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# Biochemical screening and larvicidal and ovicidal activity *Citrus limon* (L.) Burm. F. leaves against *Helicoverpa armigera* (Hub.)

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**Abstract**
To estimate the larvicidal and ovicidal activity of ethanol, methanol and aqueous extracts of important medicinal plants of *Citrus limon* (L.) Burm. f. leaves at various concentrations against most devasting agricultural field pest *Helicoverpa armigera*. Highest ovicidal and larvicidal activity of *C. limon* methanol extract was 64% and 61% against *H. armigera* at 0.5% concentration. The lowest ovicidal and larvicidal activity was recorded in aqueous extracts 11% and 15% respectively. The study reports that *C. limon* is a potential source of natural larvicidal and ovicidal properties against the lepidopteran pest *H. armigera*.

**Keywords:** Medicinal plants, *Helicoverpa armigera*, antifeedant activity, larvicidal activity

## 1. Introduction
The growing awareness of the hazards of excessive use of pesticides globally has led researchers to search for safer and more environment friendly alternative methods for insect pest control. Therefore, extensive studies are carried out to screen plants as insect growth control agents. Over the last two to three decades, greater attention has been focused on the bioactivity of phytochemicals for their potential as pesticides against phytophagous insects [1].
Lemons are the world's third most popular citrus species after orange and mandarin, which are mostly produced in Argentina and are scientifically known as *Citrus limon* [2, 3]. *Citrus limon* is a common name used for various species of lemon e.g. Lisbon lemon, Eureka lemon. *Citrus limon* is a rich source of many bioactive compounds such as flavonoids, ascorbic acid, citric acid and minerals in its various morphological parts namely: fruit, fruit peel and leaf [4].
*Citrus limon* (L.) Burm. f. is a tree with evergreen leaves and yellow edible fruits from the family *Rutaceae*. The *C. limon* fruit is source of essential oil and juice. The *C. limon* fruit stands out as having well-known nutritional properties, but it is worth remarking that its valuable biological activities are underestimated in modern phytotherapy and cosmetology [5].
*Helicoverpa armigera* is one of the serious pests of chickpea, which feeds more than 150 crops throughout the world [6]. Gram pod borer is widely distributed and a serious pest of chickpea causing heavy crop losses (20-60%) throughout the India [7]. *H. armigera* is the major and most devastating pest of chickpea which can cause crop loss up to 80 per cent under congenial weather conditions. In terms of monetary value, the estimated annual loss due to this pest in chickpea is Rs.2030 million in India [8]. It is estimated that *H. armigera* alone is responsible for losses over Rs. 3500 million annually in India, despite heavy application of pesticides inputs [9]. *H. armigera* alone accounts for the consumption of half of the total pesticides used in India for the protection of different crops [10].
The purpose of the present study was to determine larvicidal effects of crude leaves extracts of *C. limona* against eggs and fourth instar larvae of *H. armigera* and biochemical study of leaves.

## 2. Materials and Methods
### 2.1. Plant collection and extract preparation
Fresh leaves of *C. limon* were collected in Nipani and were washed in running water to remove the dust and dirt. Leaves were shade dried at room temperature for a week and then kept in the oven at 50 ºC for a while to remove the complete moisture. Dried leaves were pulverized by using the domestic grinder and sieved with 0.5-1 mm mesh size.

Each powder was separately extracted with ethanol, methanol and water by Soxhlet extraction method until clear solvent was appears in extractor. Filtered extracts were concentrated by using rotary flash evaporator under reduced pressure. The concentrated extracts residues appear in dark-green and they were kept in a refrigerator at 4 ºC for bioassay

### 2.2. Rearing of *Helicoverpa armigera*
The selected insect pests were collected from infested chickpea field at Nippani , Karnataka, India. Larvae were reared in laboratory condition at 28 ± 2 °C, 70–85% relative humidity (RH). Laboratory-reared second generation larvae were used for subsequent bioassay.

### 2.3. Ovicidal activity
25 Individuals eggs of *H. armigera* were separated and immersed in 0.1, 0.2, 0.3, 0.4 and 0.5% concentrations of leaf extract in ethanol, methanol and water. Five replicates were maintained .Number of eggs hatched in the control and the treatments were recorded. Percentage of ovicidal activity was calculated.

### 2.4. Larvicidal activity
During preliminary screening with the laboratory trial, the larvae of *H. armigera* were collected from the insect rearing. Different concentrations 0.1, 0.2, 0.3, 0.4 and 0.5% crude extracts in ethanol, methanol and water were prepared. A leaf dipping method [11] was used to evaluate the activity of the test samples. Leaf disks (6.5 cm) of castor were used for evaluating larvicidal activity of the samples against *H. armigera*. Five leaf disks per dose were separately dipped in each test solution for 30 s. Solvents were evaporated under a fume hood for 2 hours. Castor leaves were washed with 70% double-distilled alcohol and air-dried for 15 min before dipping into the required amount of plant product. The larvae were transferred individually on treated and control (disks treated with solvent) leaf disks placed in Petri plates. Treated leaves were fed to fourth-instar larvae of *H. armigera*. A total of 50 larvae were exposed in five replicates of ten larvae each. Experiments were maintained at 26±1$^0$C, 65±2% relative humidity and a photoperiod of 16:8 (L/D). Mortality was determined 24 h after larvae were placed on disks. Larvicidal activity was assessed by the procedure of WHO (1996) with some modification and following the method [12]. The numbers of dead larvae were counted after 24 hours of exposure and the percentage mortality was reported from the average of five replicates.

### 2.5. Qualitative test for phytochemicals analysis
All solvent extracts of *C. limona* were subjected to various chemical tests to identify phytoconstituents using standard methods [13, 14, 15]. For qualitative tests of all solvent extract of *C. limon* diluted to obtained mg/ml concentration then use for the phytochemical tests.

#### 2.5.1. Test for Phenolics [16]
An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent and 4 ml of sodium carbonate. The tubes were vortexed for 15 seconds and allowed to stand for 30 minutes at 40$^0$C for color development. An appearance of blue color indicates the presence of phenols

#### 2.5.2. Test for flavonoids
0.5 ml of sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture. Water was added immediately to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 min. An appearance of pink color indicates the presence of flavonoids

#### 2.5.3. Test for Tannins
The tannin tests was carried out by adding 0.5 ml of plant extract and 0.5 ml of respective solvent then add few drops of 5% $FeCl_3$. Blackish color shows presence of tannin compounds.

#### 2.5.4. Test for Terpenoids
The presence of terpenoids conformed by mixing 0.5 ml of plant extract and 0.5 ml of solvent. Further add 1 ml chloroform and then add 1 ml $H_2SO_4$. Reddish brown color confirmed the presence of terpenoids.

#### 2.5.5. Test for Alkaloids [17]
Test for alkaloids 0.5 gm of aqueous extract was stirred with 4 ml of 1% dilute hydrochloric acid. It was boiled and filtered. Mayer's test 1 ml of the filtrate was treated with few drops of Mayer's reagent. Turbidity or precipitation indicated the presence of alkaloids. Dragendorff's test 1 ml of the filtrate was treated with few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloids.

#### 2.5.6. Test for Saponins
0.5 gm of ethanolic extract was boiled and the mixture was filtered. To 2.5 ml of the filterate, 10 ml of distilled water was added in a test tube. It was shaken well for few minutes and was allowed to stand for some time. Frothing along with the formation of honey comb indicated the presence of saponins.

## 3. Results and Discussion
### 3.1. Ovicidal activity
The ovicidal activity of different crude leaf extracts of *C. limon* tested against eggs of *H. armigera*. From observation Table 1 the results shows that methanol, ethanol and aqueous extract at 0.5% concentration exhibit highest percentage of ovicidal activity i.e 64±2.1, 32±0.4 and 19±0.9 respectively. A minimum activity was recorded in 0.1% concentration of aqueous extract (11±0.1). As the concentration methanol, acetone and water extract increases, percentage of ovicidal activity was also increased.

### 3.2. Larvicidal activity
The larvicidal activity of different crude leaf extracts of *C. limon* was tested against fourth instar larvae of *H.armigera*. The perusal of the data clearly revealed that methanol extract at 0.5% concentration showed potential larvicidal effect (61±0.4%) (Table.2) followed by ethanol extract (48±1.0%) and water (23±1.4%) respectively. Whereas, very poor larvicidal effect (15±1.0%) was noted with water extract at 0.1% concentration. The positive control azadirachtin showed 43±1.0 percent mortality which was comparable to ethanolic extract of *C. limona*. Among all tests, methanolic extract showed significant mortality rate as compared to other. As seen in larvicidal activity, as extract concentration increased the larvicidal activity was also increased. The correlative results were observed in Chloroform and methanol leaves and

seeds crude extracts of *A. mexicana* in medically important vectors *Cx. pipiens* and *Ae. aegypti* [18, 19].

### 3.3. Phytochemical analysis

The results of the phytochemical screening, as shown in Table 3. revealed positive response for alkaloids, phenols and flavonoids in all extracts. Test for tannin and terpenoids shows positive in acetone and methanol and negative in aqueous extract. Test for saponins shows negative in all extracts. There are numbers of naturally occurring compounds that possess plant protection properties. So far more than 10,000 secondary metabolites have been chemically identified. In nature many plants have unpalatable substances like high content of phenols, alkaloids, flavanoids, terpenes, quinone, coumarin etc., which play a significant self-protective role against herbivorous insects. These substances possess wide range of biological activities including antifeedant, insecticidal, and insect growth regulators [20].

**Table 1:** Percent ovicidal activity of *C. limon* leaf extract against *H. armigera*. (Values were the means of five replicates ± = Standard deviation)

| Crude extract | Concentration mg/ml | | | | |
|---|---|---|---|---|---|
| | 0.1% | 0.2% | 0.3% | 0.4% | 0.5% |
| Ethanol | 21±0.4 | 21±0.4 | 26±0.2 | 26±0.0 | 32±0.4 |
| Methanol | 30±1.0 | 36±1.0 | 52±0.1 | 52±0.1 | 64±2.1 |
| Aqueous | 11±0.1 | 11±0.1 | 18±0.4 | 18±0.4 | 19±0.9 |
| Azadirachtin (0.1%) | 43±1.0 | | | | |

**Table 2:** Percent larvicidal activity of different concentrations of *C .lemon* leaf extract against *H. armigera*.

| Crude extract | Concentration mg/ml | | | | |
|---|---|---|---|---|---|
| | 0.1% | 0.2% | 0.3% | 0.4% | 0.5% |
| Ethanol | 22±0.5 | 33±0.7 | 39±0.8 | 42±0.4 | 48±1.0 |
| Methanol | 35±0.5 | 40±0.5 | 46±0.2 | 55±0.4 | 61±0.4 |
| Aqueous | 15±1.0 | 16±1.0 | 19±0.0 | 22±0.4 | 23±1.4 |
| Azadirachtin (0.1%) | 43±1.0 | | | | |

(values were the means of five replicates ± = Standard deviation)

**Table 3:** Qualitative phytochemical test for *C. lemon leaf* extract in different solvents

| Phytochemical constituents | Acetone | Methanol | Water |
|---|---|---|---|
| Phenolics | + | + | + |
| Flavonoids | + | + | - |
| Tannins | + | + | - |
| Terpenoids | + | + | - |
| Alkaloids | + | + | + |
| Saponins | - | - | - |

(+ = Present - = Absent)

## 4. Conclusion

The methanol extract of *C. limon* at 0.5% concentration confirmed highest ovicidal as well as larvicidal activity against *H.armigera* followed by ethanol, and water. The phytochemical analysis revealed the presence secondary metabolites viz. alkaloids, flavonoids, terpenoids, phenolics in *C.limon*. Hence it is inferred that *C.limon* can be used further for details phytochemical investigation to develop a new botanical formulation for the management of *H. armigera.*

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## 6. References

1. Duraipandiyan V, Ignacimuthu S, Gabriel Paulraj M. Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. Saudi Journal of Biological Sciences 2011;18:129-133
2. Gonzalez-Molina E, Dominguez-Perles R, Moreno DA, Garcia-Viguera C. Natural bioactive compounds of *Citrus limon* for food and health. Journal of Pharmaceutical and Biomedical Analysis. 2010;51(2):327-345.
3. Kawaii S, Yasuhiko T, Eriko K, Kazunori O, Masamichi Y, Meisaku K *et al*. Quantitative study of flavonoids in leaves of Citrus plants. Journal of Agricultural and Food Chemistry 2000;48:3865-3871.
4. Penniston KL, Nakada SY, Holmes RP, Assimos DG. Quantitative assessment of Citric acid in lemon juice, Lime juice and commercially-available fruit juice products. Journal of Endourology 2008;22(3):567-570.
5. Goetz P. *Citrus limon* (L.) Burm. f. (Rutacées). Citronnier. Phytotherapie 2014;12:116-121.
6. Vinutha JS, Bhagat D, Bakthavatsalam N. Nanotechnology in the management of polyphagous pest *Helicoverpa armigera.* J. Acad. Indus. Res 2013;1(10):606-608.
7. Anonymous. Directorate of Economics and Statistics, Department of Agriculture and Cooperation. Govt. of India 2013, 85-87.
8. Anonymous. Project Coordinator's Report Annual Group Meet August, 24-26th. All India Coordinated Research Project on Chickpea. IIPR, Kanpur 2013a, 20.
9. Kumar H, Kapur A. Transgenic Bt crops as a component of Integrated Pest Management. In: Biotechnological Strategies in Agro-Processing, [(Eds.) Marwaha, S.S. and Arora, J.K.] Asiatech Publishers Inc., New Delhi 2003, 85-104.
10. Suryavanshi DS, Bhede BV, Bosale SV, More DG. Insecticide resistance in field population of *H. armigera* (Hub.) (Lepidoptera: Noctuidae). Indian Journal of Entomology 2008;70(1):44-46.
11. Park BS, Lee SE, Choi WS, Jeong CY, Song C, Cho KY. Insecticidal and acaricidal activity of piperonaline and piperoctadecalidine derived from dried fruits of *Piper longum L.* Crop Protect 2002;21:249-251.
12. Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia 2000;71:553-5.
13. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd 1973, 49-188.
14. Sofowora A. Medicinal Plants and Traditional Medicine in West Africa, John Wiley and Sons, New York, NY, USA 1982.
15. Trease GE, Evans WC. Pharmacognosy, Brailliar Tiridal Can Macmillian Publishers, 11th edition 1989.
16. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels J. Agr. Food Chem 2003;51:609-614.
17. Sazada S, Verma A, Rather AA, Jabeen F, Meghvansi MK. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Adv. In Biol. Res 2009;3:188- 195.
18. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging

effects on superoxide radicals. Food Chem 1999;64:555-559
19. Zeinab S, Abou E. Strong larvicidal properties of *Argemone mexicana L.* against medically important vectors *Culex pipiens* and *Aedes aegypti*. Int. J. Mosq. Res. 2015;2:09-12.
20. Jeyasankar A, Chinnamani T, Chennaiyan V, Ramar G. Antifeedant activity of *Barleria buxifolia* (Linn.) (acanthaceae) against *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hübner (Lepidotera: Noctuidae). International Journal of Natural Sciences Research 2014;2(5):78-84.