International Journal of Natural Sciences Research
2017 Vol. 5, No. 3, pp. 43-49
ISSN(e): 2311-4746
ISSN(p): 2311-7435
DOI: 10.18488/journal.63.2017.53.43.49

EFFECT OF FRACTIONS OF *BARLERIA BUXIFOLIA* AND THEIR BIOLOGICAL ACTIVITY AGAINST ECONOMICALLY IMPORTANT LEPIDOPTERON PESTS

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Article History
Received: 14 April 2017
Revised: 18 May 2017
Accepted: 23 June 2017
Published: 20 July 2017

Keywords
Antifeedant
Insecticidal
Ovicidal activities
Spodopteralitura
Helicoverpaarmigera
Barleriabuxifolia.

ABSTRACT

Antifeedant, larvicidal and ovicidal activities of fractions isolated from ethyl acetate crude extracts of *Barleriabuxifolia* leaves were tested against fourth instar larvae of *Spodopteralitura* and *Helicoverpaarmigera*. The maximum antifeedant, ovicidal and larvicidal activity was recorded in fraction III of *B. buxifolia* against *S. litura* and *H. armigera*. Whereas significant larval mortality was observed in fraction III of *B. buxifolia*on *S. litura* (78.66%) and *H. armigera*(73.76%) at the same concentration. These results indicate that *B. buxifolia* has the potential to serve as an alternate botanical pesticide in the management of *Spodopteralitura* and *Helicoverpaarmigera*.

Contribution/ Originality: This study to approach the novel aspects of plant phytochemicals act as insecticides against economically important pest. Fractions isolated from *B. buxifolia*and tested for insecticidal activity on *S. litura* and *H. armigera* is new report in this plant. Further, it may identify the active principles which may use as potential plant derived insecticide.

1. INTRODUCTION

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 [1]. 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. Insect pests play a major role in damaging the agricultural crops and the loss varies between 10% and 30% for major crops [2]. In india, *Spodopteralitura* 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 [3]. The cotton bollworm, *Helicoverpaarmigera* (*H. armigera*) (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest worldwide that inflicts crop damage in India to the sum of one billion dollars annually and it attacks over 200 crop species belonging to 45 families [4].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 [5].

Botanical pesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages. While plant chemicals may produce toxic effects when ingested by insects, antifeeding activity may determine the extent of insect herbivory. Several papers have been published on the entomotoxic properties of crude extracts from different plant species [6, 7]**.** Plants are endowed with a potential to produce a range of secondary metabolites like alkaloids, terpenoids, flavonoids, these phytochemicals are known to protect the plants from the attack of insect-pests. Phenols, glycosides, sitosterols and tannins.*Solanummelongena.*, *Lycopersiconesculentum* And *Capsicum annuum.* (Solanaceae) are widely cultivated in India and other parts of the world. Few reports are available using *C. annuum*fruit powder [8]. However, primary work on *Barleriabuxifolia*biological properties against agricultural insect pests has been already reported [9]. Further, the present investigation was carried out to evaluate the antifeedant, insecticidal and growth inhibitory activities of isolated fractions of *Barleriabuxifolia* against economically important pests.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

The leaves of *Barleriabuxifolia* were collected from Pulliansolai, Kolli hills, namakkal District, Tamil Nadu, India during the July 2015. Collecetd plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India and The Voucher specimen (IPH 16) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India. The plant leaves were carefully washed with clean water and shade dried under room temperature (27.0 ± 2°C) at Entomology lab, PG & Research Department of Zoology, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

2.2. Extraction and Fractionation

The plant materials were thoroughly washed with tap water and shade dried under room temperature (27.0± 20C 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 (15g) was separated by silica gel (100-200 mesh) column (size 60cm x 4 cm) chromatography and eluted with hexane 100% followed by the combination of hexane : chloroform (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9), then chloroform and Similarly the column was run over chloroform, then chloroform: ethyl acetate (9:1, 8:2 and 1:9) and then ethyl acetate respectively. A total of 118 fractions were collected in 10ml test tubes and pooled into 7 fractions based on similar RF values using thin layer chromatography.

2.3. Rearing of Test Insects

Egg mass of *S. litura* and different larval stages of *H. armigera* were collected from vegetable field at Anaipatti, Musiri, Trichirappalli, Tamil Nadu, and 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.

2.4. Antifeedant Activity

Antifeedant activity of the fractions of *B.buxifolia* was studied using leaf disc no choice method [10]. Required concentration of the fractions of *B. buxifolia* (1000ppm) was prepared by dissolving in acetone and mixing with dechlorinated water Polysorbate 20 (Tween 20) at 0.05% was used as an emulsifier [11]. 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.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. [12].

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

Where

AFI = Antifeedant Index;

C = Area protected in control leaf disc;

T = Area protected in treated leaf disc.

2.5. Larvicidal Activity

For the evaluation of larvicidal activity of the fraction of *B.buxifolia* 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. 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 [13]. 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.

2.6. Ovicidal Activity

Twenty individual eggs of *H. armigera* and*S.litura* (for removal of scales from egg masses by using camel brush) were separated and dipped in various concentrations (as mentioned in antifeedant activity). Five replicates were maintained (n=100). Number of eggs hatched in the control and treatments were recorded and percent ovicidal activity was calculated according to Abbott [13] (as mentioned in larvicidal activity).

2.7. Statistical 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.LC_{50}, LC_{90} was carried out using SPSS 16.00.

3. RESULTS AND DISCUSSION

The results of the antifeedant potential of the solvent crude extracts of *B. buxifolia* investigated against S.*litura* and *H. armigera* larvae were presented in Table 1. Maximum antifeedat activity was recorded in fraction III followed by fractionVI against 74.33% and 57.32% for *S. litura* and 70.11% and 50.43% for *H. armigera* at 1000ppm concentration. Percentage ovicidal activity for fractions of *B.buxifolia*, studied at different concentration against *S. litura* and *H. armigera* was presented in table2. Maximum ovicidal activity was recorded in fraction III followed by fractionVI against 76.84% and 62.06% for *S. litura* and 73.12% and 67.02% for *H. armigera* at 1000ppm concentration. Percentage larvicidal activity for fractions of *B.buxifolia*, studied at different concentrations against *S. litura* and *H. armigera* was presented in table 3. Significantly promising larval mortality was recorded at 1000ppm concentrations of different fractions showed increased larvicidal activity in fraction fractionVI fraction III against (68.26% and78.66% for S. *litura*and (58.84% and 73.76%) for *H. armigera* respectively.

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 [14]. Antifeedant, larvicidal and insect growth inhibitory activities of *Pseudocalymma alliaceum* were studied against *S. litura* and *H. armigera*[15]. Antifeedant, larvicidal and insect growth inhibitory activities of *Barleria longiflora* were studied against *S. litura* and *H. armigera*[16]. Antifeedant, larvicidal and insect growth inhibitory activities of *Pseudocalymma alliaceum* were studied against *S. litura*and *H. armigera*. Chinnamani, et al. [15]in the present study, it was observed that III fraction of *B. buxifolia*reduced the feeding rate of *S. litura* and *H. armigera*. Jeyasankar, et al. [17] reported that the possible 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 In the present investigation, III fraction of *B. buxifolia* at 1000ppm concentration was recorded then maximum larval mortality of 78.66% *S. litura*and73.76% *H. armigera*. 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. [18]. Baskar, et al. [19]Observed that twelve fractions were collected from hexane extracts of *Couroupita guianensis* were studied against *H.armigera*. Among them, eight fractions showed maximum percentage of larvicidal (80.88%) activity against *H. armigera* at 1,000ppm concentration respectively. In the present study III fraction isolated from ethyl acetate extract of *B. Buxifolia* exhibited statistically significant larvicidal activity against fourth instar larvae of *S. litura*and *H. armigera* at 1000ppm concentrations. Present results agreed with *Atalantia monophylla* leaf extract was fractionated using silica gel column chromatography. Twelve fractions were collected and evaluated for their ovicidal activity at 125, 250, 500 and 1000 ppm concentrations. Among them, fraction 9 showed maximum ovicidal activity of 72.21% at 1000 ppm concentration with least LC 50 value of 435.92 ppm [20].

Funding: The Authors are thankful that this work was conducted in the laboratory which is financially supported by UGC, New Delhi, India (Ref No. 42-570/2013 (SR)).
Competing Interests: The authors declare that they have no competing interests.
Contributors/Acknowledgement: The authors are thankful to Principal and Head of Department of Zoology, A. A. Govt. Arts College, Musiri-621 211, Tamil Nadu, India for their support and facilities provided.

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Table-1. Antifeedant activity of ethyl acetate fractions of *B.buxifolia*against fourth instars larvae of *S.litura*and *H.armigera*

Fractions	*Spodopteralitura*				*Helicoverpaarmigera*			
	Concentrations tested (ppm)							
	125	250	500	1000	125	250	500	1000
I	7.56±3.08^{a} (15.89)	9.73±2.20^{a} (18.15)	10.38±2.22^{a} (18.72)	17.57±3.35^{a} (24.73)	8.25±3.64^{a} (16.64)	17.31±8.34^{bc} (24.58)	21.99±5.70^{b} (27.90)	26.35±7.49^{b} (30.85)
II	9.18±2.38^{ab} (17.56)	19.24±2.19^{b} (25.99)	19.64±4.35^{bc} (26.28)	24.85±4.37^{b} (29.87)	9.75±5.75^{a} (18.15)	12.30±5.19^{ab} (20.53)	13.92±7.72^{a} (21.89)	21.16±3.88^{a} (27.35)
III	21.10±4.02^{c} (27.35)	43.35±8.82^{c} (41.15)	59.42±7.31^{e} (50.42)	74.33±7.76^{e} (59.54)	23.73±4.73^{c} (29.13)	28.24±8.10^{c} (32.08)	43.99±7.41^{c} (41.50)	70.11±5.46^{e} (56.85)
IV	6.34±2.52^{a} (14.54)	9.46±1.62^{a} (17.85)	13.37±1.65^{ab} (21.39)	17.05±4.71^{a} (24.35)	12.24±9.70^{b} (20.44)	14.30±6.34^{b} (22.22)	15.66±7.49^{ab} (23.26)	20.84±7.06^{a} (27.13)
V	5.95±1.65^{a} (14.06)	13.06±4.54^{ab} (21.13)	22.35±6.26^{bc} (28.18)	28.76±8.39^{bc} (32.39)	6.36±3.56^{a} (14.54)	9.92±6.18^{a} (18.34)	11.51±5.26^{a} (19.82)	18.06±3.93^{a} (25.10)
VI	17.18±3.35^{b} (24.43)	27.88±7.94^{c} (31.82)	40.36±6.61^{d} (39.41)	57.32±11.35^{d} (49.20)	14.29±2.97^{bc} (22.14)	17.16±2.22^{bc} (24.43)	21.19±4.41^{b} (27.35)	50.43±7.06^{d} (45.23)
VII	8.83±2.75^{ab} (17.26)	14.18±7.08^{ab} (22.06)	16.45±4.78^{b} (23.89)	24.83±9.35^{b} (29.87)	7.65±3.82^{a} (16.00)	9.66±5.29^{a} (18.05)	12.76±3.47^{a} (20.88)	19.45±3.75^{a} (26.13)

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. Ovicidal activity of ethyl acetate fractions of *B.buxifolia*against fourth instars larvae of *S.litura*and *H.armigera*

Fractions	Concentration (ppm)	*S. litura*				*H. armigera*			
		Ovicidal(%)	LC_{50}	LC_{90}	X^2 value	Larvicidal (%)	LC_{50}	LC_{90}	X^2 value
I	125 250 500 1000	9.30±1.30^{a} 10.10±0.46^{a} 16.30±1.30^{a} 27.10±3.49^{a}	1636.744	3031.141	0.571	7.20±1.35^{a} 9.92±0.97^{a} 12.60±1.43^{ab} 17.10±2.77^{a}	1585.015	2821.249	0.915
II	125 250 500 1000	10.42±1.13^{a} 12.18±1.91^{a} 18.90±3.57^{b} 23.20±3.70^{ab}	1873.519	3567.500	2.463	10.84±0.70^{a} 13.60±1.98^{ab} 19.06±3.40^{bc} 29.50±2.47^{b}	1508.466	2846.093	3.615
III	125 250 500 1000	34.38±6.32^{d} 48.20±4.65^{d} 54.10±4.00^{d} 76.84±3.87^{d}	437.466	1341.279	6.817	30.82±4.37^{c} 46.14±2.77^{d} 56.40±5.41^{e} 73.12±2.48^{d}	465.191	1422.692	5.801

IV	125 250 500 1000	9.60±2.30[a] 16.98±3.23[b] 20.54±3.90[bc] 28.42±4.46[bc]	1617.994	3172.792	3.302	9.48±1.79[a] 16.14±3.06[bc] 20.50±1.39[c] 27.00±3.78[bc]	1676.816	3272.828	3.412
V	125 250 500 1000	7.88±1.86[a] 9.12±1.73[a] 10.50±1.87[a] 28.88±2.92[a]	1569.626	2798.892	1.027	8.74±1.77[a] 9.42±0.98[a] 15.00±2.09[a] 26.40±3.65[a]	1695.320	3129.784	0.189
VI	125 250 500 1000	19.40±4.74[c] 26.02±4.18[c] 40.06±5.97[c] 62.06±2.37[c]	744.960	1672.508	1.081	19.12±5.80[b] 28.42±4.99[c] 48.42±2.97[d] 67.02±3.19[c]	1657.536	3174.053	1.136
VII	125 250 500 1000	12.18±5.05[b] 16.16±4.11[b] 20.64±3.14[bc] 29.20±4.45[b]	1689.712	3417.645	0.968	10.36±1.91[a] 13.88±2.05[ab] 16.88±2.58[b] 28.20±5.46[b]	650.405	1472.027	4.322

Values are mean ± S.D of five replication; Number of larvae =10; LC_{50}=Lethal concentration 50 and LC_{90}=Lethal concentration 90; SPSS16.0. Values with different alphabet in column are statistically significant ($p<0.05$ level; DMRT).

Table-3. Larvicidalctivity of ethyl acetate fractions of *B.buxifolia*against fourth instars larvae of *S.litura*and *H.armigera.*

Fractions	Concentration (ppm)	*S. litura*				*H. armigera*			
		Larvicidal (%)	LC_{50}	LC_{90}	X^2 value	Larvicidal (%)	LC_{50}	LC_{90}	X^2 value
I	125 250 500 1000	8.60±1.14[a] 12.80±2.41[a] 17.8±1.87[a] 28.48±1.46[a]	1519.723	2791.434	3.127	8.90±2.84[a] 12.40±2.06[b] 18.20±2.58[ab] 26.70±4.40[bc]	1620.130	3025.912	2.392
II	125 250 500 1000	9.40±1.24[a] 16.40±2.51[ab] 20.30±1.63[b] 31.30±1.27[ab]	1422.045	2699.516	4.918	15.90±2.57[b] 21.20±3.81[bc] 22.80±2.48[b] 28.40±2.99[c]	1650.628	3346.041	3.759
III	125 250 500 1000	21.80±1.85[b] 30.20±2.88[d] 51.20±7.09[d] 78.60±1.61[d]	549.205	1198.750	4.317	27.10±4.52[d] 39.30±8.12[d] 51.30±3.10[c] 73.70±4.05[d]	531.660	170.776	4.784
IV	125 250 500 1000	9.40±2.07[a] 15.60±3.07[a] 16.50±3.78[a] 37.30±4.29[a]	1264.146	2335.574	3.623	9.10±0.43[a] 10.30±0.54[a] 14.00±1.93[a] 26.80±3.22[a]	1633.267	2238.010	1414
V	125 250 500 1000	12.10±2.75[ab] 17.70±1.86[b] 18.10±2.26[a] 41.00±2.06[a]	1185.616	2246.520	5.313	11.10±1.47[ab] 13.60±2.06[a] 16.80±4.54[a] 23.80±5.44[b]	1616.643	3136.691	1.045
VI	125 250 500 1000	21.60±1.20[b] 35.70±7.87[c] 47.10±2.87[c] 68.20±3.98[c]	619.307	1494.693	4.715	20.80±1.08[c] 30.90±3.88[c] 50.70±5.65[c] 58.80±4.43[c]	736.127	1767.499	5.290
VII	125 250 500 1000	10.70±1.95[a] 15.80±2.80[a] 20.20±3.78[ab] 23.00±0.76[b]	1961.831	3896.151	3.419	13.30±2.26[a] 19.40±2.41[bc] 24.20±6.25[bc] 31.30±4.98[bc]	1561.350	3254.332	2.846

Values are mean ± S.D of five replication; Number of larvae =10; LC_{50}=Lethal concentration 50 and LC_{90}=Lethal concentration 90; SPSS16.0. Values with different alphabet in column are statistically significant ($p<0.05$ level; DMRT).