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Antifeedant and larvicidal activities of ethyl acetate extract of *Jatropha integerrima* against *Spodoptera litura* and *Helicoverpa armigera*

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Abstract
Antifeedant and larvicidal activities of ethyl acetate crude extracts of *Jatropha integerrima* leaves were tested against fourth instar larvae of *Spodoptera litura* and *Helicoverpa armigera*. The maximum antifeedant activity was recorded in ethyl acetate crude extracts of *J. integrrima* against *S. litura* (74.33%) and *H. armigera* (70.11%) at 5% concentration. The maximum larvicidal activity was recorded in ethyl acetate crude extracts of *J. integrrima* against *S. litura* (76.84%) and *H. armigera* (73.12%) at 5% concentration. The antifeedant activity was recorded in chloroform crude extracts of *J. integrrima* against *S. litura* (74.33%) and *H. armigera* (70.11%) at 5% concentration. The larvicidal activity was recorded in chloroform crude extracts of *J. integrrima* against *S. litura* (76.84%) and *H. armigera* (73.12%) at 5% concentration. The antifeedant activity was recorded in hexane crude extracts of *J. integrrima* against *S. litura* (74.33%) and *H. armigera* (70.11%) at 5% concentration. The larvicidal activity was recorded in hexane crude extracts of *J. integrrima* against *S. litura* (76.84%) and *H. armigera* (73.12%) at 5% concentration. These results indicate that *J. integrrima* has the potential to serve as an alternate botanical pesticide in the management of *Spodoptera litura* and *Helicoverpa armigera*.

Keywords: antifeedant, insecticidal activities, *Spodoptera litura*, *Helicoverpa armigera*, *Jatropha integrrima*

Introduction
Even though insect pest causes agricultural crop loss of 120 billion US$ dollars worldwide and reduce the yield by 10-30%. Man suffers extensively due to the nuisance of insect populations both in agriculture and health. In agriculture, insects affect directly the growing part of the crop and causes severe damage, resulting in revenue loss. Crop loss due to insect pests is estimated between ten and thirty per cent for major crops (Ferry *et al*., 2004) [9]. An estimated one third of global agricultural production valued at several billion dollars is destroyed annually by over 20,000 species of insect pests in field and storage (Mariapackiam and Ignacimuthu, 2008) [14].

These insect pests have been controlled with the help of synthetic insecticides over the past fifty years (Kiran Gandhi *et al*., 2016) [13]. Moreover, Chemical pesticides have been used for several decades in controlling pests as they have a quick knock down effect. However, their indiscriminate use resulted in several problems such as resistance to pesticides, resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affect human health and disrupt the ecosystem, (Balaraju *et al.*, 2011) [3]. However, intensive screening is necessary to select compounds with pesticidal properties, but harmless to the environment and ecosystem. Researches on potential botanical extracts which are safe with little or no residues and naturally derived with minimal technology are urgently needed. There are more than 2400 plant species belonging to 189 plant families which are said to be rich sources of bioactive organic compounds (Rao *et al*., 2005) [17].

The Neem tree *Azadirachta indica*, belonging to the family Meliaceae originating from the Indian subcontinent, is a well-known example of one of the plants with potential to serve as an anti-feedant against insects and pests. Other examples include *Detarium microcarpum*, *Sclerocarya birrea*, *Piper guineense* as seed protectants maize (*Sitophilus zeamais*), *Cassia nigricans* Vahl oil and the plant as grain protectants of stored wheat weevil, *Tribolium castaneum*, as well as containing biologically active compounds, that may serve as candidates for new formulations in the treatment and prevention of livestock diseases and pest management (Ayo, 2010) [2]. Among current alternative strategies aiming at decreasing or minimizing the use of chemical insecticides, eco-chemical control based on plant-insect relationships is one of the most promising methods. Plant derived chemicals offer a more natural and environmentally friendly approach to pest control than synthetic insecticides. However, this plant has no report on biological properties against agricultural insect pests. Hence, in the present investigation to evaluated the antifeedant and larvicidal activity of *J. integrrima* against economically important insect pests *S. litura* and *H. armigera*.

Materials and Methods
Collection of plant materials
The leaves of *J. integrrima* leaf were collected from Pulliansolai, Trichy, Tamil Nadu, India. Plant specimen was identified was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India. The plant materials were thoroughly washed with tap water and shade dried under room temperature (27) °Cat Department of Zoology, Arignar Anna Government Arts College, Musiri.

Extraction
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. 500g of plant powder was extracted by soxhlet extraction methods with hexane, chloroform and ethyl acetate.

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, *et al*). 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 0.625%, 1.25%, 2.5% and 5%, 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) [6].

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

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 *Jatropha integerrima* 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, 250, 500, 750 and 1000ppm concentrations for fractions were tested against the freshly molted (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) [1]. 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.

Determination of lethal concentration
Lethal concentration (LC_{50} and LC_{90}) represents the concentration of the test material that caused 50% mortality of the test organisms within the specified period of exposure, and it was determined by exposing various development stages of the *H. armigera* and *S. litura* to different concentration of the extract. Based on the mortality of the test organisms recorded in the bioassays, LC_{50} and LC_{90} was calculated along with their fiducial limits at 95% confidence level by probit analysis using Microsoft Excel 2007 software (Finney, 1971). The mean values of the data were subjected to multivariate analysis (ANOVA) and the significance between and within the means were compared after test of significance (LSD, DMRT, $p < 0.05$). Then the means with statistical significance were compared and marked with different alphabets.

Results
Detailed phytochemical analysis of ethyl acetate extracts of *Jatropha integerrima* the presence of a variety of plant secondary metabolites as it is evidenced from the table 5-6. Perusal of the data clearly indicates that among the ethyl acetate extracts of *J. integrrima* showed presence of majority of secondary metabolites such as Alkaloids, Catechin, Flavonoids, Phenols Quinines, Saponins and Steroids.
Antifeedant activity of ethyl acetate extracts of *Jatropha integerrima* tested against fourth instar larvae of *S. litura* and *H. armigra* and the results pertaining to different concentrations are presented in the Table 1-2. Different concentration showed varying range of antifeedant activity. It was noted that the antifeedant activity of ethyl acetate extracts was found to be statistically significant than the other hexane and chloroform extracts tested against the fourth instar larvae of *S. litura* and *H. armigra*. Initially, at 5% concentration of ethyl acetate extracts showed 71.17 ± 2.88^{c} and 70.20 ± 1.40^{c} area of leaf protection from the larvae feeding followed by 61.33 ± 2.92^{b} and 59.40 ± 2.30^{b} chloroform extracts, 38.4 ± 1.35^{a}

and 36.20±1.50[a] hexane extracts, respectively (Table 1-2).

The previous experiment the ethyl acetate, chloroform and hexane extracts of *J. integrrima* 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 ethyl acetate extracts showed the LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) x^2 value of 3.00(2.13-4.59), 6.67(4.94-11.84) 7.368 and 3.01 (2.08-4.79), 6.90 (5.01-13.03) 7.533 against *S. litura* and *H. armigra* respectively. Likewise, for chloroform extracts showed the LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) x^2 was calculated to be 3.76 (3.31-4.37), 7.73 (6.68-9.33) 2.915 and 3.58 (2.51-6.38), 8.13(5.70-17.56) 7.031 against *S. litura* and *H. armigra* respectively. Likewise, for hexane extracts showed the LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) x^2 was calculated to be 4.71 (4.03-5.80), 6.67 (4.94-11.84) 2.262 and 5.54 (4.64-47.09), 10.53 (8.56-14.14) 1.229 against *S. litura* and *H. armigra* respectively.

Discussion

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 (Jeyasankar *et al*., 2013) [12]. 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*., 2010 [10] 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. Earlier stydies pertaining to *Couroupita guianensis* leaves extracts showed antifeedant, larvicidal, and ovicidal activities against *Helicoverpa armigera* (Baskar and Ignacimuthu, 2013) [4] and antifeedant activity against *Spodoptera litura* (Baskar *et al*., 2008). In the present investigation, various organic solvent extracts of *Jatropha integerrima* were tested for their antifeedant activity and the data obtained from the experiments clearly revealed that among the various solvent extract, the ethyl acetate extract showed promising antifeedant activity against fourth instar larvae of *S. litura* and *H. armigera*.

In the present study ethyl acetate extracts from *Jatropha integerrima* exhibited significant insecticidal activity against fourth instar larvae of *S. litura* and *H. armigera*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. Elumalai *et al*. (2004) reported that ethyl acetate leaf extract of *Acorus calamus* exhibited maximum larvicidal activity against *S. litura*. In another study, ethyl acetate extract of *Artemisia nilagirica* had induced significant larval mortality against the larvae of *S. litura* (Raja *et al*., 2003) [16]. Similarly Pavela (2004) [15] reported that leaf extract of *Marrubium vulgare* at 5.0% concentration exhibited 42.2% of larval mortality.

The extracts might have arrested the various metabolic activities of the larvae. Ultimately the larvae failed to feed and the development was arrested in various instar stages. Earlier, Elumalai *et al.*, (2010) [8] reported that the maximum larval mortality was found in the essential oil of *Zingiber officinales* tested against armyworm, *S. litura* an agricultural important lepidopteron pest (Tamhane *et al*., 2005) [18]. Jeyasankar *et al*. (2014) [11] reported that six different indigenous plants were screened for insecticidal activity against fourth instar larvae of *Henosepilachna vigintioctopunctata*. Among the plants screened, ethyl acetate extracts of *Achyranthes aspera* showed higher percentage of larval mortality against fourth instar larvae of *H. vigintioctopunctata*.

Table 1: Antifeedant activity of crude extracts of *Jatropha integerrima* against *S. litura*.

Solvent	***S. litura***			
	Concentrations tested (%)			
	0.625%	1.25%	2.5%	5%
Hexane	11.40±2.7 [a]	19.10±2.2 [a]	22.2±2.63[a]	38.4±1.35[a]
Chloroform	13.30±3.6 [a]	20.00±2.3 [b]	26.53±2.3 [b]	61.33±2.92[b]
Ethyl acetate	21.10±1.33[c]	31.10±2.98[c]	56.22±2.32[c]	71.17±2.88[c]

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: Antifeedant activity of crude extracts of *Jatropha integerrima* against *H. armigera*.

Solvent	***H. armigera***			
	Concentrations tested (%)			
	0.625%	1.25%	2.5%	5%
Hexane	10.40±3.50[a]	18.30±3.50[a]	21.20±2.30[a]	36.20±1.50[a]
Chloroform	16.20±3.65[a]	21.30±2.30[b]	30.30±1.30[b]	59.40±2.30[b]
Ethyl acetate	21.90±1.74[c]	36.23±2.80[c]	56.20±3.30[c]	70.20±1.40[c]

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 3: Larvicidal activity of plant extract of *Jatropha integerrima against S. litura.*

Extract Tested	Concentration (%)	Larvicidal (%)	LC_{50} (LCL - UCL)	LC_{90} (LCL -UCL)	X^2
Hexane	5	51.00±2.30	4.71 (4.03-5.80)	6.67 (4.94-11.84)	2.262
	2.5	31.50±2.20			
	1.25	20.00±3.20			
	0.625	16.40±1.50			
Chloroform	5	63.00±2.20	3.76 (3.31-4.37)	7.73 (6.68-9.33)	2.915

	2.5	38.30±1.40			
	1.25	23.20±1.50			
	0.625	16.10±2.60			
Ethyl acetate	5	71.30±3.20	3.00 (2.13-4.59)	6.67 (4.94-11.84)	7.368
	2.5	51.10±2.40			
	1.25	31.10±1.80			
	0.625	21.10±2.10			

Value represents mean ± S.D. of five replicationsLC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 4: Larvicidal activity of plant extract of *Jatropha integerrima against H. armigera*

Extract Tested	Concentration (%)	Larvicidal (%)	LC_{50} (LCL - UCL)	LC_{90} (LCL -UCL)	X^2
Hexane	5	48.40±1.20	5.54 (4.64-47.09)	10.53 (8.56-14.14)	1.229
	2.5	30.60±1.20			
	1.25	20.70±2.20			
	0.625	16.10±7.50			
Chloroform	5	61.20±1.20	3.58 (2.51-6.38)	8.13 (5.70-17.56)	7.031
	2.5	45.30±6.40			
	1.25	29.50±4.50			
	0.625	20.20±2.10			
Ethyl acetate	5	70.30±1.30	3.01 (2.08-4.79)	6.90 (5.01-13.03)	7.533
	2.5	50.50±4.40			
	1.25	31`60±4.80			
	0.625	23.10±5.10			

Value represents mean ± S.D. of five replications. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 5: Physical Characteristics and % Yield of Various Extracts of *Jatropha integerrima* Leaves.

Solvent	Color of the extract	Consistency	Sense Of touch	Amount of extract(gm)	% of yield (w/w)
Hexane	Brown	Semisolid	Sticky	3.80	3.8
Chloroform	Greenish yellow	Semisolid	Sticky	5.20	5.2
Ethyl acetate	Brown	Semisolid	Sticky	6.50	6.5

The % yield was maximum (6.5%) obtained with ethyl acetate and least (3.8%) with hexane.

Table 6: Preliminary phytochemical analysis of different extracts of *Jatropha integrrima*.

Phytoconstituents	Name of test	Response	Hexane	Chloroform	Ethyl acetate
Alkaloids	Mayers	Green colouration	-	+	-
Anthraquinones	Borntragers	Red colouration	-	-	-
Catechin	Salkowski	Pink colour	+	-	-
Coumarin	NaOH	Yellow colour	-	-	-
Flavonoids	Late acetate	Pure pink colour	-	-	+
Phenols	Ferric chloride	Intense red colour	-	-	+
Quinines	ConcHcl	Yellow colour	-	-	+
Saponins	Foam	Foam lather	+	-	-
Steroids	Salkowski	Green fluorescence	-	-	+
Tannins	Ferric chloride	White precipitate	-	+	-
Terpenoids	Salkowski	Purple colour	-	+	+

+ Presence of compound, - Absence of compound

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Reference
1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-266.
2. Ayo RG. Phytochemical Constituents and Bioactivities of the Extracts of Cassia nigricans Val: Journal of Medicinal Plants Research. 2010; 4(14):1339-1348.
3. Balaraju K, Ezhil Vendan S, Ignacimuthu S. Kyungseok P. Antifeedant and larvicidal activities of *Swertia chirata* Buch-Ham. Ex Wall. Against *Helicoverpa armigera* Hubner and *Spodo*ptera litura Fab. Journal of Elixir Social Science. 2011; 31:1902-1905.
4. Baskar K, Ignacimuthu S. Ovicidal activity of *Couroupita guianensis* (Aubl.) against Cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Archives of Phytopathology and Plant Protection. 2013; 46:1571-1579.

5. Baskar K, Kingsley S, Ezhil Vendan S, Paulraj MG, Duraipandiyan V, Ignacimuthu S. Antifeedant, larvicidal and pupicidal activities of Atalantia monophylla Correa against Helicoverpa armigera Hubner (Lepidoptera: Noctuidae). Chemosphere. 2009; 75:355-359.
6. Ben Jannet H, Skhiri HF, Mighri Z, Simmonds MSJ, Blaney WM. Responses of Spodoptera littoralis larvae to Tunisian plant extracts and to neo-clerodane diterpenoids isolated from Ajuga pseudoiva leaves**.** Fitoterapia. 2000; 71:105-112.
7. Elumalai A, Backiyaraj M, Kasinathan D, Mathivanan T, Krishnappa K, Elumalai K. Pesticidal activity of Rivina humilis L. (Phytolaccaceae) against important agricultural polyphagous field pest, Spodoptera litura (Fab.) (Lepidoptera: Noctuidae). Journal of Coastal Life Medicine. 2015; 3(5):389-394.
8. Elumalai K, Krishnappa K, Anandan A, Govindarajan M. Mathivanan T. Larvicidal and ovicidal activity of seven essential oil against lepidopteran pest Spodoptera litura (lepidoptera: noctuidae). International Journal of Recent Scientific Research. 2010; 1:008-014.
9. Ferry N, Edwards MG, Gatehouse JA. Plant-interaction: molecular 24. approaches to insect resistance. In Sasaki T. and Christou P. (Eds): Reviews in Current Biotechnology, 2004, 155-161.
10. Jeyasankar AN, Raja S. Ignacimuthu.. Antifeedant and growth inhibitory activities of *Syzygium lineare* against *Spodoptera litira* (Lepidoptera: Noctuidae). Current Research Journal of Biological Science. 2010; 2(3):173-177.
11. Jeyasankar A, Chinnamani T. Bioactivity of *Pseudocalymma alliaceum* (Lam.) Sandwith (Bignoniaceae) against *Spodoptera litura* Fabricius and Helicoverpa armigera Hübner (lepidoptera: Noctuidae). Journal of Coastal Life Medicine. 2014; 2(4):302-307.
12. Jeyasankar A, Elumalai K, Raja N, Ignacimuthu S. Effect of plant chemicals detterent and ovicidal activities against female moth, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). International Journal of Agricultural Science. 2013; 2:206-213.
13. Kiran Gandhi B, Patil RH, Srujana Y. Field resistance of *Spodoptera litura* (Fab.) to conventional insecticides in India. Crop protection. 2016; 88:103-108.
14. Mariapackiam S, Ignacimuthu S. Larvicidal and Histopathological effects of the oil formulation on *Spodoptera litura* In: Recent trends in insect pest management (Elite Publishing Home) Pvt -LTD. New Delhi, 2008.
15. Pavela R. Insecticidal activity of certain medicinal plants. Fitoterapia. 2004; 17**:**745-749.
16. Raja N, Elumalai K, Jayakumar M, Jeyasankar A, Muthu C, Ignacimuthu S. Biological activity of different plant extracts against armyworm, Spodoptera litura (Fab). (Lepidoptera: Noctuidae). Journal of the Entomological Research Society. 2003; 27:281-292.
17. Rao NGV, Tikar SN, Nimbalkar SA. Management of insecticide resistant *Spodoptera litura* with some ready mix formulation. *Pestology*. 2001; 25(11):36-38.
18. Tamhane VA, Chougule NP, Giri AP, Dixit AR, Sainani MN, Gupta VS. *In vivo* and in vitro effect of annum proteinase inhibitors on *Helicoverpa armigera* guts proteinases. Biochimica Biophysica Acta, 2005; 1722:156-167.