Original Article
ISSN (Online): 2582-7472

ShodhKosh: Journal of Visual and Performing Arts
July 2024 5(7), 88–93

LARVICIDAL EFFICACY OF ALCOHOL-BASED EXTRACTION OF VERNONIA AMYGDALINA WITH BACILLUS THURINGIENSIS ON THE VECTOR AEDES AEGYPTI

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DOI
10.29121/shodhkosh.v5.i7.2024.1982

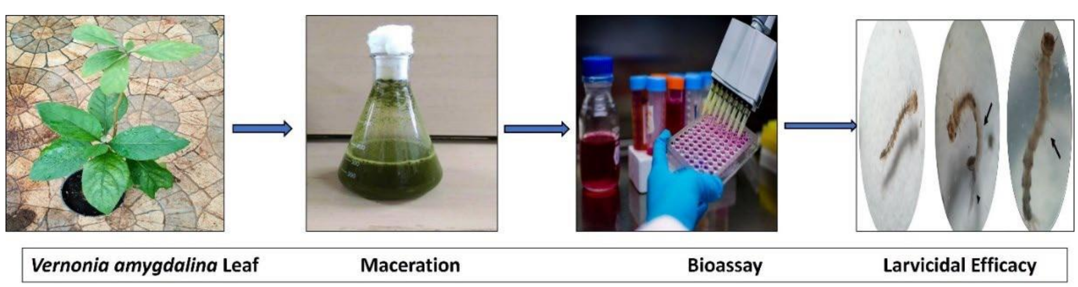
Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ABSTRACT

Mosquitoes are the most significant category of hematophagous arthropods. Traditional medicine uses numerous plants for their larvicidal properties against mosquitoes in various regions globally. The combination of bacterial toxin and plant extract has a significant impact on mosquito larvae. We evaluated the larvicidal effectiveness of the plant extract Vernonia amygdalina in combination with B. thuringiensis against third instar larvae of Aedes aegypti. We recorded the average mortality and percentage mortality of larval populations at various concentrations after 24 and 48 hours of exposure. Bti and Vernonia amygdalina extracts showed significant insecticidal efficacy, as evidenced by LC 50 and LC 90 values. The LC50 values for 24-hour and 48-hour exposure are 18.35 and 16.71, respectively. The LC 90 values at 24 hours and 48 hours of exposure are 32.47 and 29.39, respectively.

Keywords: Aedes Aegypti, Vernonia Amygdalina, Traditional Medicine, Mosquito Larvae

GRAPHICAL ABSTRACT



How to cite this article (APA): Kumari, M., Shukla, D., Dash, S.S., Dubey, A., Tiwari, A., and Sonker, A. (2024). Larvicidal Efficacy of Alcohol-Based Extraction of Vernonia Amygdalina with Bacillus Thuringiensis on the Vector Aedes Aegypti. *ShodhKosh: Journal of Visual and Performing Arts, 5*(7), 88–93. doi: 10.29121/shodhkosh.v5.i7.2024.1982

1. INTRODUCTION

Malaria, filariasis, Japanese encephalitis, dengue, yellow fever, and chikungunya are just a few infectious diseases that mosquitoes may spread (Koodalingam et al., 2009). When it comes to disease transmission, mosquitoes play a significant role (Ramaiah et al., 2006 Dubey et.al 2020). Since dengue fever has been on the rise over the past 20 years, the World Health Organization (WHO, 2023) reports that modern mosquito-borne illnesses continue to be major issues in international public health. Mosquitoes of the genus Aedes are commonplace in homes and often coexist with people. Gubler (1998) asserts that both tropical and subtropical areas can harbor dengue fever. The biting capacity of Aedes aegypti is far greater than that of Aedes albopictus, and both species are suited to laying their eggs in smaller containers with water inside (Kolivras, 2006; Winchester, 2013). The high risk of dengue in the location is determined by the high number of Aedes mosquitoes. Because of its extreme anthropophilia, frequent biting, and ability to flourish in close quarters with people, this mosquito—one of the most extensively spread in the world—is also the most effective vector for arbovirus (WHO, 2009). Mosquitoes of the genus Aedes, often known as container breeders, generally lay their eggs in man-made and semi-man-made containers, such as buckets, plastic drums, and overhead tanks, or in naturally occurring containers that contain organic materials, such as tree holes (Chadee et al., 2004). Beeker et al. (2006) and Arunachalam et al. (2008) found that Aedes mosquitoes may reproduce both inside and outdoors. Due to a lack of understanding about the dengue vector's unique breeding habits and inadequate infrastructure, mosquito control activities were ineffective. In endemic locations, the prevention of dengue hemorrhagic fever epidemics relies on long-term anti-mosquito management efforts, namely sanitary practices in the home and the environment, with a focus on reducing larval sources. *Bacillus thuringiensis var-israelensis* (Bti), a microbial control agent, has been used effectively on a global scale to decrease the emergence of adult mosquitoes (Land et al., 2019, Perez et al., 2005). Reducing mosquito breeding grounds was an early strategy for mosquito control. Plant extracts, commonly known as phytochemicals, have recently gained attention as possible control agents due to the significant environmental damage that chemicals do. Neem has a wide variety of insect-repelling properties, including those of mosquitoes.In order to effectively combat mosquito larvae and their breeding grounds, it is crucial to use a natural herbal larvicide. This study aims to eradicate dengue by examining novel eco-friendly control methods. Bacillus thuringiensis var-israelensis, most commonly known as Bti, is among these bio-insecticides for insects (Likitvivatanavong, et al.2011). According to McCie et al. (2023), mosquito control with Bti could have an especially significant impact on soil and ground-based food webs. *Bacillus thuringiensis var israelensisi (Bti)* and *Bacillus sphaericcus* are microbial mosquito larvicides that have recently been the focus of research. These bacteria exhibit larvicidal properties, prevent the insect from laying eggs, and regulate the insect's development (Wirth et al., 2005; Chatterjee et al., 2008). As an alternative to chemical treatments, Vernonia amygdalina and Bti leaf extracts work together to combat mosquito resistance.

2. MATERIAL & METHODS

The Vernonia amygdalina leaves were freshly collected from Maharan Pratap College of Pharmacy Herbal Garden. After drying them in the shade, grinding them into a powder, and then extracting 10 g using 100 ml of ethanol solvent, the mixture was shaken overnight. After passing the mixture through the Whatman No. 1 filter paper, we were able to collect the solvent residue.

3. MICROBIAL BIOASSAY PROCESS

Sumitomo Chemical India Ltd supplied the Bacillus thuringiensis. We created a range of concentrations, from 10 to 50 Gram/Liter, by thoroughly mixing the necessary amount of B. thuringiensis with distilled water.

4. PLANT EXTRACTION PROCESS

One hundred milliliters of ethanol were used to dissolve the ten grammes of dried powdered plant material. To isolate the secondary metabolites found in plants, the mixture was allowed to sit at room temperature for three days. Following that, the mixture was passed through Whatman No. 1 filter paper, and the resulting filtrate was placed in an independent container. The next step was to use a rotary evaporator set to temperatures between 40 and 60 degrees Celsius for 30 minutes to separate the solvent from the extract and concentrate it. At last, the extracts were obtained in a semisolid state. To determine the dried extract's yield, the weight of the dried content was used. The remaining extract was refrigerated and maintained at 4 ºC for future use. Extract dry weight in grammes divided by plant biomass dry weight in kilograms and then multiplied by 100 is the yield percentage Carneiro et al. (2020).

5. LARVICIDAL ACTION

We designed the experiment to test the larvicidal activity of various concentrations of the plant extract (10 mg, 20 mg, 30 mg, 40 mg, and 50 mg) and *Bacillus thuringiensis* in 100 ml of dechlorinated water. Each test concentration received twenty larvae and 0.1 mg of larval food, while the control group maintained a constant concentration. The experiment was repeated. After 24 and 48 hours, we counted how many larvae had died. To get the fatality percentage, we used the Abbott method. To determine the lethal values (LC_{50} and LC_{90}), we used probit analysis on the toxicity data Kamaraj C et al. (2011).

$$\text{Corrected mortality} = \frac{(\text{Observed mortality in treatment} - \text{Observed mortality in Control}) \times 100}{(100 - \text{Control Mortality})}$$

$$\%\ \text{Mortality} = \frac{\text{No. of dead larvae or pupae}}{\text{No. of larvae or pupae introduced}} \times 100$$

6. RESULTS

Figures 1 and 2 and Tables 1 through 3 present the findings on the effectiveness of the Vernonia amygdalina extract in killing Aedes aegypti larvae. Table 2 (Figure 4) shows that after 24 hours and 48 hours at varied concentrations, the LC_{50} value of Vernonia amygdalina with Bti was 18.35 and 16.71, respectively. After 24 hours and 48 hours at varied concentrations, the LC 90 values of the Vernonia amygdalina extract with Bti were 32.47 and 29.39, respectively. According to Table 2 and Figure 4, the proportion of deaths rises as the concentration increases.

Table: 1 The average and percentage of deaths of third-stage Ae. aegypti mosquito larvae that were treated with different amounts of *Vernonia amygdalina* and Bti leaf plants after 24 hours.

Concentration of plant extracts	Mean mortality	Mortality rate Percentage at 24 hours	Mean mortality Percentage at 48 hours	Mortality rate Percentage at 48 hours
10 milligrams Vernonia amygdalina	6±0.07	13.82051283	7+0.03	8.894736843
20 milligrams Vernonia amygdalina	9.6±1.04	36.8974358	13±0.17	37.84210527
30 milligrams Vernonia amygdalina	13±1.3	49.71794873	16±0.25	53.63157896
40 milligrams Vernonia amygdalina	14.6±0.7	62.53846155	19±1.6	72.05263157
50 milligrams Vernonia amygdalina	20±2.7	85.61538464	21±0.07	79.94736843

Table: 2 Establishing LC_{50} and LC_{90} after 24 and 48 hours

SI.No	Plant	Time	LC_{50}	LC_{90}
1	*Vernonia amygdalina*	24 hrs	18.35	32.47
2		48 hrs	16.71	29.39

Table:3 Deterministic evaluation of death rate at 24 and 48 hours.

S.No	Log C	t Analysis 24 hrs	Probit nalysis 48 hrs
1	1	4.87	4.58
2	1.301029997	5.65	5.68
3	1.477121254	5.98	5.07
4	1.602059992	5.35	5.55
5	1.698970005	7.02	6.82

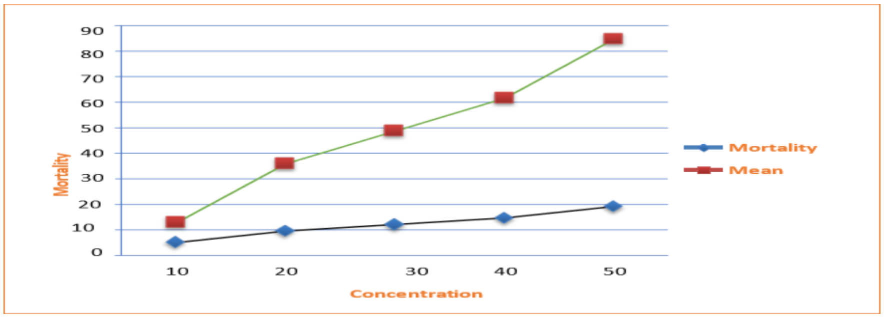


Figure 1: After 24 hours, *A. aegypti* larvae were subjected to different concentrations of *Vernonia amygdalina* leaf extract and Bti, resulting in average and percentage mortality.

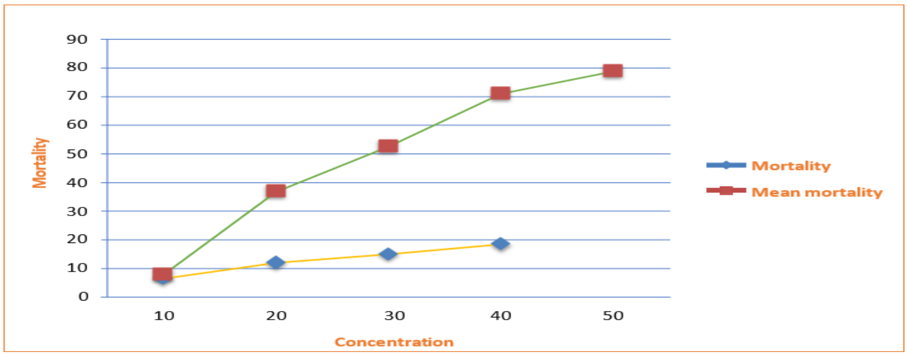


Figure 2: The results show the average and percentage of larvae that died after 48 hours of treatment with different concentrations of Bti and *Vernonia amygdalina* leaf extract.

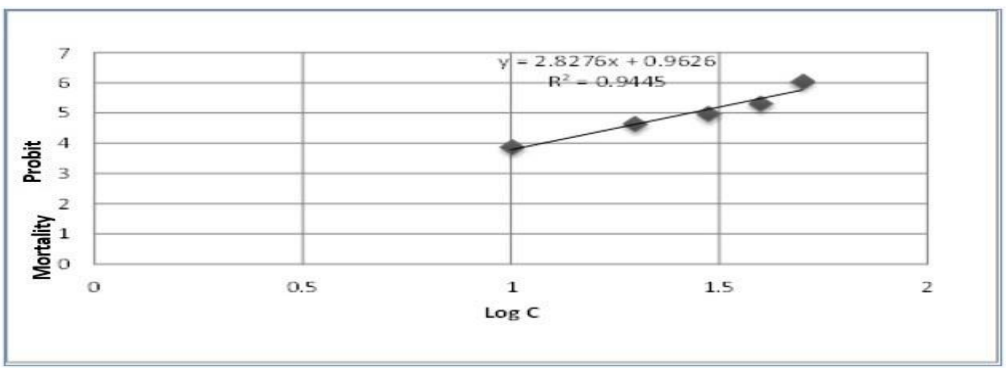


Figure 3: Probit Analysis of Percentage Mortality at 24 hours

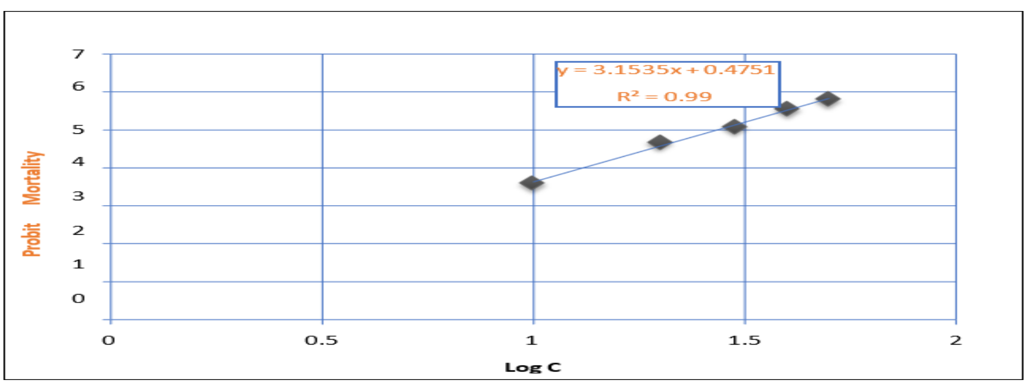


Figure 4: Probit Analysis of Percentage Mortality at 24 hours

7. DISCUSSION

Aedes aegypti developed in water, insecticides find larval mosquitoes an appealing target because they are easier to control in this environment. As mosquitoes develop resistance to traditional synthetic pesticides, vector control is in jeopardy.

Lim H et al. (2023) tested solvent extracts from three aromatic plants: turmeric (Curcuma longa), holy basil (Ocimum americanum), and parsley (Petroselinum crispum) on the dengue mosquito (Aedes albopictus). The researchers then tested the extracts for cellular toxicity and phytochemical profiles. According to the findings, the most effective larvicidal agents were the hexane extracts of Origanum americanum and P. crispum. Plant extracts are now the primary tool for controlling mosquito larvae.

M.A. Sofi, A. Nanda, M.A. Sofi et al. conducted this study in 2022 to evaluate the toxicity of methanolic and ethanolic extracts of Artemisia absinthium against arbovirus-carrying Aedes aegypti larvae. We documented the death rate of larvae after a 24-hour exposure. In our study, the ethanolic Artemisia absinthium leaf extract killed more eggs than the methanolic extract (92 ± 1.99 percent at 1000 ppm vs. 68 ± 1.99 percent at 1000 ppm). The ethanolic extract also markedly inhibited the detoxifying enzymes acetylcholinesterase and a and b-carboxylesterases. The ethanolic extract reduced the protein level against 4th instar larvae after 24 hours of exposure, with an LC_{50} of 694.3 ppm. With these findings in hand, it's easy to see how the ethanolic active extract of Artemisia absinthium might be a useful tool in the fight against pests and disease vectors.

8. CONCLUSION

The research recommends using plants to control mosquito-transmitted illnesses. Together with B. thuringiensis, these plants are very cost-effective and environmentally benign, as well as having a greater capacity to kill larvae. Therefore, we might consider Vernonia amygdalina, in combination with B. thuringiensis, as a potential resource for mosquito larvae. A technique like this would reduce pesticides' environmental impact and encourage rural populations to use local bioresources more sustainably.

ACKNOWLEDGEMENT

Authors are thankful to the College authority for providing necessary facilities to conduct the work.

DATA AVAILABILITY STATEMENT

All of the data supporting the findings of the presented study are available for corresponding author on request.

DECLARATIONS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The manuscript has not been published or submitted to another journal, nor is it under review.

SUPPLEMENTARY FILE

None.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

All authors participate equally

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