120 host plants in India including crops, vegetables, weeds and ornamental plants (Kannaiyan, 2002; Rai *et al*., 2014). The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is an agriculturally important polyphagous pest causing heavy yield loss in agricultural, ornamental, horticultural crops and worldwide that inflicts crop damage in India to the sum of one billion dollars annually and it attacks over 200 crops belonging to 45 families (Talekar *et al*.,

2006). In India, this insect occurs as a major pest in many economically important crops including cotton, pigeon pea, chickpea, tomato, okra, and black gram (Pogue, 2004; Sharma, 2005). These pests status is well justified in its polyphagy on all economically important crops and the hurdles in its management. These insect pests have been controlled with the help of synthetic insecticides over the past fifty years (Kiran Gandhi *et al*., 2016).

The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and the public in recent years. It has been estimated about 2.5 million tons of pesticides are used in crop protection for each year and the worldwide damage caused by pesticides reaches 100 billion annually (USEPA, 2011). Chemical pesticides are generally persistent in nature. The World Health Organization (WHO) estimated to have 2,00,000 people are killed worldwide (CAPE, 2009) 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). These negative impacts of chemical insecticides have forced scientists to search of alternate techniques for the management of economically important insect pests (Abudulai *et al*., 2001).

Plant derivatives are highly toxic to many insect species and more than 2000 plant species are known to possess some insecticidal properties (Krishnappa *et al*., 2010). Botanical pesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages. Essential oils and their constituents have been reported to be an effective source of botanical pesticides (Tewary *et al*., 2005; Krishnappa *et al*., 2012). Plant secondary compounds have been systematically exhibited inan effort to discover new sources of botanical insecticides. These secondary metabolites include tannins, alkaloids, polyphenols, terpenoids, polyacetylenes, flavonoids, unusual amino acids, sugars, phenylpropanoids and quinines (Ahmad 2007). The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility. Aqueous extracts of neem seed and leaf were found to extend the larval developmental period and reduced adult emergence, longevity, fecundity and fertility in polyphagous insect (Wondafrash *et al*., 2012). Botanical pesticides are highly effective, safe and ecologically acceptable (Senthil Nathan and Kalaivani, 2005). Similarly Chennaiyan *et al*. (2016a) reported that antifeedant, larvicidal and insect growth inhibitory activities of *Barleria longiflora* were studied against *S. litura* and *H. armigera*. Jadhav *et al*. (2016) reported that feeding deterrent and larvicidal activities of *Clerodendrum inerme*, *C. calamitosum*, *C. multiflorum*, *C. paniculatum*, *C. philippinum*, *C. serratum*, *C. splendens* and *C. viscosum* leaf crude extracts were evaluated against third instar larvae of *S. litura* and *H. armigera*. Repellent and larvicidal activity of *Corymbia citridora*, *Cymbopogon citrates*, *syzygium aromaticum*, *Gaultheria procumbens* and *Cymbopogon nardus*oils were tested against stored insect pests (Jeyasankar *et al*., 2016). However, primary work on *Pseudocalymma alliaceum* biological properties against agricultural insect pests has been already reported (Jeyasankar and Chinnamani 2014). Further, the present investigation was carried out to evaluate the antifeedant, insecticidal and growth inhibitory activities of isolated fractions of *P. alliaceum* economically important pests.

Materials and Methods

Collection and extraction of plant materials

The leaf of *Pseudocalymma alliaceum* was collected from Chennai, Tamil Nadu, India. Plant specimen was identified by Dr. R. Elango Mathavan, Assistant Professor, Department of Biotechnology, PRIST Uiversity, Thanjavur, Tamil Nadu, India. The plant materials were thoroughly washed with tap water and shade dried under room temperature (27) °C at Department of Zoology, Arignar Anna Government Arts College, Musiri.

Extraction and fractionation

The plant materials were thoroughly washed with tap water and shade dried under room temperature (27.0± 2^0C 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 solvent and filtered through Whatman's No. 1 filter paper. The solvent from the crude extract were evaporated to air dried at room temperature. Crude ethyl acetate extract (20g) was separated by silica gel (100-200 mesh) column (size 60cm x 4 cm) chromatography and eluted with petroleum ether 100% followed by the combination of

petroleum ether: ethyl acetate (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9), then ethyl acetate and Similarly the column was run over ethyl acetate, then ethyl acetate: methanol (9:1, 8:2 and 1:9) and then methanol respectively. A total of 199 fractions were collected in 10ml test tubes and pooled into 13 fractions based on similar RF values using thin layer chromatography.

Rearing of test insects

Egg mass of *S. litura* and different larval stages of *H. armigera* were collected from vegetable field at Anaipatti, Musiri, Trichy, Tamil Nadu, India. Larvae were reared in laboratory conditions (27.0˚C ± 2˚C; 70% RH) throughout the study period at PG & Research Department of Zoology, Government Arts College, Musiri, Tamil Nadu, India. Generally, healthy and uniform sized fourth instar larvae were used for the experiments 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). Required 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 an emulsifier (Subramonithangam and Kathiresan, 1988). 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 125, 250, 500 and 1000ppm 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 pre starved fourth instar larva of *H. armigera* and *S. litura* was introduced on each treated leaf disc. One treatment with respective solvent alone was used as positive control and one treatment with Neemazal was considered as negative control. 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).

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

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

Larvicidal activity

For the evaluation of larvicidal activity of the fraction of *P. alliaceum* against the selected pest, primarily, the plant extract was tested on a wide range of concentration, from that a narrow range of concentration was derived. Thus, 125, 250, 500 and 1000ppm 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. One treatment with respective solvent alone was used as positive control and one treatment with Neemazal was considered as negative control. 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). The larvae with no symptom of a movement or shake while touching with soft camel brush were considered as dead.

$$\text{Mortality (\%)} = \frac{\%MT - \%MC}{100 - \%MC} \times 100$$

Where,

% MT = % Larvae mortality in treatment and
% MC = % Larvae mortality in control.

Growth regulatory activity

Growth regulation activity of fractions was studied at 1000ppm concentration against fourth instar larvae of *S. litura* and *H. armigera*. Ten larvae were introduced in a Petri-plate containing tomato leaves treated with1000ppm concentrations of fractions. One treatment with respective solvent alone was used as positive control and one treatment with Neemazal was considered as negative control. 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.

Results

The results of the antifeedant potential of the solvent crude extracts of *P. alliaceum* investigated against S. *litura* and *H. armigera* larvae were presented in Table 1. Maximum antifeedat activity was recorded in fraction 9 followed by fraction 6 and fraction 2 against 88.23, 83.92 and 69.70% for *S. litura* and 86.31, 74.95 and 67.21% for *H. armigera* at 1000ppm concentration. Percentage larvicidal activity for fractions of *P. alliaceum*, studied at different concentrations against *S. litura* and *H. armigera* was presented in table 2. Significantly promising larval mortality was recorded at 1000ppm concentrations of different fractions showed increased larvicidal activity in fraction 6 i.e., fraction6> fraction 9 > and fraction 2 against (91.04, 77.42 and 69.82%) for S. *litura* and (89.14, 73.22 and 70.32%) for *H. armigera* respectively. The percentage of deformities due to the treatment of fraction of *P. alliaceum* (1000ppm) concentrations is presented in table 3. Maximum larval, pupal and adult deformities were observed in ninth fraction followed by sixth and second fractions against *S. lituta* and *H. armigera*. In addition to significant decreased adult emergence were observed in fraction 9.

Discussion

Plants have numbers of naturally occurring compounds that possess plant protection properties. The botanical extracts from the plant leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides in throughout the world. Several authors have reported that plant extracts possess similar type of antifeedant, insecticidal, oviposition deterrent, ovicidal and growth inhibition activities against lepidopteran pests (Elumalai *et al*., 2013; Jeyasankar *et al*., 2013; Maria Packiam *et al*., 2015). In the present study, it was observed that ninth fraction of *P. alliaceum* reduced the feeding rate of *S. litura* and *H. armigera*. Earlier Raja *et al*. (2005) reported that antifeedant activity of fractions isolated from ethyl acetate extracts of *Hyptis suaveolens* were tested against *S. litura* and *H. armigera*. Maximum percentage of feeding deterrent was recorded in fraction II and IV isolated from ethyl acetate extracts of *H. suaveolens* against armyworm and cotton bollworm at 2000ppm concentration. Feeding deterrent, larvicidal and pupicidal activities of hexane, chloroform and ethyl acetate extracts of *Atlantia monophylla* were studied against *H. armigera*. These three extracts showed more than 50% feeding deterrent activity. However more significant antifeedant activity was observed in hexane extract of *A. monophylla* against cotton bollworm (79.06%) at 5.0% concentrations respectively. Even though higher percentage of larvicidal (Lc_{50} and Lc_{90} values obtained at 2.46% and 5.41%) and pupicidal activities (100%) was noticed only in hexane extract at 5% concentration. Active crude hexane extract was fractionated using silica gel column chromatography. Twelve fractions were collected and evaluated again for their ovicidal activity. Among them, ninth fraction of hexane extract showed promising antifeedant, larvicidal, pupicidal activities and disrupted adult emergence in *H. armigera*. In addition to LC_{50} value was at 384.57ppm for larval mortality and 100% pupal mortality at 1000ppm concentration respectively (Baskar *et al*., 2009).

In the present investigation, ninth fraction of *P. alliaceum* at 1000ppm concentration was recorded the maximum larval mortality of 91.04% *S. litura* and 89.14% *H. armigera*. It is possible that the insecticidal property in the selected plant may arrest the various metabolic activities of the larvae during the development and ultimately the larvae failed to moult and finally died. This is in accordance with the earlier findings of (Jeyasankar *et al*., 2010) for the Antifeedant and growth inhibitory activities of crude extracts and fractions of *Syzygium lineare* were tested against *S. litura*. Antifeedant experiment clearly revealed that maximum antifeedant activity showed in ethyl acetate extract of *S. lineare* (79.4%) against armyworm at 5% concentration compared to other solvent extracts and control. Bioactive ethyl acetate extract was subjected to fractionation using silica gel column chromatography. Seven fractions were obtained. 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 92.18%). In addition to maximum percent of larval deformity (9.4 %) were observed in sixth fraction and highest pupal (11.8%); adult deformities (15.5%) and significant adult emergence inhibited in third fraction. Furthermore Baskar *et al*. (2010) observed that feeding deterrent and larvicidal activities of crude extracts of *Couroupita guianensis* were studied against *H. armigera*. Higher percentage of feeding deterrence (81.67%) was recorded in hexane extract of *C. guianensis* against cotton

Table1. Antifeedant activity of different fractions isolated from ethyl acetate extract of *P. alliaceum* against fourth instars larvae of *S.litura and H. armigera.*

Fractions tested	*Spodoptera litura*				*Helicoverpa armigera*			
	Concentrations tested (ppm)							
	125	**250**	**500**	**1000**	**125**	**250**	**500**	**1000**
Fraction 1	4.33±2.67^{a} (11.97)	15.67±5.48^{bc} (23.26)	16.91±5.75^{b} (24.27)	31.10±6.97^{c} (33.9)	7.47±4.45^{a} (15.79)	15.63±5.26^{b} (23.26)	18.10±5.02^{ab} (25.18)	23.04±6.31^{ab} (29.33)
Fraction 2	14.01±4.14^{c} (21.97)	30.74±5.74^{c} (33.65)	49.34±4.96^{e} (44.66)	69.70±7.98^{e} (56.66)	20.52±3.05^{d} (26.92)	36.31±2.28^{e} (37.05)	43.84±3.92^{e} (41.44)	67.21±3.53^{f} (55)
Fraction 3	5.84±2.26^{ab} (13.94)	10.75±3.01^{ab} (19.09)	16.69±6.02^{b} (24.04)	24.11±2.91^{b} (29.4)	9.01±1.61^{bc} (17.46)	16.35±5.33^{b} (23.81)	24.80±4.64^{bc} (29.87)	38.20±3.61^{c} (38.17)
Fraction 4	9.77±4.95^{b} (18.15)	17.03±6.92^{bc} (24.35)	21.28±7.64^{bc} (27.42)	30.88±7.17^{c} (33.71)	7.90±3.66^{bc} (16.32)	16.36±5.01^{b} (23.81)	18.35±6.60^{ab} (25.33)	28.39±7.53^{b} (32.14)
Fraction 5	20.35±3.55^{d} (26.78)	35.57±6.51^{cd} (36.57)	40.22±4.78^{d} (39.35)	60.37±5.08^{d} (50.94)	27.65±3.96^{e} (31.69)	30.22±4.31^{d} (33.34)	37.02±4.61^{d} (37.46)	56.61±5.46^{e} (48.79)
Fraction 6	23.32±5.10^{de} (28.86)	40.61±3.98^{d} (39.58)	66.00±8.23^{f} (54.33)	83.92±7.63^{f} (66.77)	31.83±1.77^{ef} (34.33)	46.40±2.55^{f} (42.94)	66.75±1.70^{f} (54.76)	74.95±3.39^{g} (59.93)
Fraction 7	4.31±1.52^{a} (11.97)	8.77±2.49^{a} (17.15)	12.18±1.14^{a} (20.36)	16.32±3.17^{a} (23.81)	2.47±1.89^{a} (8.91)	7.31±3.11^{a} (15.68)	11.54±4.50^{a} (19.82)	17.51±3.70^{a} (24.73)
Fraction 8	4.17±0.89^{a} (11.68)	9.63±1.38^{a} (18.05)	15.60±3.23^{ab} (23.26)	28.28±5.44^{bc} (32.08)	6.53±2.77^{b} (14.77)	11.92±3.29^{ab} (20.18)	17.32±4.54^{ab} (24.58)	20.48±4.13^{a} (26.85)
Fraction 9	29.32±7.19^{e} (32.77)	46.37±5.48^{de} (42.88)	66.80±7.89^{f} (54.82)	88.23±8.20^{fg} (69.91)	34.20±3.29^{f} (35.79)	57.48±4.59^{g} (49.26)	70.54±2.21^{fg} (57.1)	86.31±6.44^{h} (68.28)
Fraction 10	8.21±6.02^{b} (16.64)	13.00±3.80^{b} (21.13)	17.96±6.88^{b} (25.03)	28.82±8.10^{bc} (32.46)	17.57±2.22^{c} (24.73)	28.63±4.31^{cd} (32.33)	31.56±4.19^{cd} (34.14)	48.84±3.42^{d} (44.31)
Fraction 11	8.16±5.07^{b} (16.54)	10.79±2.91^{ab} (20)	17.47±5.24^{b} (24.65)	30.06±4.88^{c} (33.21)	6.31±3.20^{b} (14.54)	9.13±6.14^{a} (17.56)	15.64±4.32^{a} (23.26)	18.07±6.62^{a} (25.1)
Fraction 12	5.35±4.50^{a} (13.31)	14.06±5.36^{b} (21.97)	23.45±6.84^{c} (28.93)	29.74±5.30^{bc} (33.02)	3.98±1.54^{a} (11.39)	9.29±3.33^{a} (17.66)	19.35±6.50^{b} (26.66)	30.36±4.54^{b} (33.4)
Fraction 13	10.54±5.99^{bc} (18.91)	15.93±4.68^{bc} (23.5)	21.94±6.26^{c} (27.9)	31.01±3.60^{c} (33.83)	15.73±4.97^{c} (23.34)	23.84±5.71^{b} (29.2)	29.42±3.62^{bc} (32.83)	47.55±4.44^{d} (43.57)
Neemazal	45.22±4.26^{f} (42.25)	79.42±2.73^{e} (63.01)	100.00±0.0^{g} (90)	100.00±0.0^{g} (90)	52.31±3.53^{g} (46.31)	72.22±1.19^{h} (58.18)	90.32±1.00^{h} (71.85)	100.00±0.0^{i} (90)

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. Insecticidal activity of different fractions isolated from ethyl acetate extract of *P. alliaceum* against fourth instars larvae of *S.litura and H. armigera.*

Fractions tested	*Spodoptera litura*				*Helicoverpa armigera*			
	Concentrations tested (ppm)							
	125	**250**	**500**	**1000**	**125**	**250**	**500**	**1000**
Fraction 1	6.66±3.34^{ab} (14.89)	10.11±4.79^{b} (19)	13.22±5.42^{bc} (21.3)	21.44±9.35^{c} (27.56)	7.20±2.58^{b} (15.56)	11.00±3.52^{b} (19.37)	16.40±4.03^{c} (23.89)	28.80±3.89^{cd} (32.46)
Fraction 2	9.66±3.34^{bc} (18.05)	23.88±6.88^{d} (29.2)	36.33±7.90^{e} (37.05)	69.88±6.98^{e} (56.66)	15.22±4.85^{d} (22.95)	28.55±3.59^{e} (32.27)	48.33±8.44^{f} (44.03)	70.33±7.04^{d} (34.6)
Fraction 3	7.44±4.63^{b} (15.79)	15.44±7.22^{c} (23.11)	16.00±4.33^{c} (23.58)	24.00±6.90^{c} (29.33)	8.88±1.18^{b} (17.26)	13.88±5.29^{c} (21.81)	26.55±8.09^{d} (30.98)	32.22±6.39^{d} (34.57)
Fraction 4	4.00±4.68^{a} (11.54)	5.44±6.57^{a} (13.44)	6.66±4.10^{a} (14.89)	9.88±2.65^{a} (18.24)	9.22±4.02^{bc} (17.66)	10.44±6.79^{b} (18.81)	12.44±6.28^{b} (20.62)	22.44±8.59^{bc} (28.25)
Fraction 5	7.66±4.73^{b} (16)	16.44±8.11^{c} (23.89)	25.66±8.76^{e} (30.4)	48.00±6.97^{d} (43.85)	9.66±5.78^{c} (18.05)	19.66±5.99^{d} (26.28)	38.00±6.24^{e} (38.06)	61.00±8.93^{e} (51.35)
Fraction 6	12.66±8.17^{c} (20.79)	34.22±11.77^{e} (35.79)	53.90±7.72^{i} (47.24)	77.44±6.58^{f} (61.61)	9.00±4.76^{b} (17.46)	28.22±6.21^{e} (32.08)	54.88±11.18^{g} (47.75)	73.22±8.93^{f} (58.82)
Fraction 7	8.11±6.02^{b} (16.54)	11.33±8.16^{b} (19.64)	12.22±4.683^{bc} (20.44)	15.33±7.58^{b} (23.03)	3.22±2.76^{a} (10.3)	4.22±3.65^{a} (11.83)	7.44±6.97^{a} (15.79)	11.44±6.23^{a} (19.73)
Fraction 8	7.88±5.21^{b} (16.22)	12.44±4.68^{bc} (20.62)	16.33±570^{c} (23.81)	22.88±7.97^{c} (28.52)	6.80±3.34^{b} (15.12)	9.22±5.60^{b} (17.66)	10.77±3.89^{b} (19.09)	13.66±5.18^{a} (21.64)
Fraction 9	32.22±4.20^{d} (34.57)	58.66±8.18^{f} (49.95)	71.44±5.86^{j} (57.67)	91.00±6.78^{i} (72.54)	27.22±6.54^{e} (31.44)	44.00±9.04^{f} (41.55)	65.88±6.06^{h} (54.21)	89.11±7.72^{g} (70.72)
Fraction 10	3.88±3.68^{a} (11.24)	6.00±4.48^{a} (14.18)	9.66±4.01^{b} (18.05)	12.88±6.41^{ab} (20.96)	4.44±3.05^{a} (12.11)	5.22±4.28^{a} (13.18)	10.22±3.29^{ab} (18.63)	12.00±6.43^{a} (20.27)
Fraction 11	7.00±3.42^{b} (15.34)	10.00±5.10^{b} (18.43)	19.66±6.84^{d} (26.28)	25.88±9.68^{cd} (30.53)	6.00±3.68^{b} (14.18)	12.44±6.43^{bc} (20.62)	20.44±9.89^{cd} (26.85)	25.00±6.79^{c} (30)
Fraction 12	7.22±5.64^{b} (15.56)	9.88±4.16^{b} (18.24)	10.40±6.69^{b} (18.81)	18.44±4.27^{b} (25.4)	9.66±4.61^{c} (18.05)	12.66±5.50^{bc} (20.79)	12.88±3.66^{b} (20.96)	17.66±7.19^{b} (24.8)
Fraction 13	7.88±5.64^{b} (16.22)	11.22±1.81^{b} (19.55)	14.22±6.05^{c} (22.14)	22.11±8.65^{c} (28.04)	8.88±5.74^{b} (17.26)	11.00±7.12^{b} (19.37)	14.44±7.08^{bc} (22.3)	29.44±8.19^{cd} (32.83)
Neemazal	67.22±3.26^{f} (55.06)	79.44±2.23^{g} (63.01)	100.00±0.0^{k} (90)	100.00±0.0^{j} (90)	62.11±3.33^{f} (52)	81.22±1.39^{g} (64.3)	99.22±1.00^{i} (84.89)	100.00±0.0^{h} (90)

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 column indicates that the values are significantly different at p<0.05% Control group was fed with host plant without the treatment of chemicals.

Table 3 Insect growth inhibition activity of different fractions isolated from ethyl acetate extract of *P. alliaceum* against *S. litura* and *H. armigera* at (1000 ppm) concentration.

Fractions tested	*S. litura*				*H. armigera*			
	Larvae	**Pupae**	**Adult**	**Successful adult emergence**	**Larvae**	**Pupae**	**Adult**	**Successful adult emergence**
Fraction 1	8.96±2.44 (17.36)	2.44±1.12 (8.91)	6.8±1.67 (15.12)	81.80±2.77 (64.75)	4.00±1.58 (11.54)	1.32±0.64 (6.55)	3.38±1.25 (10.47)	90.80±4.08 (72.34)
Fraction 2	20.36±2.36 (26.66)	15.42±1.84 (23.11)	10.8±1.92 (19.19)	53.42±5.98 (46.94)	17.06±2.21 (24.35)	14.28±0.83 (22.14)	8.99±1.88 (17.36)	59.67±5.15 (50.53)
Fraction 3	13.64±3.04 (21.66)	10.72±2.06 (19.09)	8.51±1.11 (16.85)	67.12±3.83 (55.00)	9.71±0.95 (16.11)	10.24±2.62 (18.63)	11.89±2.87 (20.09)	68.15±0.78 (55.61)
Fraction 4	5.26±2.27 (13.18)	1.37±0.87 (6.55)	6.54±3.31 (14.77)	86.81±2.41 (68.70)	3.24±0.89 (10.30)	1.62±1.17 (7.27)	3.00±1.58 (9.97)	92.13±2.87 (73.68)
Fraction 5	10.57±3.01 (18.91)	9.10±3.38 (17.56)	8.15±1.90 (16.54)	72.16±3.40 (58.12)	7.68±1.76 (16.00)	5.58±2.12 (13.56)	10.40±1.14 (18.81)	76.36±5.41 (60.87)
Fraction 6	14.4±4.21 (22.30)	16.88±5.18 (24.20)	13.17±2.81 (21.22)	55.53±3.37 (48.16)	17.09±1.52 (24.35)	16.48±4.00 (23.89)	19.12±3.03 (25.90)	47.31±2.66 (43.45)
Fraction 7	8.42±1.66 (16.85)	5.00±1.58 (12.92)	2.57±2.36 (9.10)	84.00±5.61 (66.42)	4.24±1.26 (11.83)	5.84±1.11 (13.94)	3.75±1.79 (11.09)	86.17±4.61 (68.11)
Fraction 8	15.86±1.71 (23.42)	11.53±2.43 (19.82)	10.06±1.99 (18.43)	62.54±6.02 (52.24)	11.00±1.58 (19.37)	9.98±1.59 (18.34)	12.76±3.16 (20.88)	66.26±5.04 (54.45)
Fraction 9	34.60±4.04 (36.03)	25.08±1.95 (30.00)	19.46±5.05 (26.13)	20.84±2.53 (27.13)	29.76±6.76 (33.02)	24.71±2.91 (29.80)	28.20±5.16 (32.08)	17.84±4.03 (24.95)
Fraction 10	12.00±1.22 (20.27)	11.51±2.19 (19.82)	9.40±2.07 (17.85)	67.09±6.83 (54.94)	9.00±1.58 (17.46)	8.77±1.96 (17.15)	7.76±2.76 (16.11)	74.46±5.62 (59.60)
Fraction 11	4.13±1.12 (11.68)	3.40±2.07 (10.63)	1.20±0.83 (6.29)	91.26±4.99 (72.74)	2.00±0.70 (8.13)	3.06±1.67 (9.97)	2.48±1.30 (8.91)	92.45±2.98 (74.00)
Fraction 12	2.80±1.30 (9.63)	0.60±0.89 (4.44)	0.2±0.44 (2.56)	96.40±1.94 (79.06)	1.46±0.86 (6.80)	0.54±0.96 (4.05)	0.46±0.64 (3.63)	97.52±1.54 (80.90)
Fraction 13	0.26±0.43 (2.56)	0.40±0.54 (3.63)	1.00±0.70 (5.74)	98.33±1.00 (82.51)	0.37±0.51 (3.14)	0.49±0.69 (3.63)	0.42±0.45 (3.63)	98.71±0.44 (83.45)
Neemazal (250ppm)	50.50±1.1 (45.29)	28.50±2.54 (32.27)	21.00±1.20 (27.27)	0.00±0.00 (00.00)	61.50±2.30 (51.65)	24.00±1.54 (29.33)	25.50±2.20 (30.33)	0.00±0.00 (00.00)
Control	3.50±1.8 (10.78)			96.50±1.3 (79.22)	4.50±1.5 (12.25)			95.50±1.16 (77.75)

Values are Mean ± Standard deviation of five replications and parenthesis holds angular transformed

bollworm at 5 percent concentration. However more significant larval mortality was observed in hexane extract. Active crude hexane extract was fractionated using silica gel column chromatography. Twelve fractions were collected and evaluated again for their larvicidal activity. Among them, eight fraction showed maximum antifeedant (86.24%) and larvicidal (80.88%) activities against *H. armigera* at 1,000ppm concentration respectively. Insecticidal properties of *Pongamia pinnata* seed extracts tested against *H. armigera*. Highest percent of larval mortality was proved inmature seed extracts of *P. pinnata* against fourth instar larvae of *H. armigera* and more than 65% of feeding deterrence also recorded at 5.0% concentrations. In addition to mature seed extract exhibited a marked reduction in oviposition deterrent and egg hatchability at 2.0% concentration (Reena *et al*., 2012).

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 plant compounds 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 (Baskar *et al*., 2011). In the present study maximum percentage of deformed larvae, pupae and adults were noted in ethyl acetate extract treated larvae. The morphological deformities at larval, pupal and adult stages are due to toxic effects of crude extract on growth and development processes. Previously Jeyasankar *et al*. (2012) reported that antifeedant, insecticidal and growth inhibition activities of *Solanum pseudocapsicum* seed extracts were studied against *S. litura* and *H. armigera*. Most promising antifeedant and insecticidal activities was recorded in ethyl acetate extract against *S. litura* and *H. armigera*. Higher percentage of malformed larvae, pupae and adults were observed in treatment of ethyl acetate extract compared with other solvent extracts. In addition to significant percentage of successful adult emergence was inhibited in the same plant extract on armyworm and cotton bollworm at 5% concentrations. Biological activity of *Duranta erecta* leaves were tested against *S. litura* and *H. armigera*. Maximum antifeedant activity was recorded in ethyl acetate extract on *S. litura* (80.37%) and *H. armigera* (78.18%) at 5% concentration followed by chloroform extract and petroleum ether extract at the same concentration. Significantly greater larval mortality was observed in ethyl acetate extract on armyworm (69.88%) and cotton bollworm (63.2%) at higher concentration. However highest larval, pupal and adult deformities were noticed in ethyl acetate extract on both insects at 5% concentration respectively. In addition to deformed adult moths were recognized by their relatively poor body size, highly curled wings and under grown wings (Chennaiyan *et al*., 2016b). Ninth fraction of *P. alliaceum* showed higher percent of phytopesticidal effects against *H. armigera* and *S. litura*. Further, it may be suggested that the active fraction of *P. alliaceum* will be identify the effective compounds which will be used for controlling the economically important insect pests.

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