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Laboratory Evaluation of *Leucas lavandulifolia* Smith (Labiatae) Leaf Extracts Against Pulse Beetle, in Stored Green Gram Seed (*Vigna radiata* L.)

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ABSTRACT

A laboratory experiment was conducted to investigate the efficacy of Leucas lavandulifolia Smith against Callosobruchus chinensis L. in stored green gram seed. The leaf extracts of L. lavandulifolia with petroleum ether, methanol, ethanol and water are evaluated for their adult mortality, oviposition inhibition and F_1 adult emergence of C. chinensis. The results revealed that the extracts of the plant caused a considerable reduction of the number of beetles. The petroleum ether extract was found to be most effective over rest of the solvent extracts, registered the highest percent of mortality (94%) at 5% conc. after 96 hour of treatment followed by methanol (92%), ethanol (78%) and water extracts (76%) respectively. The LC_{50} value of petroleum ether extract of L. lavandulifolia at72 HAT recorded the lowest LC_{50} (2.309%).Highest oviposition deterrence was found in petroleum ether extract (61.00%), followed by methanol (60.01%), ethanol (56.52%) and water extract(47.59%) respectively at 5% conc. after 7days of seed treatment. In terms of the F1 adult emergence, petroleum ether extract at 5% conc. was found to be most effective (10.60%), followed by ethanol (11.60%), methanol (11.80%) and water extract (12.60%) respectively from 1st day to 10th days of adult emergence.

Key words: *Leucas lavandulifolia, Callosobruchus chinensis, Oviposition deterrence, LC_{50}.*

INTRODUCTION

Pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is one of the most devastating insect pest of all pulses causing 40-50% losses of pulses in storage[6]. In case of severe infestation, the damage caused is up to 100 % under storage[4]. The beetle is found in fields as well as in stored legume seeds, and is known to be a pest to many stored legumes. Pulse beetle being an internal feeder cannot be controlled with insecticides. Generally, management of stored product pest is done through fumigation and also is controlled by synthetic insecticides like dichlorvos, lindane, malathion, permethrin, bendiocarb, phosphine and methyl bromide etc., which pose possible health hazards to warm-blooded animals and a risk of environmental contamination. One alternative to synthetic insecticides is insecticidal plants.

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The use of plant products has assumed significance as an important component of insect pest management because of their economic viability and eco-friendly nature. They hold promise as alternatives to chemical insecticides to reduce pesticide load in the environment.

The plant kingdom contains a huge array of chemical substances; many of these are used by plants for their defense against insect attack. Phytochemicals possess a wide spectrum of biological properties against insects. They may act as antifeedants, repellents, growth inhibitors, attractants, chemosterilants or as insecticides. The naturally occurring phytochemicals are usually biodegradable and non-toxic to plants, warm-blooded animals and the environment. They offer great potential as safer, more effective and economic pesticides.Plant materials which are being traditionally used by some farmers are quite safe and appear to be the most promising grain protectants[1].*L. lavandulifolia* is an annual erect herb, 35-75cm high, pubescent or tomentose. An isopimaraner, hamnoglucoside and linifolioside have been isolated from this plant[5].

With growing awareness of the hazards associated with the use of synthetic organic insecticides, there is a greater need to explore suitable alternative methods of pest control against stored products. In view of the fact that the use of the indigenous plants will be a promising approach to reduce the bruchid population very effectively, an attempt has been made to study the adult mortality, ovipositional repellency and F_1 adult emergence of pulse beetle, *C. chinensis* after treating with extracts of *L. lavandulifolia.*

MATERIAL AND METHODS

Rearing of the Test Insect

The experiments were conducted at the Physiology Laboratory, Department of Entomology, Assam Agricultural University (AAU), Jorhat. Rearing of *C. chinensis* was maintained on green gram seed. For maintaining the culture of adult *C. chinensis*, 1kg green gram seed were put in a 5 lit capacity plastic jar and released five pairs of adult male and female in 1:1 ratio. For proper growth and development of the insect during winter season, the plastic jar containing green gram seed and *C. chinensis* were kept on BOD incubator at temperature 29±2°C.

Extraction of Bioactive Compounds

The leaves of *Leucas lavandulifolia* were collected in and around Jorhat district of Assam, India.The collected leaves were washed and dried in the shade at room temperature, grounded finely and hydro distilled in a Soxhlet apparatus as well as extracted separately with methanol, ethanol and petroleum etheras per method described by Bora *et al*[2]. The solvent were removed under reduced pressure using rotary vacuum evaporator (JSGW) and the residues were further dissolved in respective solvents on weight by volume (W/V) basis making it 100% stock solutionand stored in a sealed glass bottle at 4ºC refrigerator. Similarly, the aqueous extract was prepared grinding leaves in distilled water with weight by volume basis after washing thoroughly with running water which served as 100% stock solution.

Direct Toxicity Test

The bioassays of *L. lavandulifolia* on *C. chinensis* were performed by following the method of Talukdar and Howse[10] with some modifications. The adult insect was picked up from the stock culture and transferred to 9 cm diameter petriplate. Then 0.1ml solutions of different concentrations (1%, 1.5%, 2%, 2.5%, 3.5 %, 4%, 4.5% and 5 % W/V) were applied topically to the dorsal surface of the thorax of each insect by using hand atomizer (100ml). Released the treated insect immediately in the plastic container containing 20g green gram seeds. Insect mortality rates were recorded after 24hr, 48hr, 72hr and 96hr after treatment. Insect were examined daily and those that do not move or respond to gentle touch were considered dead. All the experiments were conducted Completely Randomized Design with five replication containing five pairs in each replication and subjected to statistical analysis.

$$\text{Mortality \%} = \frac{\text{Total no. of mortality of Pulse beetle in treated plastic container}}{\text{Total no. of insect recorded in each plastic container}} \times 100$$

Total Residual Toxicity Test

Plant extracts were mixed with 20g green gram seed @ 1%, 1.5%, 2%, 2.5%, 3 %, 3.5%, 4%, 4.5% and 5 % W/V. The treated seeds were air dried for 20 min and then put in to separate plastic pot (6cm×7cm). Fresh insect was released in each plastic pot containing 20g treated seed and closed it immediately after released of the insect. The whole experiments were replicated 5 times with 5 pairs of insects.

Oviposition Deterrence Activity

Five pairs of newly emerged beetles were released in pot containing (6cm×7cm) 20g green gram seed treated with different concentrations of each plant extracts allowed to remain in container for 7 days till they lay eggs. After one week of oviposition the number of eggs laid on treated seed (Et) and control seed (Ec) were counted and the percentage of oviposition deterrence (POD) were calculated using the following formula

$$\text{POD (\%)} = \frac{\text{Ec - Et x100}}{\text{Ec}}$$

Where, Et = No. of eggs laid on treated seed

Ec = No. of egg laid on Control seed

Adult Emergence Test

Pulse beetle starts to emerge after 30-40 days of egg laying. Theemerge beetles were count and remove every day from the container. The numbers of beetles were count daily from the date of first emergence to at least 10 days. The emergence rate was calculated and the inhibition rate (IR %) were calculated using the following formula

$$\text{IR\%} = \frac{\text{Cn - Tn x100}}{\text{Cn}}$$

Where, C_n= Number of insects in control plastic pots

T_n = Number of insects in treated plastic pots

RESULTS AND DISCUSSION

The effect of leaf and flower extracts of *L. lavandulifolia* on the adult *C. chinensis* is presented in Table 1 & 2. The result on adult mortality of *C. chinensis* revealed an increasing trend with time and varies with concentration of the plant extracts. Amongst the different solvent extracts, petroleum ether extract at 5.00 per cent concentration registered the highest mortality (94%) 96 hours after treatment (HAT), which was followed by methanol (92%); ethanol (78%) and water extract (76%).It was supported by the findings of Kovendan *et al*[8]., who reported that *L. lavandulifolia* extract showed larvicidal and pupicidal effects after 24hrs of exposure against malarial vector, *Anopheles stephensi*. They suggested that the ethanolic extracts of *L. lavandulifolia* provided an excellent potential for controlling of malarial vector, *A. stephensi*. While calculating the LC_{50} values of petroleum ether and methanol extracts against adult *C. chinensis*, it was found to be the lowest LC_{50} value, 2.309% and 2.800% respectively (Table 3).

The effects of different solvent extracts of *L. lavandulifolia* on ovipositional response of *C. chinensis* are given in Table 4. It was found that the highest oviposition deterrence (61.00%) in the seed treated with petroleum ether extract of *L. lavandulifolia,* followed by methanol, ethanol and water extract recorded 60.01%, 56.52% and 47.59%, respectively at 5.00 per cent concentrations after 7 days of seed treatment. The result of present investigation was also supported by the work of Pandey *et al*[9]., who reported that petroleum ether extract of *Azadirachta indica*, *Lantana camera*, *Agaratum conyzoides* and *Ipomoea carnea* proved as a potent oviposition inhibitor to *Callosobruchus chinensis*.

The effect of different solvent extracts of *L. lavandulifolia* on hatching success of *C. chinensis* eggs are given in Table 5. It shows that the ovicidal effect of the different solvent extracts of *L. lavandulifolia,* where all the treatments were significantly different amongst themselves. It is revealed that petroleum ether extract of *L. lavandulifolia* at

5.00% concentration was found to be most effective, inhibits the adult emergence up to 78.87%, followed by methanol (77.63%), ethanol (76.65%) and water extract (75.18%) respectively from 1 day to 10 day of adult emergence. Plant extracts and essential oils are known to possess repellent, ovicidal and insecticidal activities against various stored grain insects[3,7].

Table 1: Cumulative percentage mortality of adult *C. chinensis* treated with petroleum ether and methanol extracts of *Leucas lavandulifolia* leaf and flower

Conc. (%)	Ethanol (Mean ± SE)				Water (Mean ± SE)			
	24 HAT	48 HAT	72 HAT	96 HAT	24 HAT	48 HAT	72 HAT	96 HAT
1.00	6±2.45 (14.17)^{f}	12±3.74 (20.26)^{g}	20±3.16 (26.55)^{g}	24±2.45 (29.32)^{i}	6±2.45 (14.17)^{f}	12±3.74 (20.26)^{g}	18±2.00 (25.09)^{g}	22±2.00 (27.96)^{h}
1.50	10±3.16 (18.43)^{e}	18±4.89 (25.09)^{f}	28±3.74 (31.94)^{f}	32±3.74 (34.44)^{h}	12±3.74 (20.26)^{e}	16±2.45 (23.57)^{f}	24±2.45 (29.32)^{f}	30±0.00 (33.20)^{g}
2.00	16±2.45 (23.57)^{d}	28±4.89 (31.94)^{e}	42±3.74 (40.38)^{e}	46±2.45 (42.69)^{g}	14±2.45 (21.96)^{de}	26±5.09 (30.64)^{e}	34±5.09 (35.65)^{e}	44±3.99 (41.54)^{f}
2.50	18±3.74 (25.09)^{d}	38±4.89 (38.04)^{d}	48±5.82 (43.84)^{e}	58±5.82 (49.58)^{f}	16±2.45 (23.57)^{d}	30±0.00 (33.20)^{e}	44±3.99 (41.54)^{cd}	56±3.99 (48.43)^{e}
3.00	22±2.00 (27.96)^{cd}	44±5.09 (41.54)^{cd}	54±3.99 (47.28)^{d}	68±5.82 (55.53)^{e}	20±5.47 (26.55)^{cd}	40±4.46 (39.22)^{d}	48±7.34 (43.84)^{d}	66±5.99 (54.31)^{d}
3.50	26±5.09 (30.64)^{c}	50±7.06 (44.98)^{c}	62±5.82 (51.92)^{c}	72±4.89 (58.03)^{d}	24±5.09 (29.32)^{c}	48±5.82 (43.84)^{c}	60±4.46 (50.75)^{c}	70±3.16 (56.77)^{d}
4.00	28±3.74 (31.94)^{bc}	54±5.09 (47.28)^{bc}	70±3.16 (56.77)^{bc}	82±5.82 (64.87)^{c}	26±3.99 (30.64)^{bc}	50±4.46 (44.98)^{b}	64±2.45 (53.11)^{bc}	78±5.82 (62.00)^{c}
4.50	32±5.82 (34.44)^{b}	58±4.89 (49.58)^{b}	74±2.45 (59.32)^{b}	88±3.74 (69.70)^{b}	30±4.46 (33.20)^{ab}	56±5.09 (48.43)^{ab}	70±4.46 (56.77)^{b}	84±2.45 (66.40)^{b}
5.00	38±6.62 (38.04)^{a}	64±10.75(53.11)^{a}	86±5.09 (68.00)^{a}	94±2.45 (75.79)^{a}	36±5.09 (36.86)^{a}	62±7.99 (51.92)^{a}	80±4.46 (63.41)^{a}	92±3.74 (73.54)^{a}
Control	2±2.00 (8.13)^{g}	4±3.99 (11.53)^{h}	6±3.99(14.17)^{h}	8±3.74(16.42)^{j}	0±0.00 (1.28)^{g}	2±2.00 (8.13)^{h}	4±2.45 (11.53)^{h}	6±2.45 (14.17)^{i}
S.Ed (±)	1.51	1.96	1.52	1.64	1.59	1.49	1.32	1.42
CD (P=0.05)	2.48	3.22	2.49	2.68	2.61	2.45	2.17	2.32

*Data presented are the mean of 5 replications each having 10 nos. of insects.

* Zero and 100% mortality was corrected by using Steel &Torrie formula.

* Data within the parentheses are angular transformed value, compared by DMRT, (P<0.05)

* Means followed by same letter are not significantly different

* HAT= Hours after treatment

Table 2: Cumulative percentage mortality of adult *C. chinensis* treated with ethanol and water extracts of *Leucas lavandulifolia* leaf and flower

Conc. (%)	Ethanol (Mean ± SE)				Water (Mean ± SE)			
	24 HAT	48 HAT	72 HAT	96 HAT	24 HAT	48 HAT	72 HAT	96 HAT
1.00	6±3.99 (14.17)^{f}	10±5.47 (18.43)^{g}	16±5.99 (23.57)^{g}	22±3.74 (27.96)^{h}	6±3.99 (14.17)^{d}	8±3.74 (16.42)^{e}	16±3.99 (23.57)^{h}	20±4.46 (26.55)^{g}
1.50	14±2.45 (21.96)^{e}	24±3.99 (29.32)^{f}	32±6.62 (34.44)^{f}	34±5.09 (35.65)^{g}	8±3.74 (16.42)^{d}	20±5.47 (26.55)^{d}	22±4.89 (27.96)^{g}	26±3.99 (30.64)^{f}
2.00	18±3.74 (25.09)^{d}	32±3.74 (34.44)^{e}	42±4.89 (40.38)^{e}	46±5.09 (42.69)^{f}	16±5.09 (23.57)^{c}	26±2.45 (30.64)^{c}	34±5.09 (35.65)^{f}	38±3.74 (38.04)^{e}
2.50	26±6.77 (30.64)^{c}	36±7.47 (36.86)^{de}	44±5.99 (41.54)^{e}	52±6.62 (46.13)^{e}	18±2.00 (25.09)^{bc}	28±2.00 (31.94)^{bc}	40±4.46 (39.22)^{e}	44±6.77 (41.54)^{d}
3.00	28±4.89 (31.94)^{bc}	40±5.47 (39.22)^{d}	52±6.62 (46.13)^{d}	58±3.74 (49.58)^{cd}	22±2.00 (27.96)^{b}	32±3.74 (34.44)^{b}	50±4.46 (44.98)^{d}	54±5.09 (47.28)^{c}
3.50	30±8.93 (33.20)^{bc}	44±8.11 (41.54)^{cd}	58±7.34 (49.58)^{c}	62±3.74 (51.92)^{d}	24±3.99 (29.32)^{ab}	34±3.99 (35.65)^{b}	54±2.45 (47.28)^{c}	58±3.74 (49.58)^{c}
4.00	32±2.00 (34.44)^{ab}	48±4.89 (43.84)^{bc}	62±3.74 (51.92)^{c}	68±2.00 (55.53)^{c}	30±4.46 (33.20)^{a}	52±4.89 (46.13)^{a}	62±7.34 (51.92)^{b}	68±7.99 (55.53)^{b}
4.50	34±3.99 (35.65)^{a}	50±8.35 (44.98)^{ab}	68±7.34 (55.53)^{b}	72±5.82 (58.03)^{b}	32±3.74 (34.44)^{a}	54±5.09 (47.28)^{a}	70±3.16 (56.77)^{a}	74±5.09 (59.32)^{a}
5.00	36±6.77 (36.86)^{a}	54±2.45 (47.28)^{a}	74±2.45 (59.32)^{a}	78±3.16 (63.41)^{a}	34±5.99 (35.65)^{a}	56±5.09 (48.43)^{a}	72±2.00 (58.03)^{a}	76±3.99 (60.64)^{a}
Control	0±0.00 (1.28)^{g}	2±2.00 (8.13)^{h}	2±2.00 (8.13)^{h}	4±2.45 (11.53)^{i}	0±0.00 (1.28)^{e}	2±2.00 (8.13)^{e}	4±2.45 (11.53)^{i}	4±2.45 (11.53)^{h}
S.Ed (±)	1.61	1.82	1.75	1.32	1.64	1.40	1.35	1.55
CD (P=0.05)	2.64	2.98	2.86	2.17	2.69	2.30	2.21	2.54

*Data presented are the mean of 5 replications each having 10 nos. of insects.

* Zero and 100% mortality was corrected by using Steel &Torrie formula.

* Data within the parentheses are angular transformed value, compared by DMRT, (P<0.05)

* Means followed by same letter are not significantly different

* HAT= Hours after treatment

Table 3: LC_{50} values of *L. lavandulifolia* extract against adult mortality of *C. chinensis*

Plant Extract	Number of insect tested	Regression equation	LC_{50} (%)	95% Fiducial limit of LC_{50} Upper–Lower	X^2	LC_{95} (%)
Petroleum ether extract	150	Y=2.59x-3.72	2.309	2.80-1.90	1.80	10.035
Methanol extract	150	Y=2.43x-3.39	2.800	3.43-2.29	1.64	13.408

Table 4: Ovipositional response of *C. chinensis* female to different extracts of *Leucas lavandulifolia* on treated green gram seed

Conc. (%)	Petroleum ether		Methanol		Ethanol		Water	
	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %	No of eggs/20g seeds (Mean ± SE)	Oviposition deterrence %
1.00	88.40±2.80^{b}	25.40	90.80±6.02^{b}	22.49	92.60±3.91^{b}	21.04	93.60±4.00^{b}	18.97
1.50	84.20±4.02^{c}	29.36	86.80±3.53^{c}	25.33	89.80±2.65^{c}	23.21	92.40±5.74^{b}	19.93
2.00	80.40±3.24^{d}	32.09	83.00±4.09^{d}	28.39	84.20±4.19^{d}	28.57	88.00±5.06^{c}	24.02
2.50	77.00±2.02^{e}	35.08	78.20±5.61^{e}	32.00	80.80±3.75^{d}	30.84	84.40±5.84^{d}	26.63
3.00	67.80±4.13^{f}	42.42	69.80±5.02^{f}	39.33	76.80±4.30^{e}	34.50	82.00±4.08^{d}	27.76
3.50	66.80±4.67^{f}	44.19	64.60±3.44^{g}	44.55	72.20±4.29^{f}	37.96	78.80±4.34^{e}	31.02
4.00	59.60±3.00^{g}	49.85	56.40±4.67^{h}	50.82	66.20±4.45^{g}	43.83	75.20±5.74^{e}	34.71
4.50	48.60±3.73^{h}	58.98	49.00±3.27^{i}	57.65	59.60±5.70^{h}	48.56	67.40±4.62^{f}	41.16
5.00	45.60±5.03^{i}	61.00	47.00±4.52^{i}	60.01	50.40±508^{i}	56.52	59.60±4.43^{g}	47.59
Control	119.20±4.46^{a}	0.00	117.40±6.03^{a}	0.00	118.20±5.04^{a}	0.00	116.60±7.94^{a}	0.00
S. Ed (±)	1.71	1.59	2.12	2.25	1.98	2.21	2.38	2.34
CD (P=0.05)	2.80	2.61	3.47	3.69	3.24	3.62	3.90	3.83

*Data presented are the mean of 5 replications each having 10 nos. of insects.

* 20 g seed content approx. 560-565 seeds

* The mean values were compared by DMRT, ($P<0.05$)

* Means followed by same letter are not significantly different.

Table 5: Effect of *Leucas lavandulifolia* leaf and flower extracts on hatching success (%) of *C. chinensis* eggs on treated green gram seed

Conc. (%)	Petroleum ether		Methanol		Ethanol		Water	
	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control	No of insect emergence (% hatching) (Mean ± SE)	Hatching inhibition rate over control
1.00	37.80±0.86 (37.92)^{b}	25.23	39.80±0.66 (39.10)^{b}	24.05	38.40±1.12 (39.10)^{b}	23.98	39.40±0.75 (38.86)^{b}	23.14
1.50	33.60±0.93 (35.41)^{bc}	33.44	35.00±2.16 (36.26)^{c}	33.50	34.80±1.46 (36.26)^{c}	31.26	35.60±1.69 (36.62)^{c}	30.41
2.00	30.80±2.22 (33.70)^{cd}	38.64	32.60±2.06 (34.80)^{d}	37.86	31.60±1.91 (34.80)^{cd}	36.81	33.00±1.41 (35.05)^{cd}	35.32
2.50	29.60±1.63 (32.95)^{cd}	41.27	30.80±1.32 (33.70)^{cd}	41.35	30.40±2.83 (33.70)^{cd}	39.74	31.00±1.64 (33.82)^{d}	39.23
3.00	28.00±1.58 (31.94)^{de}	44.93	29.20±1.77 (32.70)^{de}	44.23	28.40±2.06 (32.70)^{de}	43.24	29.40±2.04 (32.82)^{de}	42.67
3.50	24.20±3.00 (29.46)^{e}	51.67	25.60±2.61 (30.38) ^{e}	50.88	25.40±2.73 (30.38)^{e}	49.79	26.60±2.33 (31.04)^{e}	47.52
4.00	16.20±0.80 (23.72)^{f}	68.04	17.20±1.07 (24.49)^{f}	67.14	17.60±1.36 (24.49)^{f}	65.62	19.00±1.22 (25.83)^{f}	63.03
4.50	13.00±0.71 (21.13)^{fg}	74.08	13.60±0.93 (21.63)^{fg}	74.00	14.60±0.51 (21.63)^{g}	71.13	16.00±1.30 (23.57)^{g}	68.85
5.00	10.60±0.75 (18.99)^{g}	78.87	11.80±0.80 (20.08)^{g}	77.63	11.60±0.75 (20.08)^{g}	76.65	12.60±0.60 (20.78)^{h}	75.18
Control	50.80±2.13 (45.44)^{a}	0.00	52.60±1.60 (46.47)^{a}	0.00	51.60±3.64 (46.47)^{a}	0.00	51.60±2.29 (45.90)^{a}	0.00
S. Ed (±)	0.48	1.63	0.47	1.44	0.92	2.45	0.47	1.76
CD (P=0.05)	0.79	2.67	0.77	2.36	1.52	4.03	0.77	2.89

*Data presented are the mean of 5 replications each having 10 nos. of insects.

* Data within the parentheses are angular transformed value, compared by DMRT, ($P<0.05$)

* Means followed by same letter are not significantly different

CONCLUSION

The results of the study have confirmed that the pulse beetle, *C.chinensis*,can be effectively controlled by leaf extract of *L. lavandulifolia*. It may be due to the presence of isopimaraner, hamnoglucoside and linifolioside in the leaves of *L. lavandulifolia*. The use of botanicals should be encouragedin small farm storage, as the cost of these botanicals is low and easily available when compared with the losses incurred in untreated seeds. Thus, the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use.

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