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BIOEFFICACY OF SOME BOTANICAL EXTRACTS AGAINST BRINJAL FRUIT AND SHOOT BORER [*LEUCINODES ORBONALIS* (GUENEE); LEPIDOPTERA, PYRALLIDAE]

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ABSTRACT

Plant-based biopesticides such as extracts of neem and eucalyptus are gaining increasing attention as potential alternatives to synthetic pesticides due to their reduced toxicity to non-target species and the environment. The present study aimed to evaluate the efficacy of ethanolic and aqueous extracts of neem (*Azadirachta indica* A. Juss.) and eucalyptus (*Eucalyptus citriodora* Hook.) as biopesticides against the brinjal fruit and shoot borer (BFSB) (*Leucinodes orbonalis*), a major pest of brinjal (eggplant) crop. Experiments were conducted to determine the lethal concentration of both neem and eucalyptus extracts against BFSB larvae. The biopesticides were applied to brinjal fruit and their efficacy was evaluated by monitoring the mortality of BFSB larvae and pupae. The results showed that both neem and eucalyptus extracts were effective in controlling BFSB larvae, with the neem extract being slightly more toxic than the eucalyptus extract. A 5% concentration of ethanolic and aqueous extracts of neem caused 82% and 64% mortality of BFSB 3rd instar larvae, while the same concentration of eucalyptus extracts caused 73% mortality with 60% mortality, respectively. BFSB 5th instar larvae showed a decreased mortality as compared to 3rd instars. It concludes that the use of aqueous and ethanolic extracts of neem and eucalyptus could help to reduce the use of synthetic pesticides to control brinjal fruit and shoot borer. Further studies are needed to assess the long-term effectiveness and sustainability of these biopesticides in controlling BFSB and protecting brinjal crops.

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INTRODUCTION

Brinjal (*Solanum melongena* L.) is one of the most important vegetables in South and South-East Asia (Thapa, 2010), where hot and wet climates prevail (NEPAL and MAINALI, 2013). It belongs to the plant

family Solanaceae and is among the most commonly grown vegetables of this family (Shah et al., 2013). The Indo-Pak Subcontinent is reported to be the native land of brinjal (Khan et al., 2019). Its worldwide cultivation is more than 2,766,181 ha and production is 96,105,332

tones (FAO, 2021). In Pakistan, it is cultivated on 7194 ha and production is 106,177 tons per annum (FAO, 2021).

Brinjal is reported to be attacked by more than 36 insect and non-insect pests like brinjal shoot and fruit borer (BFSB) (*Leucinodes orbonalis*), coccinellid beetle (*Epilachna vigintioctopunctata*), whitefly (*Bemisia tabaci*), aphid (*Aphis gossypii*), Jassid (*Amrasca bigutulla bigutulla*), hairy caterpillar (*Amsacta albistriga*), spider mites (*Tetranychus urticae*) and brinjal mealy bug (*Coccidohystrix insolita*). BFSB is the major pest of brinjal (Abhishek and Dwivedi, 2021) and is found in all the brinjal producing countries (Chandi and Chandi, 2019). A single larvae is capable to damage 4-6 healthy fruits (Rehman et al., 2017) and under severe infestation, damage reaches up to 90%, reducing the economic value of the brinjal crop (Hautea et al., 2016). The larvae bore into the fruit or shoots, causing the plant to wither (Herbst and Herbst, 2001). The adult female moth lays eggs on the undersides of flower buds, leaves or the base of growing fruits. The larvae have five to six instars and pupate on plant fragments or waste on the soil surface (Spackman and Lawson, 1991). The larvae of *L. orbonalis* reduce plant growth and size of fruits. It is imperative to adopt an environment friendly management approach to address these issues.

To manage insect pests, non-chemical methods such as biopesticides, botanicals, clean cultivation, and mechanical control, such as handpicking and destroying infected plant parts, are commonly used (Mahesh). However, pesticides residues have brought harmful effects for human's health and environment by causing environmental contamination, bioaccumulation and biomagnification. Chemical applications affect the non-target organisms as well as beneficial insects, while they can also cause many ailments in humans (Naz et al., 2023). Moreover, the overuse of pesticides increases the production cost and affects the export potential of brinjal fruits (Peng et al., 2023).

Despite several control strategies, the management of insect pests primarily relies on sequential applications of synthetic insecticides (Ramasamy et al., 2020) but new chemical pesticides have resulted hazardous to non-targeted insect pests and other living organisms (Araújo et al., 2023). Different losses caused by few insect pests of eggplant have been recognized by (Lalita and Kashyap, 2020). Contrary to the use of pesticides, the use of natural organic substances such as neem oil,

eucalyptus leaf extract and garlic bulb extract and other plant-based insecticides formulations are the best substitute now a day (Ahmad et al., 2022; Zafar et al., 2022; Maqsood et al., 2023; Mukhtar et al., 2023; Shahbaz et al., 2023). Azadirachtin is a compound found in neem plants that acts as an antifeedant and repellent (Adusei and Azupio, 2022). Garlic bulb extract is used to activate the antioxidant defense system in plants. Solvent extracts from *Eucalyptus globulus* and *Anacardium occidentale* using methanol and hexane extracts were used in the experiments as herbal preparations (Ray and Pattnaik, 2023). The present study was designed in search of promising alternative control strategies to old conventional insect control methods. For this, ethanolic and aqueous extracts of neem and eucalyptus were tested to assess the laboratory bio-efficacy against brinjal fruit and shoot borers.

MATERIALS AND METHODS

Collection and sampling

The collection was done during a visit to a vegetable area located on Multan road, Lahore on 15 June 2022. About 40 kg of infested brinjals were collected and brought back to the Insecticides and Weedicides Resistance Laboratory, Faculty of Agricultural Sciences (FAS), University of the Punjab, Lahore, Pakistan. A second survey was conducted to a farm house with an area of 70 acres near Jatti Umra, Raiwind Road, Lahore and collected about 30 kg of infested brinjals and brought them back to the laboratory. After the collection of infected brinjal fruits, these fruits were cut with knife and collected all the different instars of brinjal fruit borers and kept them separately in different Petri dishes with the help of a sterilized camel hairbrush in a plastic box. All the larvae were fed with small pieces of brinjal as shown in the Figure 1.

Mass rearing of BFSB using artificial diets

The adult population collected from Lahore was reared separately in rearing cages at Insecticides and Weedicides Resistance Laboratory. The stock of BFSB served as the source of the reference population. Fifty pupae of the reference population were taken and kept in Petri dishes and placed inside separate rearing boxes. Female and male adults at the ratio of 3:1 were placed into a glass jar (10 cm high and 7 cm diameter) for their successful mating. The open end of the jar was covered with a piece of white cloth and was tightly closed by a

rubber band. A small ball of cotton soaked in 10% sugar solution was placed inside the jar to feed the adults. After mating, the females laid eggs in clusters and singly in the inner surface of the white cloth. At least 10 to 15 newly hatched larvae were then removed from the jar and were placed on slices of the artificial diet, which was

then kept in containers with hygienic tissue paper. Fresh pieces of the diet were continuously supplied at an interval of 3 to 4 days. Only 2nd instar larvae were placed in a fresh piece of the diet. Pupae were collected and the whole procedure was repeated continually until the end of study.



Figure 1: Collecting different instars of brinjal stem and fruit borer from infested brinjal fruits.

Composition and preparation of the artificial diet

Rearing was done in a controlled environmental room (light: dark 12:12 h; relative humidity 60±5%; temperature 25±10°C). To reduce the viscosity of the agar, 150 ml double distilled water was added during the dispensing of finished diet. A mixture of 30 g of brinjal powder and coarse ground bengal gram (60 g) was used as base ingredient. About 200 g of diet was dispensed into diet plates and solidified. A modification in the agar powder quantity was made and additional 5 g of agar powder was added. The dried granules of yeast were replaced with purified extract of yeast. Diet plates (12 × 75 mm) were removed about 24 h before use and exposed to room temperature. To keep the moisture at an optimum level, lids were half removed to remove excessive moisture content. The diet was cut into pieces of 2.5 g and transferred to plastic containers. Five neonate larvae per plastic container were transferred with camel hair brush. The overall artificial diet was prepared by using the protocols of Sethi et al. (2016).

Preparation of ethanolic extracts of *E. citriodora* and neem leaves

The leaves of *E. citriodora* and neem were separated from the stem and cleaned with distilled water. The size of these leaves was reduced to 2-3 mm by using an electric grinder. One hundred grams of leaves of each plant species were taken in a 1 L round bottom flask. Condenser was attached to the flask by using a glass

bent and water supply was given. Collecting flask was attached to the other end of the condenser to collect the volatile material. The power of the microwave was settled at 60. The direction of the round bottom flask was changed after 8-10 min to stir the sample and to make sure of uniform heating. The liquid extract of the sample started accumulating in the collecting flask and extraction was carried out for about 30 min in a single extraction. The extract was collected in the collecting flask. The extract was added in the separating funnel along with 2.5 ml dichloromethane. Then it was shaken well and allowed to rest for 15 min to get a clear oil layer. Different concentrations (0%, 1%, 3% and 5%) of these ethanolic extracts were prepared separately in different small glass vials and covered them with a cork to avoid evaporation. Five larvae of 3rd and 5th instar of BFSB were kept separately in 4 different Petri dishes for each treatment. Petri dishes were lined with filter paper Whatman No. 1. Already prepared concentrations were poured into these Petri dishes with the help of pipettes. Petri dishes were covered with a perforated lid for ventilation of larvae inside. Mortality data was collected after every 8 h of applications until all larvae died.

Aqueous extraction of *E. citriodora* and neem leaves

Neem and eucalyptus leaves were collected from different trees planted in University of the Punjab, Lahore and cleaned with distilled water. The leaves were spread under shade on plastic sheets for the purpose of

good air circulation. The dried leaves were stocked in a cloth sack. According to the procedure given by (Grogan, 2021), the crushed leaves of neem and eucalyptus were ground gently to make fine powder with the help of coffee grinder (Coffee and Spice Grinder model no. SZJ-830, SAYONA Patirrier DELUXE, 220-240 V, 50HZ). The ground leaves were screened by 1 mm² wire mesh for getting the fine particles. Following the procedure of (Nair et al., 2022), the readymade leaf powder was measured at an amount of 0, 1, 3 and 5 mg in 100 ml of water and were added into four plastic buckets to make 0, 1, 3 and 5% solvent respectively. The extracts were stored under laboratory conditions at 27± 2°C throughout experiments. The muslin cloth was used to filter the extracts. Soap at rate of 1 ml L⁻¹ with no detergent was used as an emulsifier to enhance the stickiness of aqueous extracts (Fita et al., 2023).

For each concentration, at least 10 adult brinjal fruit borers were treated with three replicates in each petri plate separately and each extract was tested at three concentrations while 0% concentration was kept as control. Moist filter paper was used in each Petri dish. For control treatment, distilled water was used. Petri dishes with insects were kept at 25±1°C and 50 ± 5% RH with 12 h light in controlled condition. The collected data were subjected to Probit analysis after converting the data into percent corrected mortality as described by Henderson and Tilton (1995) given below to determine LC50.

Statistical analysis

The survival data were converted into percentage corrected mortality by using Abbot's formula (Abbott,

1925). Data obtained in various treatments were compared by ANOVA and all means were compared by using Tukey's HSD test. For the analysis of data, Statistix 8.1 software was used.

RESULTS

Insecticidal activities of neem extracts

A three-way ANOVA revealed that the effect of type of extract, instar stage and the concentrations had significant ($P \leq 0.01$ and 0.001) effects on mortality of the borer. However, their various interactions had insignificant effect on this studied parameter (Table 1). The results of our experiment demonstrated that both neem ethanolic and aqueous extracts exhibited insecticidal effects, leading to mortality in the brinjal stem and fruit borer larvae. However, it was observed that the neem ethanolic extract displayed higher efficacy as compared to the aqueous extract in controlling the larvae. The mortality percentage varied across different concentrations and larval instars. For the 3rd instar larvae, the neem ethanolic extract showed mortality ranging from 67% to 82%, while the aqueous extract displayed mortality from 4% to 64%. These findings indicate that neem-based solutions, particularly the ethanolic extract, have potential as larvicidal agents against the brinjal stem and fruit borer. During the 5th instar stage, the neem ethanolic extract exhibited even higher efficacy compared to the aqueous extract. The mortality due to neem ethanolic extract ranged from 7% to 67%, whereas the aqueous extract caused 4% to 44% as shown in Figure 2.

Table 1: Three-way ANOVA for comparison of insecticidal activity of different concentrations of aqueous and ethanolic leaf extract of neem against larvae of brinjal leaf borer at 3rd and 5th instar stages.

Sources of variation	Df	Sum of squares	Mean squares	F-values
Extract type (E)	1	1963.5	1963.52	11.17*
Instar stage (S)	1	1480.7	1480.74	8.42*
Concentration (C)	3	25582.0	8527.32	48.51**
E × S	1	92.4	92.41	0.53 ^{ns}
E × C	3	538.1	179.36	1.02 ^{ns}
S × C	3	1132.6	377.52	2.15 ^{ns}
E × S × C	3	374.2	124.72	0.71
Error	32	5624.6	175.77	
Total	47	36788.0		

*, **: Significant at $P \leq 0.01$ and 0.001 , respectively. ns: Non-significant

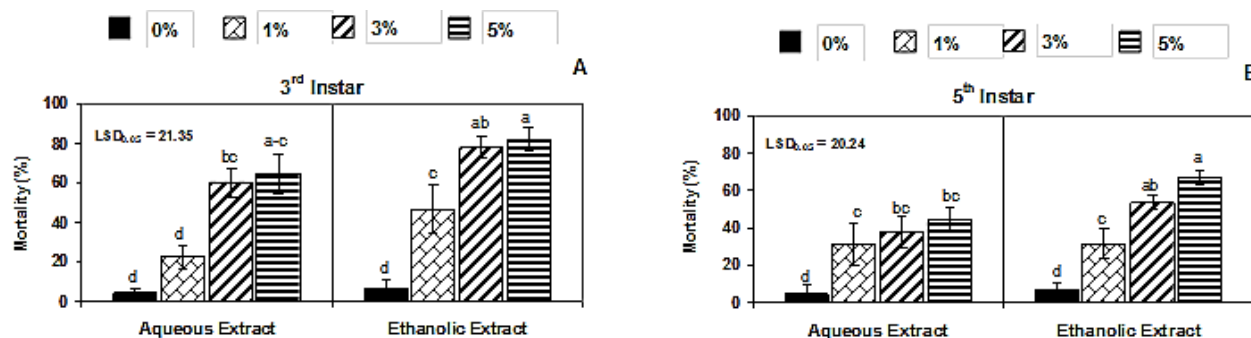


Figure 2: Effect of different concentrations of aqueous and ethanolic leaf extract of neem on mortality of brinjal fruit borer at 3rd and 5th instars. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P ≤ 0.05) as determined by LSD Test.

Insecticidal activity of eucalyptus extracts

A three-way ANOVA indicated that the effect of extract type (E), instar stage (S), extract concentration (C) as well as interactive effect of S × C was significant for mortality of the borer (Table 2).

For the 3rd instar larvae, both the eucalyptus extracts exhibited insecticidal properties, leading to larval mortality. However, the eucalyptus ethanolic extract demonstrated higher efficacy compared to the

aqueous extract. The mortality due to eucalyptus ethanolic extract ranged from 9% to 73%, while the aqueous extract resulted in 2% to 60% mortality. Similarly, in case of 5th instar larvae, the eucalyptus ethanolic extract displayed higher efficacy as compared to the aqueous extract. The mortality due to eucalyptus ethanolic extract ranged from 4% to 53%, while the aqueous extract caused 4% to 35% mortality (Figure 3).

Table 2: Three-way ANOVA for comparison of insecticidal activity of different concentrations of aqueous and ethanolic leaf extract of *Eucalyptus citriodora* against larvae of brinjal leaf borer at 3rd and 5th instar stages.

Sources of variation	Df	Sum of squares	Mean squares	F-values
Extract type (E)	1	1188.7	1188.73	7.91*
Instar stage (S)	1	3135.2	3135.20	20.87**
Concentration (C)	3	20373.7	6791.23	45.21**
E × S	1	3.2	3.17	0.02 ^{ns}
E × C	3	239.3	79.77	0.53 ^{ns}
S × C	3	1727.7	575.90	3.83*
E × S × C	3	51.8	17.25	0.11 ^{ns}
Error	32	4806.5	150.20	
Total	47	31526.1		

*, **: Significant at P ≤ 0.01 and 0.001, respectively. ns: Non-significant

Probit analysis

Probit analysis was conducted for neem and eucalyptus ethanolic extracts to determine their impact on insect populations at different stages of development (3rd and 5th instar). The probit table presents concentrations of the extracts and their corresponding probit values, which are essential in calculating lethal concentrations for a given population. By using a regression equation (Y = ax + b), it established a relationship between probit values (Y) and the log of concentration (x). The probit

analysis shows that a concentration of 1.48 of ethanolic extract would cause 50% mortality of brinjal stem and fruit and borers during the usage of aqueous extract of neem and eucalyptus. The probit table predicts that the concentration of 1.96 of aqueous extract would cause 50% mortality of the insect. These results provide valuable insights into the potential insecticidal properties of the studied extracts, which can contribute to further research and practical applications in pest management strategies.

Using the LD₅₀ values and slope values, we can calculate the probit equation, which can be used to predict the response variable for different levels of pesticide concentration. The probit equation is given by:

$$Y = \Phi (b_1 + b_2 * \log (\text{dose})),$$

Where Y is the predicted response variable, Φ is the cumulative distribution function of the standard

normal distribution, b₁ is the intercept, b₂ is the slope, and dose is the pesticide concentration. From the table, the values of b₁ and b₂ can be estimated using maximum likelihood estimation. This will allow us to determine the dose-response relationship for each pesticide, and compare the toxicities of different pesticides.

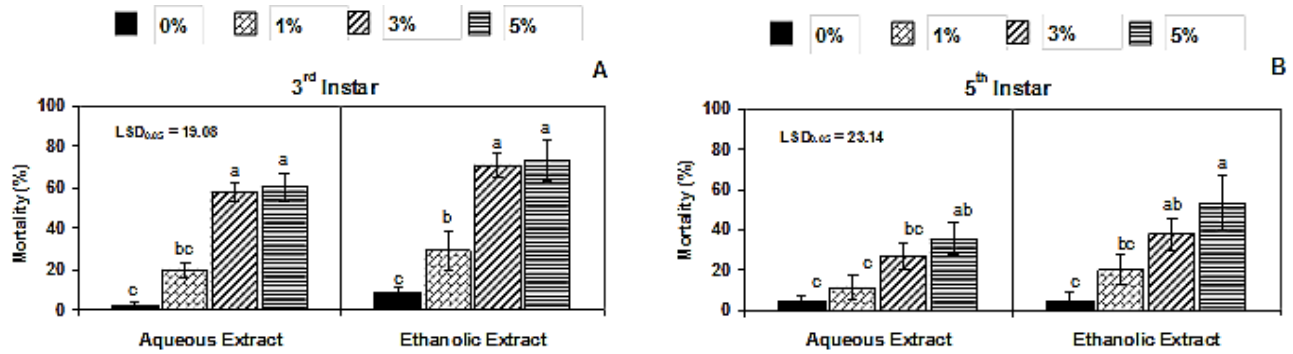


Figure 3: Effect of different concentrations of aqueous and ethanolic leaf extract of *Eucalyptus citriodora* on mortality of brinjal fruit borer at 3rd and 5th instar stages. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

Table 3: The probit analysis of ethanolic and aqueous extracts of neem and eucalyptus.

BFSF instars	Concentration (%)	Log(10)	Ethanolic Extract	Probit Values	Aqueous Extract	Probit Values
Neem 3 rd Instar	1	0	46.66	4.92	22.2	4.23
	3	0.477121	77.8	5.77	59.96	5.25
	5	0.69897	82.2	5.92	64.43	5.36
Eucalyptus 3 rd Instar	1	0	28.9	4.45	20	4.16
	3	0.477121	71.1	5.55	57.76	5.2
	5	0.69897	73.33	5.61	60.36	5.25
Neem 5 th Instar	1	0	31.33	4.5	31.1	4.5
	3	0.477121	53.3	5.08	37.76	4.69
	5	0.69897	66.66	5.44	44.43	4.85
Eucalyptus 5 th Instar	1	0	20	4.16	11.1	3.77
	3	0.477121	37.76	4.69	26.66	4.39
	5	0.69897	53.33	5.08	35.33	4.61
				LC = 1.48	LC = 1.96	

DISCUSSION

The use of synthetic pesticides in agriculture has been associated with several negative impacts on the environment and human health. In recent years, there has been increasing interest in the use of plant-based biopesticides as an alternative to synthetic pesticides (Arshad et al., 2019). Botanical extracts, such as neem and eucalyptus, are some of the most commonly studied plant-based biopesticides due to their potential efficacy

and reduced toxicity to non-target species (Ullah et al., 2022). Ten combinations of insecticides and plant extracts were evaluated against the brinjal shoot and fruit borer (Mollah et al., 2022). The foliar application of quinalphos with basal neem cake reduced borer incidence and increased brinjal yield (Owen et al., 2023). Neem products, alone or with conventional insecticides, were recommended to mitigate environmental pollution (Abhishek and Dwivedi, 2021). Field experiments on

brinjal shoot and fruit borer (*Leucinodes orbonalis*) evaluated various botanical products with an insecticide (Acharya and Ram, 2023). Previous work showed that Cypermethrin 0.05%, Pungam oil 2%, Nimbecidene 0.4%, and Iluppai oil 2% were most effective, causing lowest shoot damage (5.80-7.13%) and fruit infestation (10.29-10.63%) (Choudhury et al.). Neem oil 2% and garlic extract 3% showed moderate effectiveness with 15.12-18.82% fruit damage (Ghosh and Hasan, 2021). The results, from our experiment indicate that the ethanolic extract consistently had more mortality rates for both the 3rd and 5th instar larvae compared to the aqueous extract and this work coincides with the previous work of (Assinapol, 2020) in which leaf extract of neem showed mortality of *Tuta absoluta* Meyrick in tomato crop. In regards to the instar larvae, mortality ranged from 7% to 82% for the ethanolic extract while the aqueous extract showed 4% to 64% mortality. The difference in mortality percentages was more pronounced for the instar larvae, where the neem ethanolic extract displayed 7% and 67% mortality whereas the aqueous extract had 4% to 44% mortality. Same findings were described against *T. castenium* larvae with concentration of 0.2 mg ml⁻¹ due to neem leaf extracts showing a good repellency rates of 81.4% (Alhathloul et al., 2023). These results suggest that the ethanolic extract possesses effectiveness in eradicating brinjal fruit borer larvae when compared to the aqueous extract. This is likely due to its concentration of compounds, including azadirachtin, a well-known insecticidal compound extracted through ethanol solvent used on neem leaves extracts (Ghosh). The enhanced performance of extract in controlling brinjal fruit borer larvae holds significant implications for developing novel larvicidal agents as alternatives to synthetic pesticides, for pest control purposes.

The mortality percentages were higher, with the extract compared to the aqueous extract and it also acted more rapidly. This suggests that the ethanolic extract is better at killing larvae which is important for effective pest control. By acting, it reduces the likelihood of developing resistance to the insecticide. The study demonstrates that the ethanolic extract is an efficient agent for controlling brinjal fruit borers compared to the aqueous extract. It achieves mortality rates, acts more quickly making it less likely for resistance to develop. These findings indicate that based solutions in their ethanolic form show promise as a new and effective method for

pest control. The probit analysis conducted on the ethanolic and aqueous extracts of neem and eucalyptus against 3rd and 5th instars of Brinjal Fruit and Shoot Borers revealed valuable insights into their potential insecticidal properties and *A. indica* also has its medicinal value as it cures many diseases via regulation of many physiological and biological processes (Alzohairy, 2016). The probit table presented concentrations of the extracts and their corresponding probit values, allowing the calculation of lethal concentrations for a given population, however, the aqueous extracts of *A. indica* showed mortality to 3rd and 4th instar larvae of *A. aegypti* at concentrations of 0.0033-0.05% at 25 °C (Wandscheer et al., 2004). The results indicated that the ethanolic extracts generally exhibited higher toxicity compared to the aqueous extracts. For instance, the LD₅₀ (concentration causing 50% mortality) of the ethanolic extract of neem was found to be 1.48, while the LD₅₀ for the aqueous extract was 1.96. Similarly, for eucalyptus, the ethanolic extract had an LD₅₀ of 1.48, whereas the aqueous extract's LD₅₀ was 1.96.

CONCLUSION AND RECOMMENDATION

These findings suggest that the ethanolic extracts of both neem and eucalyptus may be more potent in controlling the brinjal fruit borer larvae compared to their respective aqueous extracts. However, further research is warranted to understand the dose-response relationship and compare the toxicities of different pesticides to optimize their practical applications in pest management strategies. Further investigations into the underlying mechanisms of action will be valuable to optimize the utilization of eucalyptus extracts, in pest control strategies.

AUTHORS' CONTRIBUTIONS

MM, AJ, MSH designed, formulated and supervised the study; AH conducted the experiments; AI, MA, MRT and MFHF provided the technical assistance; and AS and MZH collected the data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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