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Larvicidal Toxicity of *Octopus cyanea* Ink against *Aedes aegypti* Larvae

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ABSTRACT

Background: The *Aedes aegypti* mosquito is a key vector for serious diseases such as dengue, Zika, chikungunyaand yellow fever, necessitating effective control measures. Conventional chemical insecticides face challenges such as insecticide resistance and environmental impact, prompting the exploration of alternative strategies. This study investigates the larvicidal potential of *Octopus cyanea* ink against *Ae. aegypti* larvae as a natural biocontrol agent.

Methods: *O. cyanea* ink, known for its complex bioactive compounds, was tested against fourth instar larvae of *Ae. aegypti* after 24 and 48 hours of exposure at different concentrations ranged between 100-600 ppm.

Result: The study revealed a dose- and time-dependent increase in larval mortality, with the highest concentration (600 ppm) causing up to 98.33% mortality within 48 hours. Probit analysis indicated LC_{50} values of 311.31 ppm at 24 hours and 180.11 ppm at 48 hours, suggesting increased efficacy over time. The findings underscore the potential of *O. cyanea* ink as an eco-friendly, natural larvicide, offering a promising alternative to synthetic chemicals. The study highlights the need for further research, including field trials and chemical characterization of the ink, to develop new sustainable approaches for mosquito control and to combat the growing issue of insecticide resistance in *Ae. aegypti* populations.

Key words: *Aedes aegypti*, Biological control, Larvicidal, *Octopus cyanea* ink, Toxicity.

INTRODUCTION

The *Ae. aegypti* mosquito is a well-known vector for several debilitating diseases, including dengue, Zika, chikungunya and yellow fever. These diseases are responsible for significant morbidity and mortality across tropical and subtropical regions, where *Ae. aegypti* populations thrive (WHO, 2017). The control of *Ae. aegypti* is thus a critical public health objective, with the goal of reducing the incidence of these vector-borne diseases. Conventional methods for controlling *Ae. aegypti* populations primarily involve the use of chemical insecticides. However, the over-reliance on these chemicals has led to several challenges, including the development of insecticide resistance and negative impacts on non-target organisms and the environment (Hemingway and Ranson, 2000).

In recent years, the development of insecticide resistance in *Ae. aegypti* populations has become a significant obstacle to effective mosquito control. For example, widespread resistance to pyrethroids, one of the most used classes of insecticides, has been reported in several regions (Vontas *et al*., 2012). This resistance diminishes the effectiveness of chemical control measures and necessitates the exploration of alternative strategies that are both effective and environmentally sustainable. As a result, there has been a growing interest in the use of natural products as potential larvicidal agents. Natural products, particularly those derived from marine organisms, offer a promising alternative due to their rich diversity of bioactive compounds (Blunt *et al*., 2018).

Marine organisms are a prolific source of unique bioactive compounds, many of which have been shown to possess potent insecticidal properties (Davidson, 2015).

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Among these organisms, cephalopods, including octopuses, are known to produce various biologically active substances, including ink, which is traditionally used as a defense mechanism against predators. The ink of cephalopods, particularly that of *Octopus cyanea*, has been of interest due to its complex chemical composition, which includes melanin, proteins, enzymes and other organic compounds (Derby, 2007). These compounds have been studied for their antimicrobial, anticancer and antioxidant properties (Prota, 2012; Derby, 2007). However, the potential of cephalopod ink as a larvicidal agent against mosquito larvae remains largely unexplored.

Octopus cyanea, commonly known as the big blue octopus or day octopus, is a species native to the Indo-Pacific region, including the coastal waters of the Indian Ocean. This species is well-known for its behavior, adaptability and the characteristic dark ink it releases as a defensive response. The ink of *O. cyanea* contains a variety of bioactive compounds that have been demonstrated to

have potential medicinal and ecological applications (Derby, 2007). Given the chemical richness of octopus ink and the pressing need for new larvicidal agents, it is hypothesized that *O. cyanea* ink may possess larvicidal properties that could be effective against *Ae. aegypti* larvae. The potential use of *O. cyanea* ink as a larvicidal agent is of particular interest because it represents an environmentally sustainable approach to mosquito control. Unlike synthetic chemical insecticides, natural products are often biodegradable and have less harmful effects on non-target species and ecosystems (Isman, 2006). Moreover, the use of marine-derived products could offer a novel solution to the problem of insecticide resistance, as these compounds often have unique modes of action that differ from conventional insecticides (Rao *et al*., 2018).

This study aims to investigate the larvicidal toxicity of *Octopus cyanea* ink against the larvae of *Ae. aegypti*. By evaluating the mortality rates of *Ae. aegypti* larvae exposed to varying concentrations of *O. cyanea* ink, this research seeks to determine the efficacy of the ink as a potential biocontrol agent. Additionally, the study will explore the chemical composition of the ink to identify the specific bioactive compounds responsible for its larvicidal activity. Understanding the mechanisms by which *O. cyanea* ink affects mosquito larvae could provide valuable insights into the development of new, natural larvicides that are effective against *Ae. aegypti* and other mosquito species.

The outcomes of this research could contribute to the diversification of mosquito control strategies, particularly in areas where resistance to conventional insecticides is a significant concern. By identifying and characterizing the larvicidal properties of *Octopus cyanea* ink, this study aims to advance the field of biological control and offer new tools for managing mosquito-borne diseases. Furthermore, the exploration of marine-derived natural products aligns with the broader goal of developing sustainable, environmentally friendly approaches to pest management, ultimately contributing to the global effort to control vector-borne diseases.

MATERIALS AND METHODS

Collection and preparation of *Octopus cyanea* Ink

Specimens of *Octopus cyanea* was collected from the commercial markets of Jeddah, Saudi Arabia. In the laboratory, the octopuses were gently stimulated to release their ink by simulating a predatory threat using a soft probe (Derby, 2007). The released ink was immediately collected into sterile glass containers. To ensure the purity of the ink, it was filtered through a double layer of muslin cloth to remove any solid particles or debris. The filtered ink was then stored at -20°C until further use (Kim *et al*., 2013).

Preparation of ink extracts

The ink was subjected to a solvent extraction process to isolate the active compounds. A volume of 100 mL of ink was mixed with an equal volume of 100% methanol in a glass beaker. The mixture was then subjected to continuous stirring for 24 hours at room temperature to ensure thorough extraction. Following the extraction, the mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract from the solid residue. The filtrate was concentrated under reduced pressure using a rotary evaporator set at 40°C until a dark, viscous crude extract was obtained (Rao *et al*., 2018). The crude extract was stored at -20°C until further analysis.

Rearing of *Ae. aegypti* larvae

Eggs of *Ae. aegypti* were sourced from a laboratory colony maintained by the Dengue Research and Control Unit, Jeddah. The eggs were hatched in plastic trays filled with dechlorinated tap water and maintained under controlled environmental conditions at 27±2°C, 75±5% relative humidityand a photoperiod of 12:12 (light: dark) hours (WHO, 2005). The hatched larvae were fed with finely ground fish food (TetraMin®) and reared to the fourth instar stage, which was used for the larvicidal bioassays.

Larvicidal bioassay

The larvicidal activity of *O. cyanea* ink was evaluated using the standard protocol recommended by the World Health Organization (WHO) for testing mosquito larvicides (WHO, 2005). Stock solutions of the crude ink extract were prepared by dissolving the extract in dimethyl sulfoxide (DMSO) and further diluting it with dechlorinated water to obtain concentrations ranging from 100 to 600 ppm. Control solutions were prepared with dechlorinated water containing 1% DMSO.

For the bioassay, groups of 20 fourth instar *Ae. aegypti* larvae were introduced into 250 mL glass beakers containing 100 mL of the test solutions. Each concentration was tested in triplicate, along with a control group. The beakers were maintained at room temperatureand larval mortality was recorded after 24 and 48 hours of exposure. Larvae were considered dead if they did not respond to gentle prodding with a fine brush (Abbott, 1925). The percentage of larval mortality was calculated for each concentrationand the data were corrected for control mortality using Abbott's formula (Abbott, 1925). The lethal concentrations (LC_{50}) required to kill 50% of the larvae were determined using probit analysis, which provides a reliable estimate of the concentration-mortality relationship (Finney, 1971).

Statistical analysis

The experimental data from the larvicidal bioassay were analyzed using SPSS software (version 25.0). The probit analysis was also performed using SPSS to calculate the LC_{50} values and their respective confidence limits. The slope of the probit line was determined to understand the relationship between the concentration of the ink extract and the mortality rate of the larvae (Finney, 1971).

RESULTS AND DISSCUSSION

The larvicidal toxicity of *Octopus cyanea* ink against fourth instar larvae of *Ae. aegypti* was assessed at six concentrations (100-600 ppm) over 24 and 48 hours, with mortality rates presented in Table 1. The results show a clear dose- and time-dependent increase in larval mortality, confirming the potential of *O. cyanea* ink as a larvicidal agent. Mortality rates ranged from 21.66% at 100 ppm to 83.33% at 600 ppm after 24 hours of exposure and from 36.66% to 98.33% after 48 hours. No mortality was observed in the control group during the experiment, indicating that the observed effects were due to the active compounds in the ink. At the lowest concentration (100 ppm), mortality was limited to 21.66% after 24 hours and 36.66% after 48 hours. However, as the concentration increased to 600 ppm, the mortality rates increased substantially to 83.33% at 24 hours and 98.33% at 48 hours. The results indicate that *O. cyanea* ink exhibits significant larvicidal activity, with higher concentrations being more effective at inducing larval death. The dose-dependent response observed is consistent with other studies investigating marine-derived natural products for mosquito control (Rao *et al*., 2018).

The LC_{50} (lethal concentration for 50% mortality) values for *O. cyanea* ink were calculated using probit analysis, which provides a reliable estimate of the concentration required to kill 50% of the larvae. After 24 hours, the LC_{50} was calculated as 311.31 ppm, while after 48 hours, the LC_{50} dropped to 180.11 ppm, indicating a higher larvicidal efficacy over time. These values suggest that *O. cyanea* ink is more effective when larvae are exposed for longer durations, which could be due to the accumulation of active compounds or their prolonged interaction with the larvae's physiological systems (Finney, 1971).

The confidence intervals for the LC50 values ranged from 208.98 to 460.93 ppm at 24 hours and from 81.74 to 228.38 ppm at 48 hours. These intervals are relatively wide at 24 hours, indicating some variability in the effectiveness of the ink at lower concentrations. However, after 48 hours, the confidence intervals narrow significantly, reflecting a more consistent larvicidal effect over time. The slope values were 2.03±0.21 at 24 hours and 2.35±0.22 at 48 hours, indicating a steep dose-response relationship, especially after 48 hours. A steeper slope suggests a more pronounced increase in mortality with increasing concentrations, which is desirable for effective pest control (Ghosh *et al*., 2012). The larvicidal activity of *O. cyanea* ink compares favorably with other natural larvicides, particularly those derived from marine organisms. Previous studies have reported the larvicidal efficacy of other cephalopod inks, such as those from *Sepia officinalis* and *Loligo vulgaris*, which exhibited significant mortality against mosquito larvae at similar concentrations (Kim *et al*., 2013; Rao *et al*., 2018). The LC_{50} values observed for *O. cyanea* ink in this study are comparable to those of other natural larvicides, including plant extracts such as *Azadirachta indica* (neem) and *Ageratum conyzoides*, which have shown LC_{50} values ranging from 200 to 350 ppm against *Ae. aegypti* larvae (Benelli *et al*., 2016). These results highlight the potential of marine-derived biocontrol agents, such as *O. cyanea* ink, as alternatives to synthetic insecticides. The larvicidal activity of *O. cyanea* ink is likely due to its bioactive compounds, which may include melanin, tyrosinaseand other secondary metabolites with antimicrobial and insecticidal properties. Melanin, a key component of cephalopod ink, has been shown to disrupt cellular processes in insect larvae, leading to paralysis and death (Derby, 2007). Additionally, other compounds present in the ink, such as free amino acids and peptides, may interfere with larval metabolism and development, further contributing to the observed mortality (Kim *et al*., 2013). The exact mechanism of action of *O. cyanea* ink against mosquito larvae remains to be fully elucidated, but it is likely a combination of physical and chemical effects. The ink may form a physical barrier on the larvae, impairing their ability to respire and move, while the bioactive compounds disrupt critical metabolic pathways. Further phytochemical analysis and toxicological studies are needed to isolate and identify the specific compounds responsible for the larvicidal effects observed in this study (Rao *et al*., 2018). The use of *O. cyanea* ink as a larvicidal agent offers several advantages over conventional chemical insecticides. Unlike synthetic insecticides, which often have broad-spectrum toxicity and can harm non-target species, *O. cyanea* ink is a natural product with a more selective mode of action. This selectivity reduces the risk of environmental contamination and adverse effects on beneficial organisms, making it a more sustainable option for mosquito control (Blunt *et al*., 2018). Additionally, the ink is biodegradable and readily available from natural populations of *O. cyanea*, which are abundant in tropical and subtropical waters. However, further research is needed to evaluate the practical applicability of *O. cyanea*

Table 1: Larvicidal toxicity of *O. cyanea* Ink against *Ae. aegypti* 4th larval stage.

Concentrations (ppm)	Mortality (%)	
	24 h.	48 h.
100	21.66	36.66
200	31.66	46.66
300	41.66	60.00
400	50.00	73.33
500	66.66	88.33
600	83.33	98.33
Control	0.0	0.0
LC_{50}	311.31	180.11
Lower limit	208.98	81.74
Upper limit	460.93	228..38
Slope	2.03±0.21	2.35±0.22
Calculated	9.5	9.5
Tubulated	0.8	0.8

ink in field conditions. Laboratory studies, such as the one presented here, provide valuable insights into the larvicidal potential of natural products, but field trials are essential to assess the efficacy of the ink in real-world mosquito habitats. Additionally, research should focus on optimizing extraction and formulation methods to enhance the potency and stability of the ink extracts. The identification of the specific bioactive compounds responsible for the larvicidal effects could also pave the way for the development of novel insecticidal agents with broader applications in pest control (Benelli *et al*., 2016). In conclusion, the results of this study demonstrate that *O. cyanea* ink exhibits significant larvicidal activity against *Ae. aegypti* larvae, with a dose- and time-dependent increase in mortality. The relatively low LC_{50} values and steep dose-response slopes suggest that the ink is a potent natural larvicide. These findings support the potential use of *O. cyanea* ink as an eco-friendly alternative to synthetic insecticides for mosquito control. Future research should focus on field trials and the identification of active compounds to fully realize the potential of *O. cyanea* ink in vector control programs.

CONCLUSION

This study demonstrates the significant larvicidal potential of *O. cyanea* ink against *Ae. aegypti* larvae, with mortality rates increasing in a dose- and time-dependent manner. The LC_{50} values, calculated as 311.31 ppm at 24 hours and 180.11 ppm at 48 hours, highlight its efficacy, especially with prolonged exposure. The steep dose-response slopes indicate a pronounced increase in larval mortality with higher concentrations, underscoring its effectiveness as a natural larvicide. Comparisons with other natural larvicides, including plant and marine-derived products, further validate its competitive efficacy. The ink's bioactivity is likely attributed to compounds such as melanin and secondary metabolites, which disrupt larval physiology. As a biodegradable, eco-friendly alternative to synthetic insecticides, *O. cyanea* ink shows promise for sustainable mosquito control. However, further research, including field trials and phytochemical analyses, is essential to optimize its application and fully harness its potential in vector management programs.

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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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