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Larvicidal Efficacy of Sea Jellyfish (*Turritopsis dohrnii*) against *Aedes aegypti* Mosquito Larvae

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ABSTRACT

Background: The *Aedes aegypti* mosquito is a major vector for several viral diseases, including dengue fever, Zika virus, chikungunya and yellow fever, posing significant global health challenges. Traditional control methods rely heavily on chemical insecticides, leading to widespread resistance and environmental concerns.

Methods: This study explores the larvicidal potential of *Turritopsis dohrnii* jellyfish extract as a sustainable alternative for mosquito control. The extract was tested against fourth instar *Ae. aegypti* larvae at concentrations ranging from 1% to 10%.

Results: Mortality rates increased with both concentration and exposure time, with the highest concentration (10%) achieving 98.8% mortality after 24 hours. The LC_{50} value decreased from 22.50% at 2 hours to 2.06% at 24 hours, indicating increased potency over time. The results demonstrate that *T. dohrnii* extract is highly effective as a larvicide, with potential to reduce reliance on chemical insecticides. The findings suggest that *T. dohrnii* extract could be integrated into existing mosquito management programs, offering a novel and environmentally friendly approach to controlling *Ae. aegypti* populations. Further research is recommended to isolate the bioactive compounds responsible for this effect and to assess the extract's efficacy under field conditions.

Key words: *Aedes aegypti*, Jeddah, Mosquito, Sea Jellyfish, *Turritopsis dohrnii*.

INTRODUCTION

The *Aedes aegypti* mosquito is a principal vector for several severe viral diseases, including dengue fever, Zika virus, chikungunya and yellow fever. These diseases impose a substantial global health burden, particularly in tropical and subtropical regions where *Ae. aegypti* is endemic. The World Health Organization (WHO) estimates that dengue fever alone affects over 390 million people annually, with around 96 million experiencing symptomatic infections (WHO, 2023). The spread of these diseases has been exacerbated by rapid urbanization and climate change, which have expanded mosquito habitats and increased the frequency of outbreaks (Bhatt *et al*., 2013). Consequently, there is an urgent need for effective mosquito control strategies to manage and mitigate the spread of these vector-borne diseases.

Traditional mosquito control methods predominantly involve the use of chemical insecticides targeting either adult mosquitoes or their larvae in breeding sites. These chemicals, including organophosphates, carbamates and pyrethroids, have been effective in reducing mosquito populations and disease transmission (Zaim *et al*., 2000). However, the extensive and repeated use of these insecticides has led to significant challenges, including the development of resistance among mosquito populations and environmental contamination (Hemingway *et al*., 2020). Resistance mechanisms in *Ae. aegypti* are well-documented, with populations exhibiting resistance to multiple classes of insecticides, thus diminishing the effectiveness of conventional control efforts and necessitating higher doses or more frequent applications (Moyes *et al*., 2017). This resistance crisis underscores the need for alternative, sustainable control methods that can circumvent these issues.

Biological control methods, which utilize natural predators, pathogens, or compounds derived from natural sources, represent a promising alternative to chemical insecticides. These methods are generally considered safer for non-target organisms and can be integrated into existing vector management programs to enhance their efficacy (Benelli and Mehlhorn, 2016). Among the various sources of biological control agents, marine organisms have recently gained attention for their potential in mosquito management due to their diverse and unique bioactive compounds. One such organism is the sea jellyfish *Turritopsis dohrnii*.

The jellyfish *Turritopsis dohrnii*, commonly known as the "immortal jellyfish," has attracted interest due to its remarkable biological properties and potential for producing

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novel bioactive compounds. This species is known for its ability to revert to its juvenile form after reaching maturity, a unique trait that allows it to potentially produce a range of bioactive substances (Peyton *et al*., 2020). Recent research has suggested that jellyfish may produce compounds with insecticidal properties, making them potential candidates for mosquito control (Graham *et al*., 2021). Despite this potential, the larvicidal efficacy of *T. dohrnii* against *Ae. aegypti* larvae has not been extensively studied.

The application of marine-derived compounds in mosquito control is still relatively new, with limited research exploring their full potential. However, the unique chemical properties of these compounds, coupled with their relatively low toxicity to non-target species, make them attractive candidates for further investigation (Li *et al*., 2020). The larvicidal effects of *T. dohrnii* against *Ae. aegypti* larvae could provide valuable insights into the development of sustainable mosquito control strategies and contribute to reducing reliance on chemical insecticides.

This study aims to evaluate the larvicidal activity of *Turritopsis dohrnii* jellyfish extracts against *Aedes aegypti* mosquito larvae. By examining the mortality rates of larvae exposed to various concentrations of jellyfish extract, this research seeks to determine the efficacy of *T. dohrnii* as a potential biocontrol agent. Additionally, the study will investigate the mechanisms through which *T. dohrnii* extract affects larval mortality, contributing to a better understanding of its potential role in integrated mosquito management programs.

Understanding the larvicidal potential of *T. dohrnii* can pave the way for more sustainable mosquito control methods, particularly in areas where chemical control measures are becoming less effective due to resistance. The results of this study could also offer new insights into the broader application of marine-derived compounds in vector control, potentially expanding the range of tools available for combating mosquito-borne diseases.

MATERIALS AND METHODS

Collection and maintenance of *Ae. aegypti* larvae

Ae. aegypti larvae were collected from natural breeding sites in Jeddah, Saudi Arabia. The larvae were carefully gathered using a dipper and transferred to plastic containers filled with water from the collection sites. To ensure the integrity of the samples, the larvae were promptly transported to the laboratory, where they were maintained under controlled conditions. The laboratory environment was kept at a temperature of 27°C±2°C, with a relative humidity of 70%±5% and a 12:12 hour light-dark cycle, following World Health Organization (WHO) guidelines (WHO, 2005). The larvae were reared until they reached the third and fourth instar stages, which are considered most suitable for larvicidal bioassays due to their higher metabolic activity and sensitivity (Hemingway *et al*., 2020). During rearing, the larvae were fed a diet of finely ground fish food, provided twice daily, to ensure optimal growth and development.

Collection and preparation of *T. dohrnii* extract

Specimens of the jellyfish *T. dohrnii* were collected from coastal waters near Jeddah, Saudi Arabia. The jellyfish were harvested using hand nets to minimize damage and contamination. Upon collection, the specimens were rinsed with distilled water to remove any surface debris or contaminants. The cleaned specimens were then blotted dry with filter paper and immediately processed for extraction. To prepare the jellyfish extract, the specimens were first dried in an oven at 45°C for 48 hours. Once completely dehydrated, the dried jellyfish were ground into a fine powder using a mechanical grinder. The powdered material was stored in airtight containers at -20°C until further use to preserve the bioactive compounds (Li *et al*., 2020). For the extraction process, 100 grams of the dried jellyfish powder were mixed with 1 liter of 70% methanol (w/v) in a conical flask. The mixture was subjected to continuous stirring at room temperature for 48 hours to facilitate the extraction of bioactive compounds. After the extraction period, the mixture was filtered through Whatman No. 1 filter paper to remove solid residues. The methanol solvent was then evaporated under reduced pressure using a rotary evaporator set at 40°C. The resulting concentrated extract was further dried using a freeze dryer to obtain a fine powder, which was stored at -20°C until use in the larvicidal bioassays (Xie *et al*., 2023).

Larvicidal bioassay

The larvicidal activity of *T. dohrnii* extract against *Ae. aegypti* 4th larval stage was evaluated according to the WHO standard procedures for testing mosquito larvicides (WHO, 2005). The dried jellyfish extract was reconstituted in distilled water to prepare a series of concentrations: 1, 2, 3, 5, 7 and 10% (w/v). Each concentration was prepared by dissolving the appropriate amount of extract in 100 mL of distilled water in separate disposable plastic cups. For each bioassay, 30 fourth instar *Ae. aegypti* larvae were introduced into each cup containing the different concentrations of the jellyfish extract. A control group was maintained using distilled water without the extract. All treatments, including the control, were conducted in triplicate to ensure statistical reliability. The larvae were exposed to the jellyfish extract for 24 hours, during which no additional food was provided. Mortality was recorded at 4, 6, 12 and 24-hours post-exposure. Larvae were considered dead if they showed no movement when probed with a needle (Finney, 1971). The percentage of larval mortality was calculated for each concentration and the data were corrected for control mortality using Abbott's formula (Abbott, 1925).

Statistical analysis

The lethal concentrations required to kill 50% (LC_{50}) and 90% (LC_{90}) of the exposed larvae were calculated using

probit analysis. The mortality data were transformed to probits and plotted against the logarithm of the extract concentration to obtain a dose-response curve. The LC_{50} and LC_{90} values, along with their 95% confidence intervals, were estimated using SPSS software version 25.0. This analysis provided a measure of the larvicidal potency of the *T. dohrnii* extract (Finney, 1971). The data from the larvicidal assays were analyzed using one-way analysis of variance (ANOVA) to determine the significance of differences in mortality rates between the different concentrations of *T. dohrnii* extract. Tukey's post-hoc test was applied for multiple comparisons to identify specific differences between the treatment groups. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software version 9.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Table 1 summarizes the mortality rates of *Ae. aegypti* larvae exposed to different concentrations of *T. dohrnii* extract over time. At the lowest concentration (1%), the mortality rate was 32.2% after 24 hours, while the highest concentration (10%) resulted in 98.8% mortality. The control group, which was exposed to distilled water, showed no mortality throughout the experiment, confirming that the observed effects were due to the *T. dohrnii* extract. The concentration-dependent increase in mortality is evident, with higher concentrations leading to greater larval death across all time points. For instance, at 2 hours post-exposure, the mortality rate ranged from 3.3% at 1% concentration to 32.2% at 10% concentration. By 24 hours, mortality had significantly increased across all concentrations, with the highest concentration nearly achieving complete larval mortality (98.8%). This trend suggests that *T. dohrnii* extract exhibits potent larvicidal properties that are both dose- and time-responsive.

Fig 1 summarizes the LC_{50} and LC_{90} values, which represent the concentrations required to kill 50% and 90% of the exposed larvae, respectively, were calculated for each time point (Table 1). The LC_{50} value decreased from 22.50% at 2 hours to 2.06% at 24 hours, indicating increased potency over time. Similarly, the LC_{90} value also decreased, from 197.5% at 2 hours to 9.50% at 24 hours. These decreasing LC_{50} and LC_{90} values over time reflect the cumulative toxic effect of the extract, with prolonged exposure leading to higher mortality at lower concentrations. The slope of the concentration-response curve was consistent across the time points, with values ranging from 1.08±0.1 at 12 hours to 1.9±0.1 at 24 hours. A steeper slope, as observed at 24 hours, indicates a more rapid increase in mortality with increasing concentration, further supporting the effectiveness of *T. dohrnii* extract as a larvicide.

The resistance ratio (RR) and index values, which were calculated relative to the 24-hour time point, provide additional insight into the extract's efficacy (Table 1). The RR values decreased from 10.8 at 2 hours to 1 at 24 hours, suggesting that the larvae become increasingly susceptible to the extract over time. The index values, which measure the relative efficacy of the extract compared to the 24-hour exposure, showed a similar trend, with a value of 100 at 24 hours, confirming the extract's maximum effectiveness at this time point.

The results of this study clearly demonstrate the larvicidal potential of *T. dohrnii* extract against *Ae. aegypti* larvae, with significant mortality observed even at relatively low concentrations and short exposure times. The concentration- and time-dependent mortality patterns observed align with previous studies that have explored

Table 1: Larvicidal activity of *T. dohrnii* extract *against Ae. aegypti* after different time exposure hours.

Concentrations (%)	Mortality (%)			
	2	4	12	24
1	3.3	8.8	21.1	32.2
2	7.7	14.4	27.7	46.6
3	12.2	21.1	35.5	61.1
5	17.7	27.7	45.5	68.8
7	24.4	37.7	54.4	82.2
10	32.2	44.4	58.8	98.8
Control	0.0	0.0	0.0	0.0
LC_{50} (Lower limit-Upper limit)	22.50 (14.8-48.1)	13.3 (9.6-22.4)	6.1 (4.9-8.5)	2.06 (1.2-2.7)
LC_{90} (Lower limit-Upper limit)	197.5 (79.2-1115)	141.3 (63-599.8)	92.7 (43.8-357.2)	9.50 (7.6-22.3)
Slope	1.3±0.2	1.2±0.1	1.08±0.1	1.9±0.1
Calculated	9.5	9.5	9.5	9.5
Tubulated	0.8	0.8	0.8	0.8
RR	10.8	6.4	2.9	1
Index	9.1	15.5	33.4	100

RR: resistance ratio compared to 24 h. Index compared with 24 h.

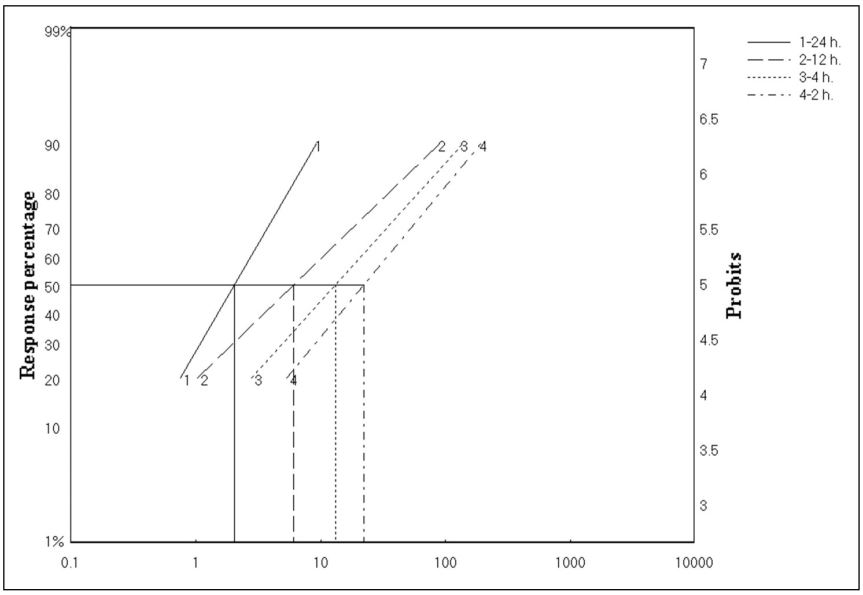

Fig 1: LDP lines of *T. dohrnii* larvicidal activity *against Ae. aegypti* after different time exposure hours.

the use of marine-derived compounds for mosquito control. For instance, marine invertebrates have been shown to produce bioactive compounds with strong insecticidal properties, which may act by disrupting larval cell membranes, leading to osmotic imbalance and eventual death (Li *et al*., 2020; Xie *et al*., 2023). The decreasing LC_{50} and LC_{90} values over time suggest that prolonged exposure to *T. dohrnii* extract enhances its larvicidal effect, making it a promising candidate for integrated mosquito management programs. This is particularly relevant in the context of increasing resistance to conventional chemical insecticides, which has become a major challenge in mosquito control efforts globally (Hemingway *et al*., 2020). The ability of *T. dohrnii* extract to induce high mortality at lower concentrations after extended exposure may offer a viable alternative to chemical insecticides, reducing the risk of resistance development and minimizing environmental impact. The RR and index values further support the extract's efficacy, indicating that, *Ae. aegypti* larvae do not develop significant resistance to the extract over the short term. In general, *T. dohrnii* may produce bioactive compounds such as Nematocyst toxins which produced from their stinging cells with larvicidal properties through its natural defense mechanisms. These compounds likely target the larvae's nervous or digestive systems, disrupting key metabolic functions. Proteins, peptides, or toxins from *T. dohrnii* might inhibit enzyme activity, damage cellular structures, or interfere with neurotransmission, ultimately leading to mosquito larval death. However, specific studies identifying and characterizing these bioactive compounds are limited and more research is needed to pinpoint their exact mode of action against mosquito larvae. However, long-term studies are needed to fully assess the potential for resistance development and to determine the optimal application strategies for field use. The findings of this study have significant implications for public health, particularly in regions where *Ae. aegypti* is endemic and chemical control methods are becoming less effective. The high larvicidal activity of *T. dohrnii* extract, combined with its potential environmental safety, makes it a promising candidate for inclusion in integrated mosquito management programs. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the larvicidal activity observed, as well as conducting field trials to evaluate the efficacy of *T. dohrnii* extract under real-world conditions.

CONCLUSION

The study demonstrates the potent larvicidal effects of *T. dohrnii* extract against *Ae. aegypti* larvae, showing concentration- and time-dependent mortality. The highest concentration (10%) led to nearly complete larval death (98.8%) within 24 hours, while even the lowest concentration (1%) caused significant mortality (32.2%). Decreasing LC_{50} and LC_{90} values over time indicate increasing potency with prolonged exposure and the resistance ratio values suggest no significant resistance development. These findings highlight the extract's potential as a natural alternative to chemical insecticides, particularly considering rising resistance to conventional methods. Further research is necessary to isolate the bioactive compounds responsible and evaluate the extract's effectiveness in field applications. This study suggests that *T. dohrnii* extract could be a valuable tool in integrated mosquito management programs, offering an environmentally friendly solution to combatting *Aedes aegypti* populations.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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