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Evaluation of Larvicidal Properties of *Zingiber officinale* Rhizome and *Allium Sativum* Bulbs against *Aedes aegypti*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluates the larvicidal potential of methanolic extracts derived from *Zingiber officinale* (ginger) rhizome and *Allium sativum* (garlic) bulbs against the 4th instar larvae of *Aedes aegypti*, a key vector responsible for transmitting diseases such as dengue, yellow fever, chikungunya, and Zika. Due to the increasing resistance of mosquitoes to conventional insecticides, there is a pressing need for alternative, eco-friendly vector control methods. Botanical extracts, known for

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their pesticidal properties, offer a promising solution. Methanolic extracts were collected from the selected test plant parts and different test solutions were prepared to test their larvicidal efficacy against the fourth instar larvae of *Ae. aegypti.*
The results indicated a significant dose-dependent increase in larval mortality for both extracts. The extracts of *Z. officinale* consistently showed better larvicidal effects than outperformed the extracts of *A. sativum* at all tested concentrations, with mortality rates ranging from 14.29% at 62.5 ppm to 79.22% at 500 ppm. In contrast, *A. sativum* exhibited mortality rates from 11.69% at 62.5 ppm to 76.62% at 500 ppm. The LC_{50} values further highlighted the greater efficacy of ginger extract, with an LC_{50} of 219.10 ppm compared to 237.39 ppm for garlic. These results suggest the usage of extracts of ginger rhizomes and garlic bulbs as eco-friendly larvicides against *Ae. aegypti* mosquitoes.

Keywords: Aedes aegypti; larvicidal activity; Zingiber officinale; Allium sativum; mosquito control; botanical insecticides.

1. INTRODUCTION

Aedes aegypti, also known as the "yellow fever mosquito," is a major carrier of diseases such as yellow fever, dengue fever, chikungunya, and Zika viruses. It has been responsible for pandemics of these viral diseases over the years, causing immense human suffering. The historical spread and impact of *Ae. aegypti* provide insight into the human populations at risk for diseases transmitted by this mosquito, and understanding its distribution is crucial for public health and disease control [1,2]. Dengue hemorrhagic fever (DHF) incidences have risen cyclically recently. In the absence of an effective vaccine and with the clinical challenges of treating DHF, vector control remains crucial for preventing dengue transmission [3].

Aedes mosquito control measures include chemical interventions, habitat management, non-chemical larviciding, population replacement methods, and genetic techniques, with the efficacy of each requiring investigation [4]. Insecticide resistance in these *Aedes* species poses a significant challenge to vector control efforts. Larval source reduction and improving access to reliable, clean piped water are important strategies for *Aedes* control, especially in urban areas with common artificial aquatic habitats [5]. Plant secondary metabolites, such as terpenes, phenolics, nitrogen- and sulfur-containing compounds, hold promise as potential alternatives to synthetic chemicals for pest and disease control in crop production [6]. They are effective in controlling pests, particularly when applied early, and offer advantages such as being less hazardous to human health, environmentally friendly, and safe for natural predators [7]. The secondary metabolites of the plant extracts can inhibit insect reproduction and other processes, and some may even be toxic. The mixture of secondary metabolites in plant extracts can provide a deterrent effect for a longer period than a single compound [8].

The extracts of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) are well-documented for their pesticidal properties against many pests. Rizvi, et al. [7] have reported the larvicidal potential of ginger, garlic, and tobacco extracts against the cabbage looper. Garlic extracts were superior in controlling the cabbage looper with the lowest infestation rates (8.53%), followed by ginger extracts (10.14%) and tobacco extracts (11.02%). The untreated control plot had the highest infestation rate (182.02%). Ginger extract was used for controlling okra flea beetles and cowpea bruchid. Higher ginger concentrations (20%, 25%, 30%) notably increased okra yields, with fruit weights improving by 29% to 44%. In the lab, higher ginger residue concentrations effectively suppressed cowpea bruchid emergence [9]. Increasing the concentration of Ginger shoot extract (GSE) reduced aphid litter size, longevity, and molting while increasing mortality [10].

Oil extracted from the fresh ginger rhizomes (*Z. officinale*) demonstrated moderate insect growth regulatory and antifeedant effects against *Spilosoma obliqua*, as well as significant antifungal activity against *Rhizoctonia solani* [11]. The oils extracted from *A. sativum* and *Z. officinale* extended larval and pupal durations, increased pupal weight, and reduced egg hatchability, especially at the LC_{50} concentration of ginger oil against *Spodoptera littoralis* (African cotton leafworm). Additionally, catalase enzyme (CAT) activity was significantly altered only with ginger oil at LC_{50} concentration [12].

As the results of the previous studies reported the pesticidal effects of ginger and garlic extracts, the present study hypothesized that the methanolic extracts of ginger rhizomes and garlic bulbs would be effective in controlling the fourth instar larvae of *Ae. aegypti*.

2. MATERIALS AND METHODS

Plant Materials: Fresh rhizomes of *Z. officinale* and bulbs of *A. sativum* were collected from organic fields in the Vikarabad region of Telangana State, India. They were thoroughly washed, dried over 15 days, and then ground into a fine powder. This powder was stored in a sealed container until further use.

Preparation of Extracts: To prepare the extracts, 100 grams of the prepared powders were each soaked in 250 mL of methanol, with frequent shaking, for four days. On the fifth day, the mixtures were filtered using Whatman filter paper no. 1. The filtrates were then left to evaporate under a rotating fan for one day, resulting in a semi-solid extract, which was stored in a refrigerator at 4°C until use.

Test solutions: Stock solutions of 1000 ppm were prepared by mixing 1 gram of the extracts in 10 ml of methanol and then by adding 990 ml of distilled water. From this stock solution, test solutions with concentrations of 62.5, 125, 250, and 500 ppm were prepared by serial dilution method. A control solution was also prepared using the same solvents but without the extracts.

Mosquito Larvae: *Ae. aegypti* eggs were collected by placing egg traps on the campus of Tara Government College, Sangareddy, Telangana State, India. The collected eggs were hatched in the Department of Zoology and the larvae were raised on a diet of dog biscuits and dry yeast powder. Fourth instar larvae from this population were used for the larvicidal bioassay.

Larvicidal Bioassay: The larvicidal activity of the methanolic extracts of *Z. officinale* and *A. sativum* against the 4th instar larvae of *Ae. aegypti* was assessed following WHO [13] guidelines. Twenty larvae of uniform size were placed in 100 mL of each test solution in 250 mL test cups. Mortality was recorded after 12, 24, 36, and 48 hours of exposure. The average percentage mortality was calculated from five replicates using Abbott's [14] formula:

> Percentage Mortality (PM) = (Number of dead larvae / Total larvae population) × 100

Abbott's [14] formula was applied to obtain corrected mortality rates.

Statistical Analysis: The data were analyzed using Microsoft Excel, where regression and probit analyses were conducted to determine the LC_{50} values. A significance level of $p<0.05$ was used for all statistical tests.

3. RESULTS AND DISCUSSION

The larvicidal bioassay results demonstrate that both *Z. officinale* and *A. sativum* methanolic extracts exhibit significant larvicidal activity against the 4th instar larvae of *Ae. aegypti*, with a clear dose-dependent relationship. As the concentration of the extracts increased, so did the mortality rate of the larvae, indicating that higher concentrations of these plant extracts are more effective at killing the larvae.

At the lowest test concentration of 62.5 ppm, the mortality rate for *Z. officinale* was 14.29%, slightly higher than the 11.69% observed for *A. sativum*. This trend continued across all concentrations tested, with *Z. officinale* consistently producing higher mortality rates than *A. sativum* at each concentration level. At 125 ppm, *Z. officinale* induced 33.77% mortality, while *A. sativum* caused 29.87% mortality. At 250 ppm, *Z. officinale* resulted in 50.65% mortality, whereas *A. sativum* produced 46.75%. Finally, at the highest concentration of 500 ppm, *Z. officinale* exhibited 79.22% mortality, surpassing the 76.62% mortality achieved by *A. sativum*.

Probit analysis results indicated the LC_{50} values of these extracts. The LC50 of *Z. officinale* was determined to be 219.10 ppm, while that of *A. sativum* was 237.39 ppm. This suggests that *Z. officinale* is more potent, requiring a lower concentration to achieve 50% larval mortality compared to *A. sativum*. The lower LC_{50} value for *Z. officinale* indicates it could be a more effective botanical insecticide at lower doses.

The regression equations and corresponding R^2 values for both extracts reflect strong correlations between concentration and mortality. For *Z. officinale*, the regression equation is $y = 0.1746x + 11.745$ with an R^2 value of 0.9645, while for *A. sativum*, the equation is $y = 0.1759x + 8.2439$ with an R^2 value of 0.9738. *Z. officinale* extracts appear to be more effective than *A. sativum* in killing *Ae. aegypti* larvae at the same concentrations, with a lower LC_{50} and higher mortality rates across all concentrations.

Table 1. Results of Larvicidal Bioassays of *Z. officinale* & *A. sativum* extracts against the fourth instar larvae of *Ae. aegypti*.

Tested Plant extracts	Mortality % against Test Conc. in PPM					LC_{50} in PPM	Regression Equation	R^2 Value
	0	62.5	125	250	500			
Z. officinale	0.00 ± 0.47	14.29 ± 0.87	33.77 ± 0.82	50.65 ± 1.29	79.22 ± 0.67	219.10	y = 0.1746x + 11.745	0.9645
A. sativum	0 ± 0.47	11.69 ± 0.63	29.87 ± 0.40	46. 75 ± 0.98	76.62 ± 0.40	237.39	y = 0.1759x + 8.2439	0.9738

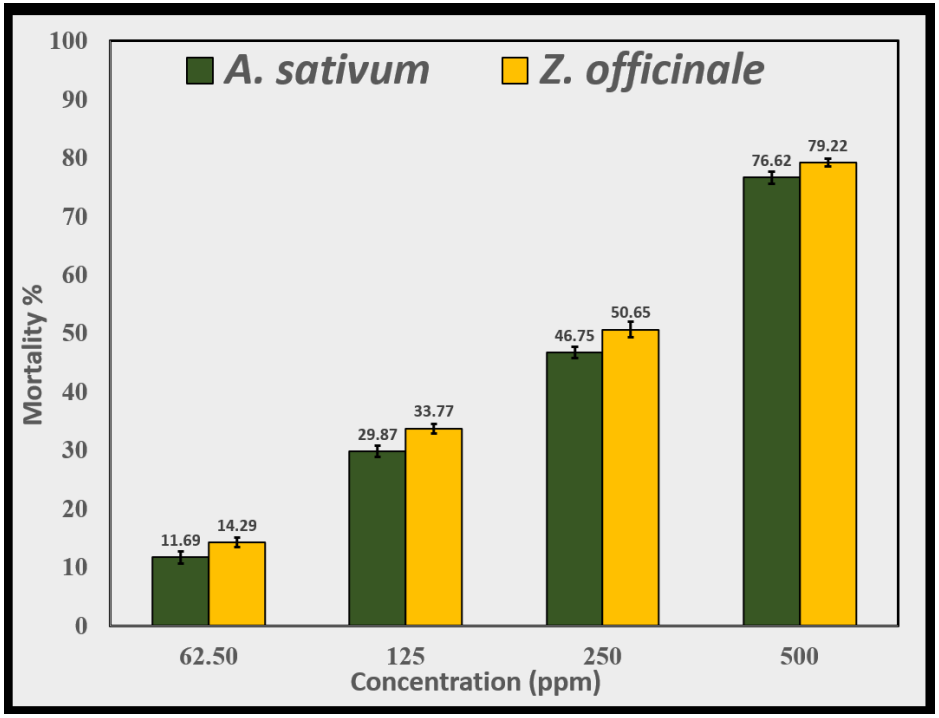

Fig. 1. Graphical representation of the results of Larvicidal Bioassays of *Z. officinale* & *A. sativum* extracts against the fourth instar larvae of *Ae. aegypti*.

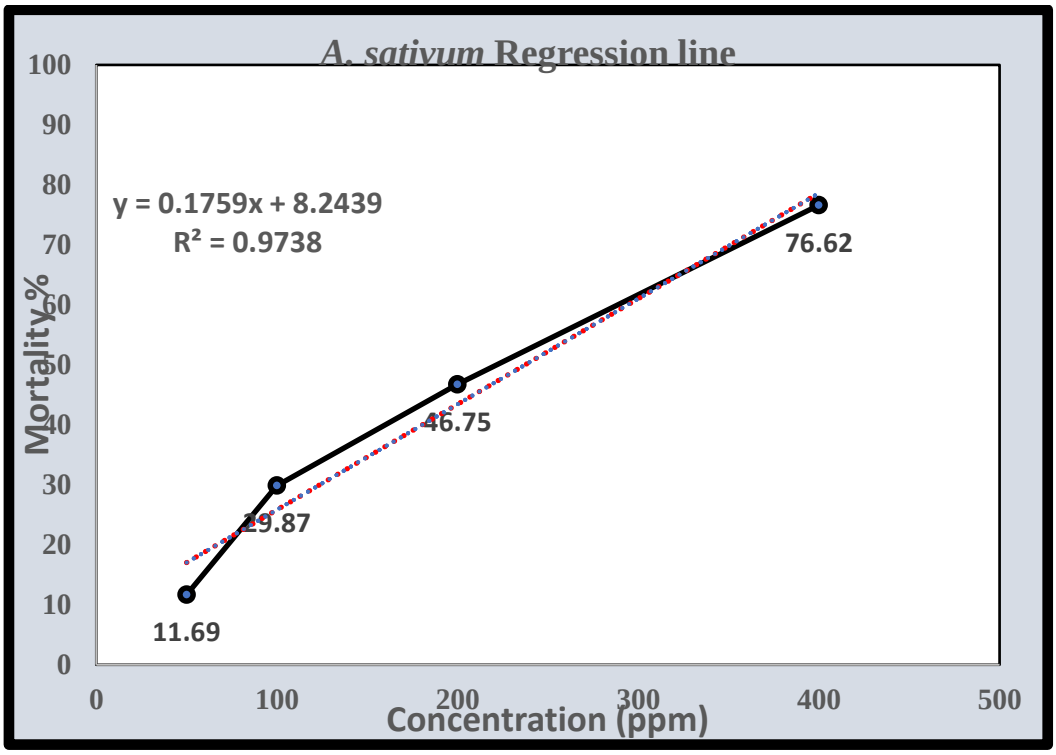

Fig. 2. Regression analysis of Larvicidal Bioassays of *A. sativum* extracts against the larvae of *Ae. aegypti*

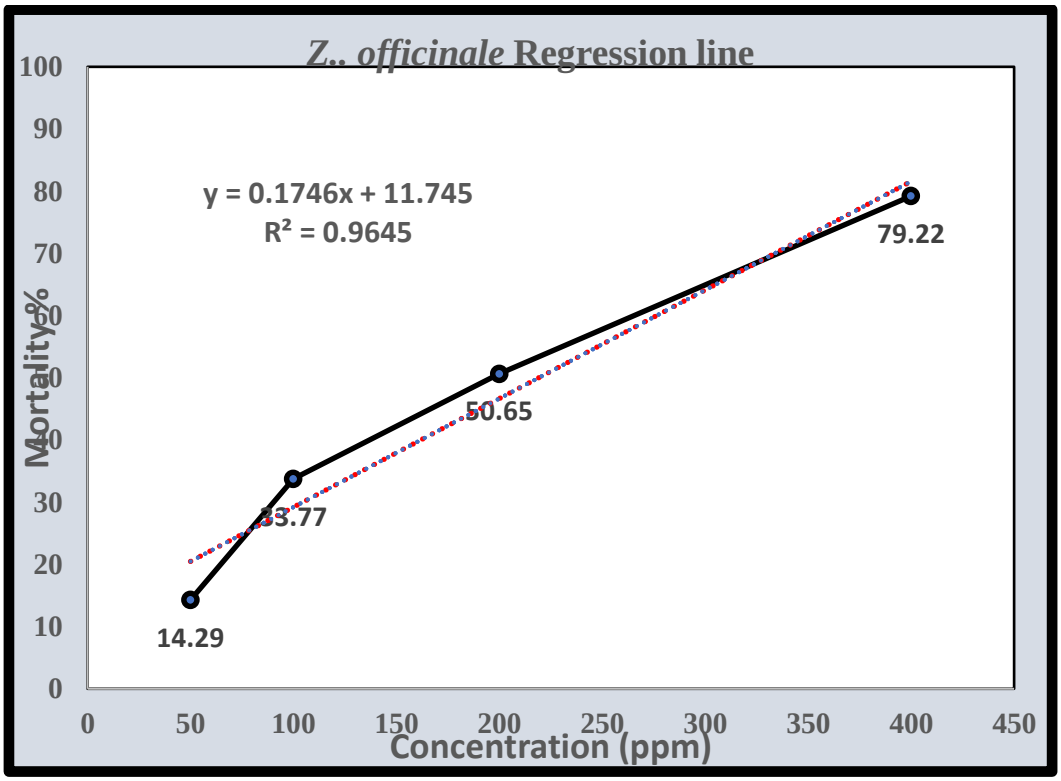

Fig. 3. Regression analysis of Larvicidal Bioassays of *Z. officinale* extracts against the larvae of *Ae. aegypti*

The results of previous studies support the present study results. Loni, et al. [15] tested the efficacy of *Z. officinale* essential oil vapours on the egg hatchability and larval and adult mortality of *Callosobruchus maculatus*. LC_{50} was 1.151, 2.336, and 2.183 µl/l air for the eggs, larvae, and adults, respectively. In other studies, active components identified in ginger extracts are zingiberene, shogaol, gingerol, curcumene, quercetin-3-O-rutinoside, quercetin-3-O-rutinoside, and dehydroshogaol [11,10,16]. The bioactive compounds of garlic extracts include organic sulfides, saponins, phenolic compounds, and polysaccharides. The group of sulfur compounds identified in garlic includes allicin (AC), alliin, S-allyl cysteine (SAC), diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl sulfide (DAS), and ajoene [17].

The bioactive compounds present in ginger extracts exhibit their effects by modifying the enzymatic actions in the host body. Ginger shoot extracts significantly inhibited the activities of pepsin, lipase, and α-amylase in aphids, while activating superoxide dismutase. Peroxidase and catalase activities initially increased but later decreased. Among detoxification enzymes, carboxylesterase activity was notably increased, acetylcholinesterase activity was significantly inhibited, and glutathione S-transferase activity first increased and then decreased [10]. The mode of action of garlic extracts is not fully understood, but it is believed to be related to the presence of various bioactive compounds such as allicin, alliin, S-allyl cysteine, diallyl disulfide, diallyl trisulfide, diallyl sulfide, and ajoene [18]. These extracts also possibly exert their pesticidal effects on the arthropod pests by interfering with the enzyme functioning.

In the present study, the test solutions of ginger and garlic exhibited larvicidal efficacy against the fourth instar larvae of *Ae. aegypti*. In light of the previous reports by Agarwal, et al. [11], Liu, et al. [10], Sinha & Ray [16], Bar, et al. [17], Liu, et al. [10], and Loni, et al. [15], the bioactive compounds present in the methanolic extracts of ginger and garlic are responsible for the larvicidal effects of these extracts against the fourth instar larvae of *Ae. aegypti*. These extracts interfere with the functioning of enzymes such as pepsin, lipase, α-amylase, peroxidase, catalase, carboxylesterase, acetylcholinesterase, and glutathione S-transferase and cause the death of the mosquito larvae.

4. CONCLUSION

The methanolic extracts of *Z. officinale* and *A. sativum* demonstrated significant larvicidal activity against the 4th instar larvae of *Ae. aegypti*, with a clear dose-response relationship. *Z. officinale* proved more potent, achieving higher mortality rates and a lower LC_{50} value compared to *A. sativum*. These results suggest that both extracts, particularly ginger, could serve as effective, environmentally sustainable alternatives for controlling *Ae. aegypti*, contributing to the broader efforts in vector control and disease prevention. To know the exact mechanism of action of these extracts, further studies are required.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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