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Larvicidal potential of methanolic leaf extracts of *Citrus limon* and *Aloe vera* against Brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee

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Abstract
The brinjal shoot and fruit borer, scientifically known as *Leucinodes orbonalis* Guenee, is a major insect pest that causes significant damage to eggplant crops in the Indian subcontinent. The use of synthetic pesticides to control this pest has raised concerns due to their negative impact on the environment and human health. Consequently, there is a growing interest in adopting environmentally friendly pest management strategies, including the utilization of plant extracts. This research aimed to evaluate the effectiveness of crude methanolic leaf extracts obtained from *Citrus limon* and *Aloe vera* against the 4th instar larvae of *L. orbonalis*. Phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in both extracts. The larvicidal bioassay demonstrated that both *C. limon* and *A. vera* extracts exhibited concentration-dependent larvicidal activity against *L. orbonalis*. Notably, *C. limon* extracts exhibited the highest mortality rate of 82.61% at a concentration of 400 ppm, while *A. vera* extracts showed a mortality rate of 78.26% at the same concentration. Probit analysis was conducted to estimate the LC50 and LC90 values for *C. limon* extracts (227.71 ppm and 423.50 ppm, respectively) and *A. vera* extracts (246.61 ppm and 451.00 ppm, respectively). These findings contribute to the exploration of environmentally friendly alternatives for managing the brinjal shoot and fruit borer.

Keywords: Eggplant, brinjal shoot, and fruit borer, *Leucinodes orbonalis*, synthetic pesticides, plant extracts, *Citrus limon*, *Aloe vera*, larvicidal activity, phytochemical analysis, environmentally friendly pest management

Introduction

Eggplant, also known as brinjal (*Solanum melongena* L.), is a vegetable native to India and holds significant popularity and economic importance in Asia (Harish *et al.*, 2011 [12]; Pareet, 2006 [19]; Alam *et al.*, 2003 [2]). Unfortunately, eggplant crops in the Indian subcontinent are commonly plagued by the brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee), which is a primary insect pest (Chakraborti and Sarkar, 2011) [6]. The larvae of *L. orbonalis* have a detrimental impact on plant growth, as they reduce the number and size of fruits (Atwal and Dhaliwal, 2007) [4]. Furthermore, the tunnels created by these larvae render the fruit unsuitable for marketing, leading to substantial economic losses (Alam *et al*., 2003) [2]. In fact, in various states of India, eggplant crops have suffered damage ranging from 37% to 63% (Dhankar, 1988) [9].

To combat agricultural pests like the brinjal shoot and fruit borer, insecticides play a crucial role, with significant implications for both agriculture and public health (Diego Valbuena *et al.*, 2021) [8]. Chlorpyriphos 50% EC and Cypermethrin 5% EC are among the commonly used synthetic pesticides to manage the brinjal shoot and fruit borer (Sharma *et al*., 2012). However, the indiscriminate and improper use of insecticides can lead to several issues, such as a loss of effectiveness (Alam *et al*., 2003) [2], the development of insect resistance, pollution, and health hazards (FAO, 2003) [11]. Additionally, the presence of residual pesticides in brinjal poses a threat to vegetable exports in foreign markets (Islam *et al*., 1999) [14]. Therefore, it is essential to adopt environmentally friendly management approaches. Non-chemical methods like biopesticides, botanicals, clean cultivation, and mechanical control (e.g., handpicking and removal of infected plant parts) are commonly employed to manage insect pests (Hassan, 1994) [13].

In recent years, there has been a growing concern about the harmful effects of synthetic pesticides on the environment and human health, leading to an increased interest in the use of plant extracts for pest control in agriculture (Isman, 2000) [15]. Plant extracts contain natural compounds that possess the ability to repel or eliminate pests, making them a promising alternative to conventional pest control methods.

Several plant extracts have shown effectiveness against *L. orbonalis.* Extracts from neem leaves and seeds (*Azadirachta indica*) have been found to reduce the number of eggs laid and increase larval and pupal mortality, leading to reduced damage in eggplant crops (Kumar and Thakur, 2017) [16]. Similarly, marigold flowers (*Tagetes erecta*) and papaya leaves (*Carica papaya*) extracts have also demonstrated potential in controlling the brinjal shoot and fruit borer (Calumpang & Ohsawa, 2015 [5]; Sangma *et al*., 2019) [20]. In a recent study, Madhavi *et al*. (2023) [18] investigated the efficacy of *Murraya paniculata* leaf extracts in controlling the larvae of *L. orbonalis.* Their findings contribute to the growing body of research on environmentally friendly pest management strategies for eggplant crops.

The present study aims to assess the larvicidal efficacy of crude methanolic leaf extracts of *Citrus limon* and *Aloe vera* against the 4th instar larvae of *L. orbonalis.*

Materials & Methods

Plant Material Collection

Fresh leaves of *Citrus limon* and *Aloe vera* were collected from Sangareddy town, Telangana State, India. They underwent thorough washing and were subsequently dried for a period of 15 days. After drying, the leaves were powdered using a grinder and stored in a sealed bag until they were used.

Extracts Preparation

To prepare the extracts, 100 grams of the prepared powders were soaked in 250 mL of Methanol separately with frequent shakings for four days. On the fifth day, the solutions were filtered using Whatman filter paper no.1 and allowed to evaporate under a rotating fan for one day. After one day a semi-solid extract was obtained and preserved in the refrigerator at 4^0C until usage. Prior to the larvicidal bioassays, a stock solution of 1000 ppm was prepared by mixing 1 gram of the extract with 10 mL of methanol and later with 990 mL of distilled water. From this stock solution, various test solutions with concentrations of 100, 200, 300, and 400 ppm were prepared through dilution method. Control stock solution was made with the same solvents in the same ratios excluding the extracts.

Phytochemical Analysis

Secondary metabolites in the prepared extracts were identified by conducting several tests. To identify alkaloids, the Mayor's Test was employed. This test involved the addition of the Mayor's reagent and the subsequent observation of a cream-colored precipitate. The Alkaline Reagent Test was utilized to detect flavonoids, which exhibited a yellow color upon the addition of NaOH. The presence of terpenoids was determined through the Salkowski Test, where the addition of concentrated H2SO4 led to the development of a red or orange color. For the identification of saponins, the Froth Test was employed, wherein the formation of foam was observed upon vigorous shaking. The Keller-Killiani Test was conducted to detect glycosides, and it involved the addition of HCl and FeCl3 resulting in a red or violet color. Lastly, the NaOH Test was performed to identify polyphenols, where the addition of NaOH resulted in yellow color.

Insect Culture

Larvae of *L. orbonalis* were obtained from infested eggplant fruits grown in an organic garden located in Zaheerabad, Sangareddy District, Telangana State. These larvae were reared on organic fruits. Fourth instar larvae from the reared population were selected for the larvicidal bioassay.

Larvicidal Activity

The larvicidal activity of the crude methanolic extracts of *C. limon* and *A. vera* against the 4th instar larvae of *L. orbanolis* was evaluated using the immersion method. Five larvae of the same size were selected for each test batch and immersed in the respective extract solutions for a duration of 5 seconds. The treated larvae were then placed in Petri dishes with a diameter of 12 cm, along with sliced eggplant fruit for larval feeding. The larvae were observed until all of them died, or the control larvae molted into the next instar. The entire experiment was repeated five times, and the number of dead larvae was recorded. Larvae exhibiting abnormal symptoms such as body contraction, feeding cessation, or paralysis were considered dead. The percent mortality was calculated, and the mortality data were adjusted using the formula proposed by Abbott (1925) [1].

Statistical Analysis

The obtained results were subjected to probit analysis using MS Excel software. Regression and probit analysis were carried out to calculate LC50 and LC90 values. A significance level of ≤ 0.05 was set for the analysis.

Results

The phytochemical analysis results of the two extracts (Table 1), revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in high quantities in both extracts. Saponins were present in slight quantities in the two tested extracts. Glycosides were identified in *C. limon* extracts whereas they were absent in *A. vera* extracts.

The larvicidal bioassay results of the present study (Tables 2 and 3; Figures 1) indicated that both the plant extracts exhibited larvicidal activity against *L. orbanolis* in a concentration-dependent manner. Comparably, crude methanolic leaf extracts of *C. limon* were slightly more efficient than *A. vera* extracts. *C. limon* extracts exhibited the highest larval mortality percentage of 82.61 ± 0.34 at 400 ppm concentration. At 300 and 200 ppm concentrations the observed mortality percentages were 65.22 ± 0.46 and 47.83 ± 0.51, respectively. The lowest mortality was 26.09 ± 0.48 at 100 ppm concentration. Probit analysis results following the regression analysis (Figure 2) indicated that LC50 and LC 90 values are 227.71 and 423.50 ppm, respectively.

A. vera methanolic leaf extracts also showed the same trend in the present study. The highest mortality observed was 78.26 ± 0.41 at 400 ppm. 60.87 ± 0.49 and 43.48 ± 0.51 percentages of mortalities were observed at 300 and 200 ppm concentrations, respectively. The lowest mortality obtained was 21.74 ± 0.46 at 100 ppm concentration. Probit analysis results following the regression analysis (Figure 3) indicated that LC50 and LC 90 values are 246.61 and 451.00 ppm, respectively.

Table 1: Identified secondary metabolites in the selected plants' methanolic leaf extracts. Absent: -; Slightly Present: +; Moderately present: ++; Heavily present: +++.

extracts	alkaloids	flavonoids	saponins	terpenoids	polyphenols	glycosides
C.limon	+++	++	+	++	++	+
A.vera	++	++	+	+	++	-

Table 2: *C. limon* Methanolic leaf extracts larval mortality percentages ± Standard Deviations against the 4th Instar larvae of *L. Orbonalis.* LC50 – 50% Lethal Concentration; LC90 – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Conc. In ppm	C. limon	LC50 95% CL (LCL – UCL)	LC90 95% CL (LCL – UCL)
0	0 ± 0.28	227.71 (227.54 - 227.95)	423.50 (423.33 - 423.74)
100	26.09 ± 0.48		
200	47.83 ± 0.51		
300	65.22 ± 0.46		
400	82.61 ± 0.34		

Table 3: *A. vera* Methanolic leaf extracts larval mortality percentages ± Standard Deviations against the 4th Instar larvae of *L. orbonalis*, LC50 – 50% Lethal Concentration; LC90 – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Conc. In ppm	*A. vera*	LC50 95% CL (LCL – UCL)	LC90 95% CL (LCL – UCL)
0	0 ± 0.28	246.61 (246.43 - 246.82)	451.00 (450.83 - 451.22)
100	21.74 ± 0.46		
200	43.48 ± 0.51		
300	60.87 ± 0.49		
400	78.26 ± 0.41		

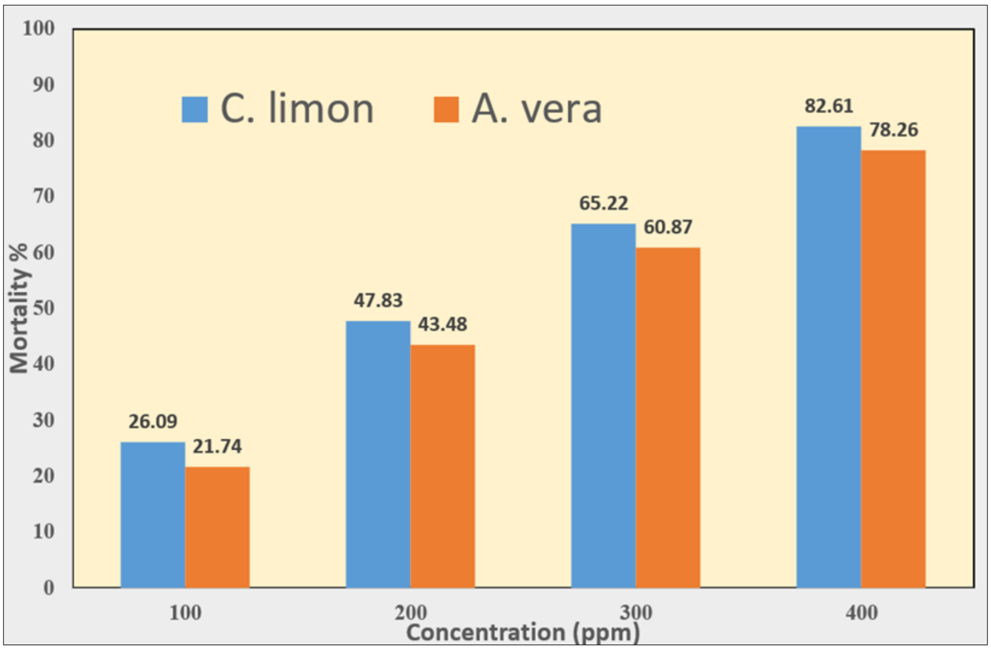

Fig 1: Larvicidal efficacy of crude methanolic leaf extracts of *C. limon* and *A. vera* against the 4th instar larvae of *L. orbonalis*

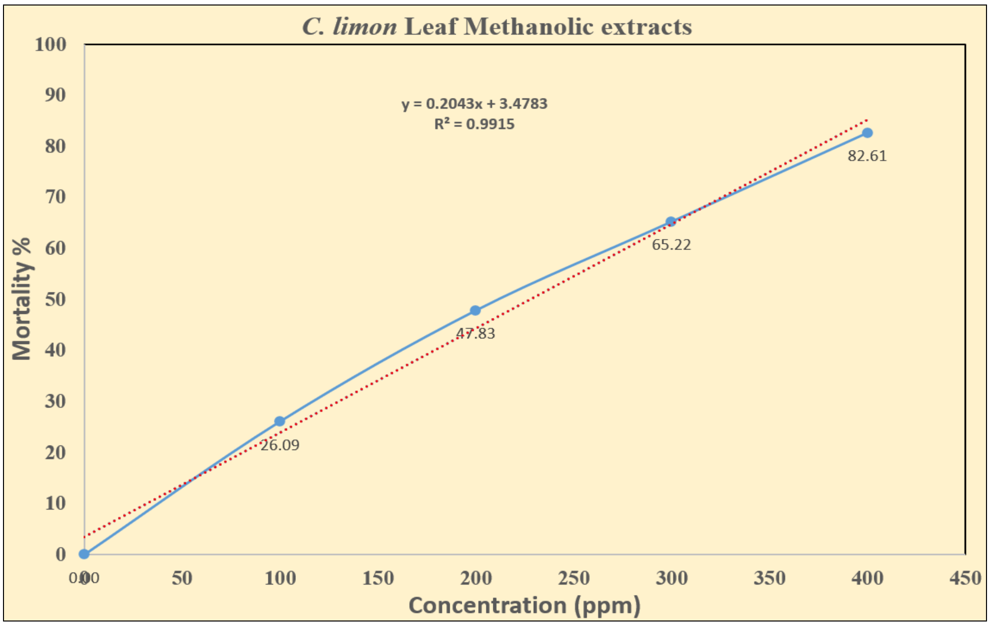

Fig 2: Regression analysis of the larvicidal bioassay of crude methanolic leaf extracts of *C. limon* against the 4th instar larvae of *L. orbonalis*

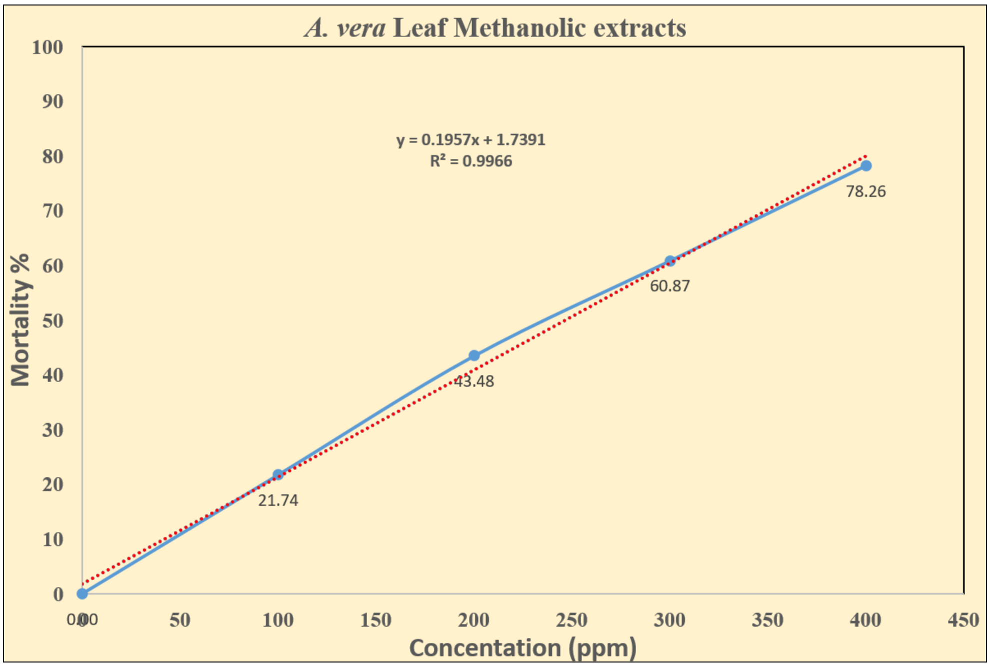

Fig 3: Regression analysis of the larvicidal bioassay of crude methanolic leaf extracts of *A. vera* against the 4th instar larvae of *L. orbonalis*

Discussion

Phytochemical analysis of both the test compounds in the present study confirmed the presence of bioactive compounds that have pesticidal potential. Similar results were reported in previous studies. Ehiobu, *et al*. (2021) [10], reported the presence of Alkaloids, Saponins, flavonoids, and Phenols in the extracts of *C. limon* leaves. Asker, *et al*. (2020) [3] reported various terpenoids such as Sabinene, 3-carene, Limonene, and β-ocimene by GC-MS analysis of *C. limon* leaf extracts. High phenolic content was identified from the methanolic leaf extracts of *A. vera* by Kumar, *et al*. (2017) [17]. Dharajiya, *et al*. (2017) [17], reported alkaloids, flavonoids, phenols, and saponins from the methanolic leaf extracts of *A. vera*.

This study highlights the effectiveness of *C. limon* and *A. vera* in controlling *L. orbanolis* larvae. The results of the present study are in line with the previous studies. Madhavi, *et al*. (2023) [18] tested the efficacy of *Murraya paniculata*, *Parthenium hysterophorus*, and *Acacia arabica* leaf extracts against the 4th instar larvae of *L. orbonalis*. At the highest tested concentration 400 ppm, *M. paniculata* exhibited 100 percent mortality whereas 86.96 and 78.26 % mortalities were shown by *P. hysterophorus* and *A. arabica*, respectively. The presence of secondary metabolites which are proven to possess pesticidal and various biological effects might be responsible for the mortality of the *L. orbonalis* larvae.

Overall, the study demonstrates the potential of the crude methanolic leaf extracts of *C. limon* and *A. vera* in controlling the larvae of *L. orbanolis*. To know which specific secondary metabolites and their working mechanisms in causing larvicidal effects, further studies are required.

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