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## Antifeedant activity of essential oil *Lantana camara* L. against *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) and *Plutella xylostella* Curtis (Lepidoptera: Plutellidae)

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### ABSTRACT

The main volatile components of leaf extracts from *Lantana camara* by gas chromatography–mass spectrometry (GC-MS) were identified as  $\beta$ -caryophyllene (29.67%), caryophyllene oxide (2.69%),  $\alpha$ -humulene (10.24%) and germacrene D (1.72%). The antifeedant activity of *Lantana camara* essential oil was tested against *Spodoptera litura* and *Plutella xylostella* at different concentrations. Results indicated that 50%–60% antifeedant activity induced by the oil was recorded on *Spodoptera litura* and *Plutella xylostella* second instar larvae and gave significant difference compared to the control ( $P=0.0000$ ) in no-choice test and choice-test experiments, respectively; the essential oil of *Lantana camara* affected the ratio of pupation and adult emergence of both *S. litura* and *P. xylostella*.

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## 1 INTRODUCTION

In Vietnam, developing biopesticides from natural plant-derived products plays an important role through promoting sustainable agriculture. Therefore, increasing the efficiency of Integrated Pest Management (IPM) program, particularly using biopesticides to manage Lepidopteran pests is paid attention nationally and worldwide. Moreover, *Spodoptera litura* and *Plutella xylostella* are the most devastating insect pests of brassicaceous crops and have developed resistance to many chemical pesticides.

Many different plant species have useful compounds that can be used as biopesticides. Scientists have reported that the leaf extracts from *Elsholtzia cristata* have antifeedant activity to *Pieris rapae* (Nguyen Ngoc Hoa *et al.* 2012). *Lantana*

*camara* distributes to subtropical and tropical regions of the world which contains phenolics, flavonoids, alkaloids, triterpenes, saponin, terpenoids, etc. having the ability to kill mosquito 3<sup>rd</sup> and 4<sup>th</sup> instars (Kalita *et al.* 2012). It was reported that this plant has been used in many places for treating various human illnesses, and the plant extracts have exhibited a broad range of biological activities (Sharma *et al.* 2007). Besides, Ogendo *et al.* (2004) reported that the plant has been shown to have toxic and repellent effects against stored grains pests. Essential oils and their constituents from leaves and flowers of *L. camara* showed fumigants against stored product insects (Zoubiri and Baaliouamer, 2012). Some scientists have reported the insecticidal activities of *L. camara* essential oil on *Sitophilus* spp. (Mohamed and Abdelgaleil, 2008; Zoubiri and Baaliouamer, 2011) and

*Tribolium castaneum* (Coleoptera: Tenebrionidae) (Mohamed and Abdelgaleil, 2008). The objective of the study was to determine the basic volatile components of essential oil from leaves and fumigant toxicity of essential oil of *L.camara* growing in Binh Duong province against *S. litura* and *P. xylostella*.

## 2 MATERIALS AND METHODS

### 2.1 Plant materials and essential oils

The fresh leaves of *L. camara* were collected in Binh Duong province from their natural habitats. Collected leaves were washed and kept in plastic bags inside a refrigerator until the time for oil extraction. The essential oils were obtained by hydrodistillation method using a Clevenger type apparatus.

### 2.2 Insect culture

*Plutella xylostella* larvae were collected at *Brassica integrifolia* vegetable fields in Hoc Mon and *Spodoptera litura* at swamp morning-glory (*Ipomoea aquatica*) in Cu Chi, Ho Chi Minh City and were reared until the second generation on leaves of *Brassica integrifolia* at room temperature ( $28\pm 2^\circ\text{C}$ ) with 16-8 L:D condition. Second instar larvae of *P. xylostella* and *S. litura* were used for experiments.

### 2.3 Gas chromatography

The analyses of the essential oil compounds were sent to Vietnam National University Ho Chi Minh City, University of Science, 227 Nguyen Van Cu Street, Ward 4, District 5, Ho Chi Minh City, Viet Nam for GC-MS system analysis.

### 2.4 Antifeedant activity of essential oils

The experiments were conducted with 5 treatments of essential oil 1, 5, 10, 100 and 500  $\mu\text{L}$ , and 50  $\mu\text{L}$  acetone was used as control treatment.

No-choice test method was conducted to study the antifeedant activity of crude extracts. Fresh *B. integrifolia* and swamp morning-glory leaf discs of 1 cm diameter were punched using the cork borer and then treated each with 1, 5, 10, 100 and 500  $\mu\text{L}$  of essential oil in 50  $\mu\text{L}$  acetone treatments. Larvae were starved 3-4 hours prior to experimentation, then second instar larvae were introduced to plastic petri dish (1.5 cm x 9 cm) containing wet filter paper to avoid early drying of the leaf discs. No-choice test method was calculated using the leaf disc method (Zandi-Sohani *et al.* 2012):

$$\text{Antifeedant index (\%)} = [1 - (C - T) / (C)] \times 100]$$

where “C” is the weight of leaf disc and “T” is the weight of leaf discs remained in the treatment after 24 hours.

Choice test method was conducted by the method as previously described. Half of filter paper (5 leaves,  $1\text{cm}^2/\text{each leaf}$ ) was impregnated with 50  $\mu\text{L}$  acetone and another half were treated with acetone solutions of different concentrations of essential oil treatment (1, 5, 10, 100 and, 500  $\mu\text{L}$ , respectively) and dried for 10 minutes at room temperature. Three replicates were maintained for each treatment. Five larvae of *S. litura* and 10 larvae of *P. xylostella* per replicate were exposed separately. The antifeedant activity was calculated using the formula of Caasi (1983):

$$\text{Antifeedant index (\%)} = [(C_o - C_i) / (C_o)] \times 100$$

where “C<sub>o</sub>” is the weight of leaf disc consumed in the control and “C<sub>i</sub>” is the weight of leaf discs consumed in the treatment.

### 2.5 Statistical analysis

The antifeedant, larvicidal and pupicidal activities were subjected to analysis of variance (ANOVA) followed by Duncan, Statgraphics plus 3.0 software.

## 3 RESULTS AND DISCUSSION

### 3.1 Chemical composition of essential oil

**Table 1: Volatile composition of the essential oil from *Lantana camara* leaves**

Volatile compounds	Concentration (% peak area)		
	In this study	Ref *	Ref **
$\alpha$ -Pinene	0.79	0.25	-
Sabinene	6.90	1.56	-
$\beta$ -Pinene	1.01	-	-
$\beta$ -Myrcene	0.48	0.67	-
$\delta$ -3-Carene	1.24	0.04	-
1,8-Cinevole	8.71	-	-
$\beta$ -Elemene	0.84	6.41	2.40
$\beta$ -Caryophyllene	29.67	35.70	-
$\alpha$ -Humulene	10.24	3.72	3.46
Germacrene D	1.72	2.85	4.11
Germacrene B	6.95	-	3.18
$\delta$ -Cadinene	1.07	-	2.40
Danavone	2.90	0.30	-
Spathulenol	1.88	-	4.08
Caryophyllene oxide	2.69	10.04	1.13

(\*): Zoubiri and Baaliouamer (2012); (\*\*): Murugesan *et al.* (2012)

The main volatile composition of *L. camara* by GC-MS in this study was identified as:  $\beta$ -caryophyllene (29.67%), caryophyllene oxide (2.69%),  $\alpha$ -humulene (10.24%) and germacrene D (1.72%)

which were also reported responsible for the insecticidal activity (Zoubiri and Baaliouamer, 2012; Zandi-Sohani *et al.*, 2012) (Table 1). Zandi-Sohani *et al.* (2012) indicated that genetic, climatic, geographical and seasonal variations caused the difference in quality and quantity of essential oil composition.

### 3.2 Antifeedant activity

Results of the study indicated that the essential oil of *L. camara* gave toxic and repellent effects against both *S. litura* and *P. xylostella*. It produced high mortality to the 2<sup>nd</sup> instar larvae of both *P. xylostella* and *S. litura*. Antifeedant activity was found to increase with the essential oil concentration in no-choice test experiment. The essential oil at 500 µl concentration showed significant difference to other treatments (P<0.05) and caused almost 50% antifeedant activity on the exposed larvae (Table 2).

Pupicidal activity of T3-10 treatment was recorded as of 0% for *S. litura* and of T4-100 treatment for *P. xylostella*, respectively. Adult activity was increased corresponding with the increased concentrations of essential oil and showed

significant difference to other treatments (P<0.05) (Table 3).

**Table 2: Antifeedant activity of essential oil from *Lantana camara* on *S. litura* and *P. xylostella* (no-choice test)**

Name of the treatments	Antifeedant index (%)	
	<i>S. litura</i>	<i>P. xylostella</i>
T1-1	29.63 <sup>e</sup>	31.17 <sup>d</sup>
T2-5	35.39 <sup>d</sup>	38.98 <sup>c</sup>
T3-10	42.15 <sup>c</sup>	48.06 <sup>b</sup>
T4-100	50.67 <sup>b</sup>	51.28 <sup>b</sup>
T5-500	57.07 <sup>a</sup>	62.42 <sup>a</sup>
T6-acetone	27.38 <sup>e</sup>	25.18 <sup>e</sup>
cv %	4.38	5.75

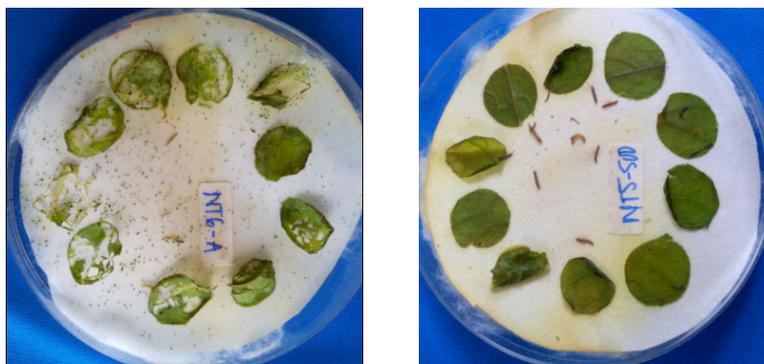
Means within column followed by the same letter were not significantly different by Duncan test. Data were transferred to arcsine prior ANOVA (95%) and comparison of means.

T1-1: (1 µL essential oil + 50 µL acetone); T2-5: (5 µL essential oil + 50 µL acetone); T3-10: (10 µL essential oil + 50 µL acetone); T4-100: (100 µL essential oil + 50 µL acetone); T5-500: (500 µL essential oil + 50 µL acetone); T6-acetone: 50 µL acetone.



**Fig. 1: Antifeedant activity of essential oil from *Lantana camara* on *S. litura* (no-choice test)**

Left: Control; Right: T5-500 treatment



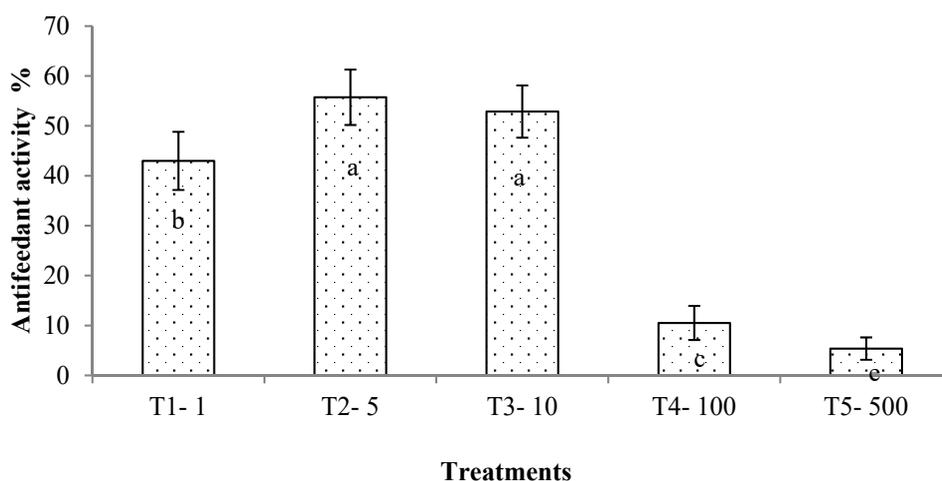
**Fig. 2: Antifeedant activity of essential oil from *Lantana camara* on *P. xylostella* (no-choice test)**

Left: Control; Right: T5-500 treatment

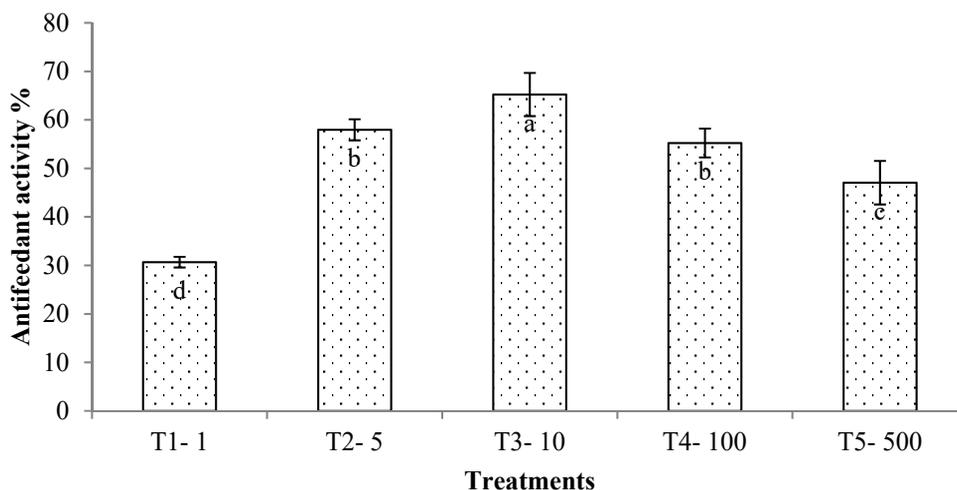
**Table 3: Percentage of pupicial activities and adult activity observed after treatment of essential oil from *Lantana camara* on *S. litura* and *P. xylostella* (no - choice test)**

Name of the treatments	<i>Spodoptera litura</i>		<i>Plutella xylostella</i>	
	Pupicial activity (%)	Adult activity (%)	Pupicial activity (%)	Adult activity (%)
T1-1	39.23 c	30.78 b	36.93 c	26.07 c
T2-5	30.78 b	30.78 b	23.85 b	21.15 bc
T3-10	0.00 a	0.00 a	21.15 b	18.44 b
T4-100	0.00 a	0.00 a	0.00 a	0.00 a
T5-500	0.00 a	0.00 a	0.00 a	0.00 a
T6- Acetone	43.08 c	43.08 c	45.00 c	37.22 d
cv%	21.43	28.80	24.88	22.43

Data were transformed into  $\arcsin\sqrt{x}$  and were analyzed using one-way analysis of variance (ANOVA) followed by Duncan. Means within column followed by the different letters indicate statistically significant differences among groups ( $P < 0.05$ )



**Fig. 3: Antifeedant activity of essential oil to *S. litura* (choice test)**



**Fig. 4: Antifeedant activity of essential oil to *P. xylostella* (choice test)**

In choice test experiment (Fig. 3 and Fig. 4), the essential oil of *L. camara* also produced high antifeedant activity to the 2<sup>nd</sup> instar larvae of *S. litura* and *P. xylostella* from T2-5 and T3-10 treatments. However, antifeedant activity was found to decrease as the essential oil concentration increased. This result can be explained as the concentration of essential oil of *L. camara* was high at T4-100 and T5-500 treatments and active compounds effected to the control leaves when leaves were put together in choice test experiment.

*Lantana camara* is toxic and feeding deterrent to both *S. litura* and *P. xylostella*. The presence of  $\beta$ -caryophyllene, caryophyllene oxide, germacrene B/D were analyzed in this study (Table1) indicated the effectiveness of essential oil of *L. camara*, as the same result was also obtained by Zoubiri and Baaliouamer (2012). Studies have been reported the deterrent activity of plant extracts and the essential oil of *L. camara* against *Reticulitermes flavipes* (Yuan and Hu, 2012) and *Sitophilus granarius* adults (Zoubiri and Baaliouamer, 2012), respectively. The ability of antibacterial, antimicrobial and antifungal activities of essential oil of *L. camara* have been intensively studied by Siddiqui *et al.* (1995). The plant also has been shown to have toxic and repellent affects against insect pests of stored grains (Ogendo *et al.*, 2004) and adult termite workers (Verma and Verma, 2006).

Many plant secondary metabolites are known as antifeedant and toxic to insect larvae such as triterpenes, sesquiterpene lactones and alkaloids (Paul and Saha, 2012). Baskar *et al.* (2009, 2010) reported that the antifeedant activity was due to the presence of alkaloids, some plant extracts against *P. xylostella* such as *Acorus calamus* L. (Arecaceae), *Azadirachta indica* A. Juss, *Melia azedarach* L. (Meliaceae), and *Acalypha fruticosa* F. (Euphorbiaceae) (Lingathurai *et al.*, 2011). There are seventy-four triterpenoids have been isolated from different *Lantana* species, especially lantadenes are considered as the main bioactive constituents (Sharma *et al.*, 2007). However, scientists have not conducted experiments to understand the insecticidal mechanisms of the actions of these chemical constituents.

#### 4 CONCLUSIONS

In this study, significant insecticidal and antifeedant activities against *S. litura* and *P. xylostella* were first observed in essential oil of *L. camara* suggesting a future exploitation for the isolation of active molecules and to develop a new botanical formulation for sustainable agriculture in Vietnam. The results described the role of biopesticide of essential oil of *L. camara* on insect pests. More experiments on other

lepidopteran pests are needed to conduct in the future.

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