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Biological Control of *Aedes aegypti* Larvae using Sea Shell Powder

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Abstract

Background: The *Aedes aegypti* mosquito is a primary vector for several arboviral diseases, including dengue, Zika, chikungunya and yellow fever, posing significant public health challenges, especially in tropical and subtropical regions. Traditional chemical insecticides have been the mainstay for controlling *Aedes aegypti* populations, but their extensive use has led to the development of insecticide resistance, environmental pollution and adverse effects on non-target organisms. This study explores the potential of sea shell powder as a natural larvicide against *Aedes aegypti* larvae.

Methods: Sea shell powder, derived from the calcified exoskeletons of marine mollusks, was tested for its larvicidal activity against fourth instar larvae of *Ae. aegypti* at concentrations ranging from 100 to 600 ppm.

Result: The results demonstrated a concentration- and time-dependent increase in larval mortality, with LC_{50} values of 407.01 ppm at 24 hours and 327.55 ppm at 48 hours. The findings suggest that sea shell powder could serve as an effective, environmentally sustainable alternative to chemical insecticides, offering advantages such as biodegradability, low cost and reduced risk of resistance development. This study supports the potential inclusion of sea shell powder in integrated mosquito management programs, particularly in areas with prevalent insecticide resistance.

Key words: *Aedes aegypti*, Biological control, Larvae, Sea shell powder.

Introduction

The *Aedes aegypti* mosquito, a principal vector for several arboviral diseases, including dengue fever, Zika virus, chikungunya and yellow fever, poses significant public health challenges, particularly in tropical and subtropical regions. These diseases result in high morbidity and mortality rates globally, particularly in areas with dense human populations and favorable breeding conditions for mosquitoes (WHO, 2017). Given the public health impact of *Ae. aegypti*, effective control measures are crucial for reducing the incidence of these vector-borne diseases.

Traditional methods for controlling *Ae. aegypti* populations have largely relied on the use of chemical insecticides. These chemicals are effective at reducing mosquito populations; however, their extensive use has led to several significant challenges, including the development of insecticide resistance, environmental pollution and negative impacts on non-target organisms (Hemingway and Ranson, 2000). For instance, resistance to commonly used insecticides such as pyrethroids, organophosphates and carbamates has been documented in *Ae. aegypti* populations across different geographical regions (Vontas *et al.*, 2012). This resistance diminishes the efficacy of chemical control strategies and underscores the need for alternative, more sustainable approaches to mosquito management.

In response to these challenges, there has been a growing interest in the use of biological control methods as environmentally friendly alternatives to chemical insecticides. Biological control involves the use of natural enemies or substances derived from natural sources to suppress mosquito populations. This approach is considered more sustainable and less harmful to non-target species and ecosystems. Among the various biological control agents explored, natural products derived from marine organisms have shown great promise due to their rich diversity of bioactive compounds with insecticidal properties (Blunt *et al.*, 2018).

One such promising natural product is sea shell powder, derived from the calcified exoskeletons of marine mollusks. Sea shells are composed primarily of calcium carbonate ($CaCO_3$), a compound known for its wide range of applications, including in agriculture, medicine and industry (Mann, 2001). The use of sea shell powder in pest management is based on its potential to act as a physical and chemical barrier to insect development. Studies have shown that the abrasive nature of calcium carbonate can damage the cuticle of insects, leading to dehydration and death (Subramanian and Shrinivasa, 2007). Additionally, calcium carbonate may interfere with the physiological

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processes of insect larvae, including respiration and molting, further contributing to its insecticidal effects (Kamel *et al.*, 2018).

The use of sea shell powder as a larvicide offers several advantages over conventional chemical insecticides. First, it is a natural product that is readily available and biodegradable, making it an environmentally friendly option. Second, sea shell powder is relatively inexpensive and can be produced locally, reducing the reliance on imported chemical insecticides. Third, unlike synthetic chemicals, sea shell powder is unlikely to contribute to the development of resistance in mosquito populations, as its mode of action is primarily physical rather than chemical (Isman, 2006). Furthermore, the use of sea shell powder aligns with the principles of integrated pest management (IPM), which advocates for the use of multiple control strategies to achieve sustainable pest management (Ehler, 2006).

Despite the potential benefits of using sea shell powder as a biological control agent, research on its efficacy against *Ae. aegypti* larvae is still in its early stages. Previous studies have explored the use of calcium carbonate and other mineral powders against various insect pests, but few have specifically focused on Aedes aegypti (Kamel *et al.*, 2018). This research aims to fill this gap by investigating the larvicidal activity of sea shell powder against *Ae. aegypti* larvae. By evaluating the mortality rates of mosquito larvae exposed to different concentrations of sea shell powder, this study seeks to determine the potential of this natural product as a biocontrol agent.

Moreover, this study will explore the mechanisms by which sea shell powder exerts its larvicidal effects. Understanding these mechanisms is essential for optimizing the use of sea shell powder in mosquito control programs. For instance, the particle size of the powder, the method of application and the environmental conditions may all influence its efficacy. Additionally, this research will assess the potential non-target effects of sea shell powder, ensuring that its use does not negatively impact other organisms in the ecosystem.

The outcomes of this study could contribute to the development of new, sustainable approaches to mosquito control, particularly in regions where resistance to conventional insecticides is a growing concern. By providing scientific evidence on the efficacy and safety of sea shell powder as a larvicide, this research could support its inclusion in IPM programs and help reduce the reliance on chemical insecticides. Ultimately, this study aims to advance the field of biological control and contribute to the global effort to control vector-borne diseases through environmentally sustainable methods.

MATERIALS AND METHODS

Collection and preparation of sea shell powder

Sea shells were sourced from local fisheries in Jeddah, Saudi Arabia, where they were collected from Al-Cornish region from February to June. The collected shells were thoroughly cleaned with fresh water to remove any organic matter and impurities as in Fig 1. After cleaning, the shells were dried at 60°C for 24 hours to ensure complete removal of moisture (Mann, 2001). Following drying, the shells were ground into a fine powder using a laboratory grinder (Moulinex Grinder Orange 180W) until a particle size of less than 100 micrometers was achieved. The powdered sea shells were then sieved through a 100-mesh screen to ensure uniform particle size distribution and stored in airtight containers at room temperature until use (Smith and Jones, 2019).

Fig 1: Sea shell.

Preparation of sea shell powder suspensions

The sea shell powder was suspended in dechlorinated tap water to prepare stock solutions at different concentrations. Stock solutions of 1 g/L were prepared by adding 1 g of sea shell powder to 1 liter of dechlorinated water and stirred continuously for 1 hour to ensure complete dispersion. The suspension was then filtered through a Whatman No. 1 filter paper to remove undissolved particles, resulting in a clear solution used for further dilutions. For the larvicidal bioassay, various concentrations of the sea shell powder suspension were prepared by serial dilution, ranging from 100 to 600 ppm (parts per million) (Mann, 2001).

Rearing of *Ae. aegypti* larvae

Ae. aegypti larvae were obtained from a laboratory colony maintained by the Dengue Research and Control Unit, Jeddah, King Abdulaziz University. The larvae were reared in plastic trays filled with dechlorinated tap water under controlled conditions of 27 ± 2°C, 75 ± 5% relative humidity and a 12:12 light photoperiod (WHO, 2005). The larvae were fed daily with finely ground fish food (TetraMin®) until they reached the fourth instar stage, which was used for the larvicidal assays (Jansen and Lounibos, 2001).

Larvicidal bioassay

The larvicidal activity of sea shell powder was evaluated using a standard bioassay procedure adapted from the World Health Organization (WHO) guidelines for testing larvicides (WHO, 2005). Groups of 20 fourth instar *Ae. aegypti* larvae were introduced into 250 mL glass beakers containing 100 mL of the sea shell powder suspension at various concentrations (100, 200, 300, 400, 500 and 600 ppm). Each concentration was tested in triplicate, along with a control group containing only dechlorinated water. The beakers were maintained at room temperature (27 ± 2°C) throughout the experiment. Larval mortality was recorded at 24 and 48 hours after exposure. Larvae were considered dead if they did not respond to gentle prodding with a fine brush (Abbott, 1925).

Data analysis

The percentage of larval mortality was calculated for each concentration. Lethal concentration (LC_{50}) values, the concentrations required to kill 50% of the larvae, were estimated using probit analysis. This analysis provides a reliable estimate of the concentration-mortality relationship (Finney, 1971). The slope of the probit line was also determined to assess the dose-response relationship. All statistical analyses were performed using SPSS software (version 25.0) (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The results showed a clear concentration- and time-dependent increase in larval mortality, indicating that sea shell powder possesses larvicidal properties that can be effective against *Ae. aegypti* larvae (Table 1). The concentration range of 100 to 600 ppm ensures a gradient to evaluate varying larvicidal efficacy of the sea shell powder. At 100 ppm, mortality rates were relatively low, with 3.33% mortality observed after 24 hours, increasing slightly to 8.33% after 48 hours. As the concentration increased to 600 ppm, the mortality rates rose significantly, reaching 86.66% at 24 hours and 96.66% at 48 hours. The data suggest that sea shell powder can induce substantial mortality in *Ae. aegypti* larvae, especially at higher concentrations. The absence of mortality in the control group confirms that the observed larvicidal effects were directly attributable to the sea shell powder, rather than other environmental factors. This supports the potential use of sea shell powder as a natural larvicidal agent, aligning with the growing interest in environmentally sustainable methods for vector control (Isman, 2006). The lethal concentration required to kill 50% of the larvae (LC_{50}) was calculated using probit analysis. After 24 hours, the LC_{50} was determined to be 407.01 ppm, which decreased to 327.55 ppm after 48 hours. This reduction in LC_{50} over time indicates that prolonged exposure to sea shell powder enhances its larvicidal efficacy. The LC_{50} values are consistent with those reported for other natural larvicides, demonstrating that sea shell powder is a promising candidate for mosquito control (Rao *et al.*, 2018). The slope of the probit line, which describes the dose-response relationship, was found to be 3.98 ± 0.34 at 24 hours and 3.71 ± 0.29 at 48 hours. These relatively steep slopes suggest a strong correlation between the concentration of sea shell powder and the mortality of *Ae. aegypti* larvae. A steeper slope indicates that small increases in concentration result in significant increases in mortality, which is a desirable characteristic for an effective larvicide (Finney, 1971).

The larvicidal activity of sea shell powder compares favorably with other natural larvicides. For instance, previous studies have demonstrated the effectiveness of plant-based larvicides, such as neem oil and eucalyptus extract, which have similar LC_{50} values against *Ae. aegypti* larvae (Benelli *et al.*, 2016). The comparable efficacy of sea shell powder highlights its potential as a viable alternative to chemical insecticides, particularly in regions where insecticide resistance is a growing concern. The exact mechanism by which sea shell powder exerts its larvicidal effects is not fully understood, but it is likely related to its physical and chemical properties. The powder may cause physical abrasions to the larvae's cuticle, leading to desiccation and death. Additionally, the mineral content of the shells, including calcium carbonate, may disrupt the larvae's osmoregulatory processes, further contributing to mortality (Derby, 2007). The use of sea shell powder as a larvicidal agent offers several environmental advantages over synthetic insecticides. Being a natural product, it is biodegradable and poses minimal risk to non-target organisms and the environment. The widespread availability of sea shells as a byproduct of the seafood industry also makes this approach economically viable and sustainable (Blunt *et al.*, 2018). The findings of this study indicate that sea shell powder is an effective natural larvicide against *Ae. aegypti* larvae, with significant mortality observed at higher concentrations. The reduction in LC_{50} over time suggests that sea shell powder's efficacy increases with prolonged exposure. These results support further exploration of sea shell powder as a component of integrated mosquito management strategies, particularly in areas where resistance to conventional insecticides is prevalent. Future research should focus on field trials to assess the practical applicability of sea shell powder in real-world mosquito habitats. Additionally, studies investigating the specific compounds and mechanisms responsible for its larvicidal activity could lead to the development of more

Table 1: Larvicidal toxicity of sea shell powder against *Ae. aegypti* 4th larval stage.

Concentrations (ppm)	Mortality (%)	
	24 h.	48 h.
100	3.33	8.33
200	11.66	20.00
300	23.33	31.66
400	38.33	50.66
500	63.33	78.33
600	86.66	96.66
Control	0.0	0.0
LC_{50}	407.01	327.55
Slope	3.98±0.34	3.71±0.29
Calculated	9.5	9.5
Tubulated	0.8	0.8

refined and potent formulations. In general, sea shell powder could be integrated into mosquito control programs by applying it to breeding sites, creating larvicidal traps, or mixing it with soil in stagnant water areas. Its natural composition could serve as an eco-friendly alternative, particularly in regions facing insecticide resistance, reducing dependency on chemical insecticides.

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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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