

# Antifeedant Activity of Ethanolic Leaf Extract of *Lantana camara* Against *Crocidolomia pavonana* and *Spodoptera litura*

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Type of the Paper (Article)

Received: 2019-01-08; Accepted: 2019-08-03; Published: 2019-08-07

<https://doi.org/10.29253/jptafm.1.1.2018.55>

**Abstract.** Chemical insecticides that have been intensively used to control cabbage caterpillars have become the main causes of pest resistance, high chemical residues in crop products and the decrease of biodiversity of natural enemies in the environment. Secondary metabolites from plant extract provide opportunities to be developed as bioinsecticides. The objective of this research was to evaluate the antifeedant effects of ethanolic leaf extract of *L. camara* on *Crocidolomia pavonana* and *Spodoptera litura* larvae for pre preliminary study. The crude extract was prepared by leaf maceration of *L. camara* leaf using ethanol, evaporation of the solvent, followed by antifeedant bioassay test. Leaf disc dipping method with choice and no choice tests used to find out the effective minimum concentration on feeding deterrent activity. The mean leaf area consumed were analyzed by using nonparametric statistics of Mann-Whitney U. The results showed that the minimum effective concentration of ethanolic leaf extract that deter the feeding activity of *C. pavonana* larvae was 2000 ppm (choice test) and 1000 ppm (no choice test) ( $P < 0.05$ ) and consider as medium antifeedant category. The minimum concentration showing antifeedant activity to *S. litura* larvae was 500 ppm (choice test) but result no choice test was showed not significant antifeedant activity ( $P > 0.05$ ). This performance consider as medium antifeedant category on *S. litura* larvae at 500 ppm, in concentrations 1000ppm-5000ppm, the extract of *L. camara* were tended as attractant on *S. litura* larvae.

**Keywords:** Antifeedant, *L. camara*, leaf extract, *C. pavonana*, *S. litura*

## 1. Introduction

The increase in cabbage production in Indonesia is constrained by various factors including attacks by two main Lepidoterian insect pests *Crociodolomia pavonana* and *Spodoptera litura* (Rauf, 2004 ; Dono et al., 2010). The control of these two insect pests still relies on the application of chemical insecticides which spend 10% -30% of the total cost of cabbage production [1]. However, careless and overuse of these chemicals in agricultural ecosystems cause various detrimental effects to human health and the environment [2]. This also encourages the development of pest resistance to the most commonly used pesticides. In addition to the population of biocontrol agents, honeybees and earthworms can also be affected [3]. Plant protection efforts that minimize the use of pesticides can be carried out using biopesticides, plant extract, cultural techniques, resistant plants as integrated pest management (IPM) (Cespedes 2014). Plant-based insecticides have many advantages such as low risk on the development of pest resistance, target-specific and hence they are not harmful to human and beneficial insects, not persistent in nature and hence they are environmentally friendly [4].

In the present research, an attempt has been made to evaluate widely distributed medicinal plants at Arboretum of Universitas Padjadjaran, for their bioactivity against pest insect of cabbage. *Lantana camara* is a selected plant of the family Verbenaceae known as an important medicinal plant, a type of weed shrub plant that is high in abundance and rich in active phytochemical compounds [5] [6]. Phytochemical screening of methanolic plants extract of *Lantana camara* showed the presence of glycosides, carbohydrates, phenolic compounds, saponins, alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids as major phytochemical groups [7]. Organic extract and essential oils of *Lantana camara* have shown a wide variety of biological activities [8][9]. The chloroform leaf extract of *L.camara* had excellent repellent and moderate toxic and antifeedant activities against *Reticulitermes flavipes* (Kollar), types of termite which is an important structural pest [10]). Hexane extract of *L. camara* leaf caused mortality, inhibited the growth, and resulted in developmental anomalies of *Dysdercus koenigii* (Heteroptera) [9]. The n-hexane and dichloromethane crude extract of *L. camara* flowers showed contact toxicity and antifeedant effects on the second instar of *S. litura* [11]. Extract and fraction of *L. camara* leaf are known to have antifeedant effect against cabbage pest, *Plutella xylostella* larvae (Lepidoptera) [12]. The effect of feeding-deterrent activity can weaken and inhibit the development of the target pests, thereby increasing the risk to be preyed by natural enemies[12]. Thus, the plant extract with antifeedant effect can reduce the pest populations with environmentally-friendly ways [13].

So far, the antifeedant activity of ethanolic leaf extract of *L. camara* on cabbage pests other than *P. xylostella*, namely *C. pavonana* and *S. litura*, has not been reported. Whereas *C. pavonana* has been known to be resistant to synthetic insecticides Profonefos [14] and therefore the search for environmentally-friendly insecticide is interesting to be studied. Antifeedant compounds are sublethal toxicity for pest insects while lethal toxic compounds with high board spectrum negatively impacts on natural enemies, pollinators a non-target organism, and continuous use of lethal toxic compounds has frequently resulted in the development of resistance for population suppression [15]. The aims of this research was to determine the bioactivity particularly antifeedant effect of ethanolic extract of *L. camara* leaf against *C. pavonana* and *S. litura* larvae. The results of the study can be used as consideration on the use of *L. camara* as a biopesticides source for environmentally friendly cabbage cultivation.

## 2. Materials and Methods

### 2.1. Preparation and Rearing of *C. pavonana* and *S. litura*.

The instar larvae of *C. pavonana* and *S. litura* were obtained from Indonesian Vegetable Research Institute (BALITSA) Lembang, West Java, Indonesia. The main equipment for rearing was a rearing cabinet with the size of 63.5 × 64 × 186.2 cm<sup>3</sup> and equipped with the automatic control system to the humidity, temperature, and day-night [16] fig.1(a). The *C. pavonana* and *S. litura* were fed with cabbage leaf (*Brassica oleracea* var capitata). Honey 10% was also used as additional nutrition. The larval and imago cage places in containers were inserted in a rearing cabinet with setting temperature at 25 °C and humidity at 70% and

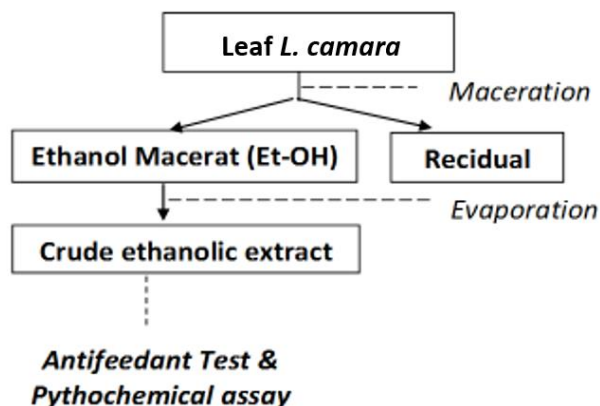
photoperiodism 12 day-light: 12 night-light [17]. *C. pavonana*'s life cycle is shown in fig. 1(b). The larva of fourth instar stage was used for the antifeedant bioassay test.



**Figure.1.** (a) Insect rearing cabinet (b) *C. pavonana*'s morphology from eggs to imago

### 2.2. Preparation of Ethanolic Leaf Extract of *L. camara*

The extract preparation was initiated by soaking the leaf simplicial into 95% ethanol solvent for 72 hours for maceration. Ethanol is a polar solvent and can attract polar compounds including active compounds of *L. camara*'s leaf extract. The instruments of extraction were used glasswares and vacuum evaporator type Buchi Rotavapor R-300. The macerate was concentrated with a low pressure (40°C) to obtain a viscous extract in the form of paste (Figure.2)



**Figure.2.** The procedure of ethanolic *L. camara* leaf extraction

### 2.3. Phytochemical assay of Ethanolic of *L. camara* Leaf Extract

The phytochemical constituents of ethanolic *L. camara* leaf extract was investigated by phytochemical analysis method (qualitative assay) and this has been analyzed in Analytical Test Laboratory of Research Center Padjadjaran University.

#### 2.4. Antifeedant Bioassay Test

Research method applied was bioassay guided tests with leaf disc method-choice and no choice test [15]. The completely randomized design with 6 treatments : 0 ppm (as a control), 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm, 5000 ppm concentration of leaf extract were applied [18]. Each treatment was repeated five times ( $r_i$ ). Further, antifeedant bioassay with choice and no choice test were undertaken with observation on testing nonparametric design, this is due to a variety of eating behaviors resulting in a non-homogeneous distribution of data. The leaf disc ( $\emptyset$  5cm) was dipped into the extract (3 seconds) and air dried. The treated leaf disc was put into petridish ( $\emptyset$  10cm) which is exposed inside two larvae had been fasted for 3 hours [18].

The choice antifeedant test involved two different leaves, that were with extract and without extract (control). This method was based on the real condition when the larvae could choose between treated and untreated leaves. The no choice test the leaf that given extract separately from the leaf control. The larvae were fed only with the treated or untreated (control) leaf discs.

#### 2.5. Data Analysis and Evaluation

The investigated parameter for the antifeedant bioassay was the leaf area consumed by *C. pavonana* and *S. litura* 3<sup>rd</sup> instar larvae for both in the treatment and control at 24-hours after treatment [18]. The response and feeding behavior of each individual larva were varied and caused non-homogeneous data. Data analysis was performed using the Mann-Whitney U statistical test, it is a non-parametric for abnormal data distribution, which is comparing two non-homogeneous data (from the same population) with the average controls ( $P < 0.05$ ).

Image J software from digital camera photo images was used to measure the mean leaf area consumed by the larvae. The antifeedant coefficient was calculated from the number of the percentage of relative and absolute antifeedant activities [19]. Relative activity is calculated by measuring the leaf area of the choice test results based on the formula (1)

$$\% R = (C-T)/(C+T) \times 100 \text{ (choice test)} \quad (1)$$

[R = Relative antifeedant activity (%), C = Area of leaf consumed in the control ( $\text{mm}^2$ ), T = Area of leaf consumed in the choice test treatment ( $\text{mm}^2$ )].

Absolute antifeedant activity was calculated by measuring the leaf area consumed by the test larvae with no choice, based on the formula (2)

$$\% A = (CC-TT) / (CC+TT) \times 100 \text{ (no-choice test)} \quad (2)$$

[A = Absolute antifeedant activity (%), CC = Area of leaf consumed in the control ( $\text{mm}^2$ ), TT = Area of leaf consumed in the no choice test treatment ( $\text{mm}^2$ )].

Feeding deterrent activity was measured from the total value of the coefficient based on the formula (3). The total antifeedant coefficient value will determine the category of antifeedant activity according to [19] shown in table.1.

$$T_o = A + R \quad (3)$$

[To = Total coefficient of antifeedant activity (%), A = Absolute antifeedant activity (%), R = Relative antifeedant activity (%)].

**Table.1.** Antifeedant Category (Gabrys et al., 2006)

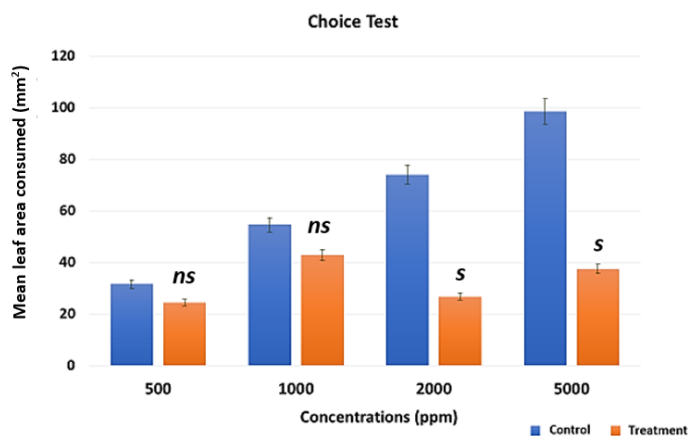
T <sub>o</sub> Value	Antifeedant Category
Negative T value	Attractant
0 < 50	Low antifeedant activity
51 – 100	Medium antifeedant activity
101 – 150	Strong antifeedant activity
151 – 200	Very strong antifeedant activity

### 3. Results and Discussion

#### 3.1. Mortality and Feeding behavior of *S. litura* and *C. pavonana* Larvae

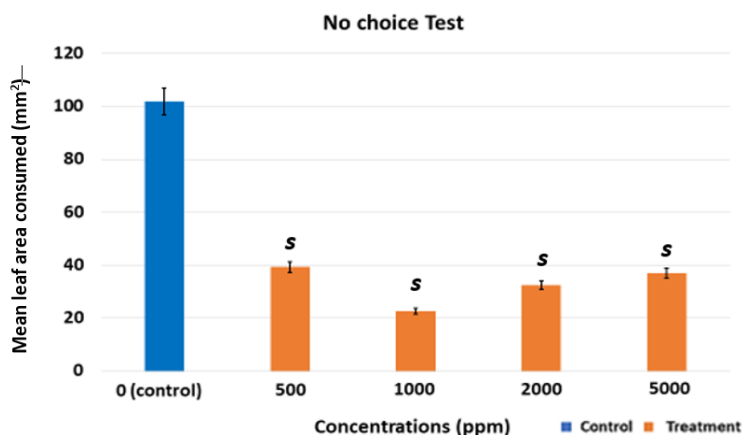
##### Antifeedant Test on *C. pavonana* Larvae

**Figure 3.1.** showed the results of the choice test of ethanolic leaf extract of *L. camara* on *C. pavonana*. Antifeedant activities were shown by the significantly different leaf area consumed by the third instar of *C. pavonana* larvae in the treatments compared to control, at minimum concentration of 2000 ppm. Then the result of no choice test showed the minimum concentration led to significant antifeedant effect was 1000 ppm (**Figure 3.2**).



**Figure 3.1.** The choice antifeedant test result on *C. pavonana* larvae

(s = significantly different between treatment and control at p < 0.05 ;  
 ns = not significantly different between treatment and control)



**Figure 3.2.** The no choice antifeedant test result on *C.pavonana* larvae

(s = significantly different between treatment and control at  $p < 0.05$  ;  
 ns = not significantly different between treatment and control )

Both antifeedant test results (choice and no choice) showed the higher concentration of the extract given, the less the leaf area consumed. The effective minimum concentration of no choice test was 1000 ppm, which was lower than the choice test (2000 ppm), that means smaller antifeedant concentrations have been effective to deterrent feeding activity of *C. pavonana* larvae.

Larval feed preferences are influenced by their choice to consume less leaf containing antifeedant compounds, in contrast the larvae in no choice test were only given one choice of fed, so that the results of their consumption were not affected by alternative feed. Based on Juraz, 2014' s research it was found that larval consumption preferences influence the tendency of feeding activities of *S. litura* larvae. Tasting a small amount of antifeedant can deterrent the larval feeding activity and make it look for another preferred fed [20].

Ethanollic leaf extract of *L. camara* at each concentration had different categories of antifeedant activity. The concentration of the extract at 5000 ppm had strong antifeedant activity category. The category of medium antifeedant activities were obtained at the concentration's of 1000 ppm and 2000 ppm, while the low antifeedant activity was at 500 ppm (Table 3.1)

**Table 3.1.** Antifeedant category of ethanollic leaf extract *L. camara* on *C. pavonana* larvae

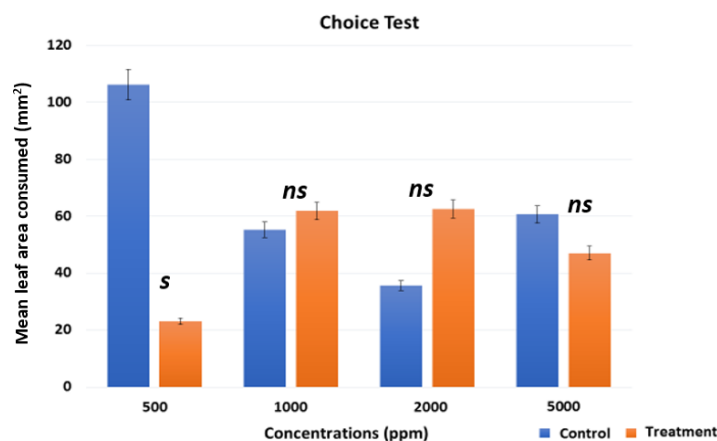
Concentration (ppm)	Deterrent coefficients		T	Antifeedant Category
	R (%)	A (%)		
500	12	9	21	Low antifeedant activity
1000	11	66	77	Medium antifeedant activity
2000	46	50	96	Medium antifeedant activity
<b>5000</b>	<b>44</b>	<b>60</b>	<b>104</b>	<b>Strong antifeedant activity</b>

Ethanollic leaf extract of *L. camara* had stronger antifeedant activity against *C. pavonana* larvae in proportion to the higher concentration, at concentrations of 1000ppm and 2000ppm in the category of

medium antifeedant activity and at concentration of 5000ppm was in the category of strong antifeedant activity. The antifeedant effect is more or less concentration dependent, this was stated in the study antifeedant activities of the chloroform extract of *L. camara* leaf against the eastern subterranean termite (*Reticulitermes flavipes*) [10]. Exposure to the treated higher concentration of *L. camara* leaf extract also resulted in greater antifeedant activity against termite. Insecticidal properties include feeding deterrent activity (antifeedant) can also cause growth disruption (weight less), inhibition develops and fecundity [6] [9]. Disruption of larval feeding activities generally correlates with nutrient deficiencies and thus affects insect metabolism, causing an imbalance of hormones that regulate to the next instar stage to pupation and imago.

#### Antifeedant test on *S. litura* larvae

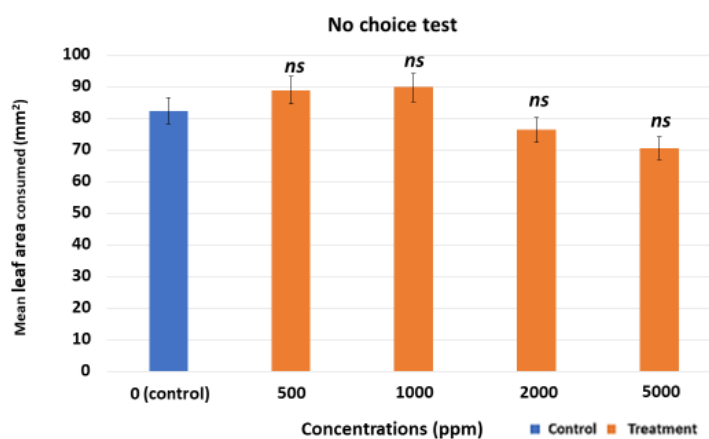
The ethanolic leaf extract of *L. camara* showed different response to the *S. litura* and *C. pavonana* larvae. The tested *L. camara* extract tended to show antifeedant and phagostimulant activity on *S. litura* with choice and no choice test. The choice antifeedant test result shows that the ethanolic leaf extract *L. camara* was considered as antifeedant only at 500ppm concentration. While the results of the choice and no choice test the concentration of 2000 ppm were considered as phagostimulant (Figure 3.3. & Figure 3.4. ). A wide variety of plant extract constituents possess varying degrees of bioactivity to insects [6]. The antifeedant and phagostimulant activities of compounds extracted from *Hymenoxys robusta* against *Spodoptera exigua*, were reported by Juarez *et al* [20]. The crude methanolic leaf extract and fractionated chloroform extract of *H. robusta* has been shown to have antifeedant activity against *Spodoptera exigua*. Neither antifeedant nor deterrent activities, but a phagostimulating activity was observed when the larvae were fed on the n-hexane extract. Another study of flowerheads extract of *Anacyclus cyrtolepidioides*, fraction of partition chromatography crude extract can modify the specificity of antifeedant activity and allow the localization of antifeedant and phagostimulant activity towards *Tribolium confusum* [21]. Thus, it is necessary to investigate further the specific antifeedant and phagostimulant bioactivities contained in the ethanolic leaf crude extract *L. camara* through partitioning into fractionation using polar and semipolar solvents.



**Figure 3.3.** The choice antifeedant test result on *S. litura* larvae

(s = significantly different between treatment and control at  $p < 0.05$  ;  
 ns = not significantly different between treatment and control)





**Figure 3.4.** The no choice antifeedant test result on *S. litura* larvae

(*s* = significantly different between treatment and control at  $p < 0.05$  ;  
*ns* = not significantly different between treatment and control)

**Table 3.2.** Antifeedant category of ethanolic leaf extract *L. camara* on *S. litura* larvae

Concentration (ppm)	Deterrent coefficients		T	Antifeedant Category
	R (%)	A (%)		
500	64.40%	5.63%	70.03%	<b>Medium antifeedant activity</b>
1000	-5.64%	-3.46%	-9.11%	attractant (phagostimulan)
2000	-27.35%	-41.88%	-69.22%	attractant (phagostimulan)
5000	12.64%	-27.50%	-14.86%	attractant (phagostimulan)

Based on coefficients antifeedant analysis, the ethanolic leaf extract *L. camara* at 500 ppm against *S. litura* larvae had medium antifeedant activity category. The negatively total coefficient shows the attractant (phagostimulant) category [19]. The category of attractant (phagostimulant) activities were obtained by ethanolic leaf extract *L. camara* against *S. litura* larvae at the concentrations of 1000ppm, 2000ppm, and 5000ppm (**Table 3.2**).

### 3.2. Phytoconstituents of ethanolic leaf extract of *L. camara*

Antifeedant bioactivity is affected by the active compounds contained in the leaf extract of *L. camara*. The phytochemical analysis results shows that the ethanolic leaf extract of *L. camara* contained alkaloids, saponins, triterpenoids, and steroids **Table 3.3**.



**Table 3.3.** Phytoconstituents detected in ethanolic leaf extract of *L.camara*

Compound	Present (+) or Absent (-)
Alkaloids	+
Flavonoids	-
Phenolic compounds	-
Saponins	+
Tannins	-
Triterpenoids	+
Steroids	+

Plants produce a diverse of secondary metabolites that is important in their defense against herbivores [22]. Terpenoids, steroids and saponins were the compounds that have been known to be antifeedant activities against a number of insect pests such as *Putela xylostella* and *S.litura* [12][23]. Triterpenoids often occur as glycosides (conjugated with sugars) also had antifeedant effects on Lepidoptera [15].

Referring to the results of the study,ethanolic leaf extract of *L. camara* has stronger antifeedant activity against *C. pavonana* 3<sup>rd</sup> instar larvae especially at concentration of 5000ppm. Mode of action antifeedants is directed at the taste cells of *C. pavonana* larvae. A typical gustatory sensillum in an insect contains receptors selective for deterrents and others for stimulants (such as sugars and amino acids). Antifeedants stimulate a deterrent receptor, that in turn sends a signal “do not feed” to the feeding center in the larva’s central nervous system, some antifeedants block the perception of feeding stimulants, preventing the *C. pavonana* larvae from acquiring appropriate taste information that interfere with feeding behavior [15].

Antifeedant have benefits as much as pesticides for plant protection, selectivity towards the target pest (and thus non-toxic to mammals and other non-target organisms such as natural enemies and pollinators). However, the constraints on the application of plant-based pesticides affect the effectiveness and cost efficiency. Antifeedant compounds do not directly kill the target insect pests but suppress pest populations through feeding deterrent bioactivity which weakens pest insects, inhibits growth and development, slowing down the resistance. The weak pests are easily attacked by natural enemy. This concept is in line with the natural control mechanism that maintains the balance of the ecosystem food chain.

Future research is planned to develop *L. camara* leaf extract with the prospect of being feasible as a botanical insecticide. Bioinsecticides are used in large quantities to maintain their effectiveness from environmental degradation. Active compounds in smaller size are known to have higher bioactivity so with less use it has been effectively applied. *L.camara* leaf extract through nano-based formula is expected to increase effectiveness and efficiency of applications, especially into *C. pavonana* larvae which have been proven to be potentially antifeedant activity. Prospects for development application of *L. camara* extract against *S. litura* larvae as attractant are different from the potential antifeedant compounds to control *C. pavonana*, where attractant can be developed as bait compounds to trap pest insects. Nano encapsulation techniques can be formulated with slow release compounds so that attractants can be more durable. However, this concept is also an effort to control environmentally friendly pests.

#### 4. Conclusion

The ethanolic leaves extract *L. camara* at 5000 ppm concentration was considered as strong antifeedant category to *C. pavonana* larvae. Minimum concentration of the ethanolic leaf extract of *L. camara* that was effective as antifeedant on *C. pavonana* larvae was 2000 ppm (choice test) and 1000 ppm (no choice test),



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