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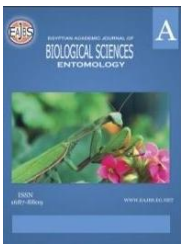

Investigating the Insecticidal and Repellent Potential of Oriental Hornet, (*Vespa orientalis*, Linnaeus, 1771, Vespidae) Venom against the Common House Mosquito (*Culex pipiens*, Linnaeus, 1758, Culicidae)

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ABSTRACT

Background: Mosquitoes are notable public health threats because of their role as disease vectors, causing considerable morbidity and mortality worldwide. Current mosquito control methods face challenges (insecticide resistance, ecological impacts, etc). Bioinsecticides, particularly those from natural sources like hymenopterous venoms, offer a promising alternative due to containing bioactive compounds that can disrupt vital physiological processes in insects. **Aim of the Study:** Assess the efficiency of *Vespa orientalis* venom as a larvicidal, pupicidal, and repellent agent against *Culex pipiens* mosquitoes, as a natural alternative to synthetic mosquito control methods. **Materials and Methods:** Vespid hornets were collected from nests near honeybee colonies using sweep nets. Venom was extracted by electroshock method, purified via gel filtration, and analyzed with HPLC. *C. pipiens* were reared under controlled conditions. Larvicidal and pupicidal assays were conducted by exposing 3rd instar larvae and newly-moulted pupae to venom concentrations. Mortality was recorded at intervals up to 72 hours. Repellency was assessed by applying venom to pigeon hosts and exposing them to starved female mosquitoes, using distilled water and DEET as controls. **Results:** *V. orientalis* venom showed significant larvicidal, pupicidal, and repellent activities against *C. pipiens*. Larvicidal assays indicated increased mortality with higher venom concentration and longer exposure. Lethal concentrations (LC_{25}, LC_{50}, and LC_{95}) decreased over time, showing cumulative toxic effects. Pupicidal assays revealed moderate effectiveness, with higher mortality at increased concentrations and longer exposure. The repellent effect was dose-dependent, with higher venom concentrations achieving greater repellency compared to DEET. **Conclusion and Recommendations:** The venom of *V. orientalis* demonstrated significant larvicidal properties and moderate effectiveness as a pupicidal agent against *C. pipiens*, alongside a moderate level of repellency towards adult females. Its efficacy was enhanced at elevated concentrations and extended exposure durations. The environmentally friendly characteristics of the venom suggest its viability as a sustainable substitute for chemical pesticides within integrated pest management strategies. Additional research is essential to refine its application methods, improve its effectiveness, and guarantee safety for the environment and non-target organisms.

INTRODUCTION

In addition to being notorious pests, mosquitoes hold particular medical significance due to their capacity to transmit several viral and parasitic diseases to humans, making them a substantial public health concern (Manikandan *et al.,* 2023; Nosrat *et al.*, 2021). Diseases transmitted by *Culex* sp. mosquitoes include Rift Valley fever, Zika virus, West Nile virus, lymphatic filariasis, St. Louis encephalitis and Japanese encephalitis. These diseases pose a substantial threat to human health, causing significant morbidity and mortality (Manikandan *et al.,* 2023). As a result, efficient mosquito control plays a crucial role in controlling the transmission of these diseases while preserving public health. However, mosquito control faces various limitations that require careful consideration and innovative solutions (Anoopkumar & Aneesh, 2022; Arias-Castro *et al.*, 2020; Karunaratne & Surendran, 2022). Insecticide resistance, ecological impact, vector complexity, and urbanization challenges demand a multifaceted approach. The potency of bioinsecticides offers a promising solution to the challenges posited by insecticide resistance and environmental impact. By utilizing the power of bioinsecticides derived from microbial, botanical and animal sources, sustainable mosquito control strategies can be developed. Integrated mosquito management, incorporating bioinsecticides alongside other approaches, provides a comprehensive framework to combat mosquitoes and reduce the burden of mosquito-borne diseases on society (Hanif *et al.*, 2022; Irsad *et al.*, 2023; Şengül Demirak & Canpolat, 2022). Wasps, known for their ability to deliver painful stings, have long been regarded as unwelcome insects. However, recent scientific investigations have shed light on the fascinating properties of wasp venom, especially as a natural insecticide (Rádis-Baptista & Konno, 2024; Sadek *et al.*, 2022; Smith *et al.*, 2013). Wasp venom comprises an intricate mixture of bioactive compounds produced and injected through their stingers. While the makeup of the venom varies across species, it typically contains proteins, enzymes, and other bioactive molecules, serving various purposes, including potent neurotoxic properties, leading to paralysis and immobilization. Certain venom components have shown the ability to disrupt essential physiological processes in insects, such as feeding, reproduction, and development. Also, different wasp species have evolved venom compositions that are specialized for their specific prey, allowing selective incapacitation of particular insect species while minimizing the impact on non-target organisms (Konno *et al.,* 2016; Moreau, 2013; Schmidt, 1982). Such characteristics of wasp venom make it an intriguing avenue for the formulation of eco-friendly pest control solutions. By harnessing the power of nature's own insecticidal weapon, effective pest management strategies can be devised while minimizing harm to the environment (Oguh *et al.,* 2019).

The aim of the current research was to assess the efficiency of *Vespa orientalis* venom as a larvicidal, pupicidal, and repellent agent against *Culex pipiens* mosquitoes, as a natural alternative to synthetic mosquito control methods.

MATERIALS AND METHODS

1-Collection of the Oriental Hornet *Vespa orientalis*:

The collection of the *V. orientalis* hornets used in the current study was achieved during the summer (July to August, 2023) to ensure the highest potency of the collected venom as suggested by Dias *et al.* (2014) and Schmidt *et al.* (1983). Live hornets were collected from naturally occurring nests in close proximity to honey bee colonies at the Honeybee Research Department, Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt. Collection was achieved via sweep nets. The hornets selected for

collection (age: 25-45 days) were transferred into plastic containers before being exposed to the venom collection apparatus.

2-Venom Collection by Electroshock Therapy:

Extracting the vespid hornet venom was achieved using an electroshock venom collection device along the guidelines described by Feás *et al.* (2021); Mueller *et al.* (1981), and Turillazzi *et al.* (2022). Such device was reported to minimize harm to the insects, while at the same time providing an efficient and relatively safe approach for venom extraction.
The electroshock apparatus worked on the principle of intermittent pulse oscillation and made use of the characteristic, mostly aggressive, biology of stinging Hymenoptera.

The device was placed adjacent to the container harboring the hornets, Afterwards the device was activated for 3-7 seconds to deliver a controlled electric shock, followed by a 2 second rest between impulses. As a response to the shock, the hornet extended its stinger and attempted to sting the glass plate of the device, depositing droplets of the venom.

The venom collection procedure was performed in dark conditions to avoid the excessive exposure of venom to direct sunlight, and the subsequent risk of denaturation of the proteomic venom components (Mukund & Manjunath, 2017).
At the end of the collection, the glass plate was removed out of the wooden frame and left till the venom dried by air.
Using the same hornet colony for multiple extractions was avoided to prevent stress or injury.

3-Purification of *Vespa orientalis* Venom:

After the collection procedure, the resulting venom was scrapped up from the glass plate with a plastic scraper, then the collected venom powder was weighed and washed with 1 :1 0.1% (v/v) trifluoroacetic acid in water : acetonitrile to solubilize venom peptides, afterwards it was centrifuged (12,000 ×g, 10 min) to eliminate insoluble materials; then it was stored at (-5◦C) for later use.

The venom purification was performed at the Micro Analytical Center, Faculty of Science, Cairo University. Along the general working guidelines of Hoffman (1985) and Xu *et al.* (2006), the collected venoms were dissolved in 0.1 M phosphate buffer solution (pH 6.5). Afterwards, the samples were filtered in a Sephadex G-50 (Sigma Aldrich, 2.6 × 100 cm) gel column. Elution was performed at a flow rate of 0.1 ml/min, with fractions of 1 ml collected every 10 min. The absorbance of the elute was monitored at 280 nm. The venom was then diluted in sterile distilled water to produce 0.1, 0.2, 0.4, 0.8, 1 and 1.5 mg/ml concentrations for the insecticidal bioassay.

Furthermore, 10 mg samples of the collected hornet venom samples were weighed and dissolved thoroughly in 1 mL double distilled water, using a standard lab vortex mixer. Afterwards, the dissolved samples were situated into an ultrasonic bath (32 kHz) for 15 min at 25◦C, followed by centrifugation (10,000 rpm for a duration of 10 min). Afterwards, membrane filtration (0.22 µm filter) was used to filter the supernatant before being injected into the HPLC device for analysis.

4-HPLC Analysis of *Vespa orientalis* Venom:

Analysis using High-performance liquid chromatography (HPLC) was later applied to determine the major components present in the purified venom samples, generally following the procedures reported by Hoffman (2006); Wu *et al.* (2022) and Zhou *et al.* (2019). The chromatographic analysis was achieved on a YL900 HPLC system at 25◦C on a C18 column (250 x 4.6 mm, 5 µm pore size) at a solvent flow rate of 1 ml/min. Eluents A (acetonitrile with 0.1% trifluoroacetic), and B (water with 0.1% trifluoroacetic) were used to separate venom components. The injection volume was 10 µL, and the UV absorbance was observed at 215 nm. The gradient program was structured as follows: 0–45 min, 5–75% A; 45–60 min, 75–95% A. Each run was followed by a 5-min equilibration time.

5-Rearing of *Culex pipiens* Mosquitoes:

The egg rafts of the *C. pipiens* mosquito were obtained from and reared in the insectary affiliated with the Entomology Department, Faculty of Science, Ain Shams University. The rearing procedure closely followed the protocols reported by Alto *et al.* (2012) and Meuti *et al.* (2023). Upon collection, the egg rafts were transferred to clean plastic trays filled with dechlorinated water. The rearing environment was maintained under carefully controlled conditions, including a thermal range of 25–27◦C, relative humidity of 70 ± 10%, and a photoperiod of 12 hours light and 12 hours dark to simulate natural day-night cycles. Adequate ventilation and proper air circulation were ensured to promote optimal development and prevent fungal/bacterial contamination. The newly-hatched larvae were transferred to larger pans containing dechlorinated water. A shallow water level was kept in the pans to facilitate larval access to the surface for respiration. The larvae were fed daily with finely ground TetraMin™ fish food flakes, provided in small quantities to avoid water fouling. The amount of food was adjusted according to the larval density and developmental stage to ensure proper nutrition without overfeeding.

Regular monitoring was conducted to assess larval health, growth rate, and water quality. Any debris or unconsumed food was removed daily to maintain a clean environment. Dead larvae were promptly removed to prevent contamination. Pans were cleaned and the water replaced every 2–3 days to maintain optimal rearing conditions.

Upon reaching the 4th instar, the larvae were carefully transferred to separate containers (for pupation) containing clean water using a fine pipette to ensure safe handling. After pupation, these containers were placed in woodmen cages (80cm x 60cm x 60cm) with a mesh-covered opening. The cages were maintained under the same controlled conditions of temperature, humidity, and photoperiod.

Adult mosquito emergence was monitored, and cotton wicks dipped with 10% sucrose solution were provided as a carbohydrate source for their initial feeding.

This standardized rearing protocol ensured the production of healthy and uniform mosquito populations for subsequent experimental investigations.

6-Screening the Insecticidal Effects of the *Vespa orientalis* Venom against the *Culex pipiens* Mosquito:

Investigating the insecticidal effects of the vespid hornet venom against *C. pipiens* involved observing the direct larvicidal and pupicidal effects of the venom.

The larvicidal bioassay of the *V. orientalis* venom was performed on 3rd instar *C. pipiens* larva, while the pupicidal bioassay was performed on newly-moulted pupa. Both assays were performed according to the guidelines reported by Subarani *et al.* (2013) and Yagoo *et al.* (2023), with slight modifications adopted due to the physico-chemical properties of hornet venom. The bioassay was achieved in 250 ml glass beakers containing 100 ml of each given venom concentration. Five experimental groups were used (one for each given concentration of hornet venom) in addition to a negative (untreated) control. This experimental trial was repeated three times.

For the larvicidal bioassay, each group involved thirty mosquito larvae. Mortality was recorded after 12, 24, 36, 48, 60 and 72 hours after treatment.

The pupal bioassay involved twenty young pupae for each experimental group. Mortality was recorded after 12, 24 and 36 hours after treatment.

7-Assessing the Repellence Effect of *Vespa orientalis* Venom on Adult Female *Culex pipiens* Mosquitoes using Pigeon Host (*Columba livia domestica*):

Standard rearing cages were used to detect the repellent activity of the *V. orientalis* venom, after the guidelines reported by El-Naggar & Hasaballah (2018) and Khan *et al.* (2018). The adult female *C. pipiens* mosquitoes were starved for 24 hours prior to the repellency assay so as to enhance their attraction to the host. Selected concentrations (0.1,

0.2, 0.4, 0.8 and 1 mg/ml) were prepared with a drop of Tween 20. An aliquot of 1 ml was applied directly onto the abdominal region of the pigeon for a duration of 10 minutes. Afterwards, the pigeons were accommodated in cages containing 10 starved female adult *C. pipiens* mosquitoes for a timespan of 2 h. Distilled water served as a negative control treatment, while 15% DEET, a commercial repellent purchased from Johnson Wax, Egypt, was employed as a positive control.

The repellency percentage using Abbott's formula:

$$\text{Repellency (\%)} = \left(\frac{(C-T)}{C}\right) \times 100$$

where T: the percentage of unfed *Culex* sp. females in treatment group.

C: the percentage of unfed *Culex* sp. females in control group.

8-Statistical Analysis:

The mortality (larvicidal and pupicidal) and repellency data were presented as mean ± standard deviation from independent triplicates, post-treatment. The recorded data were statistically analyzed using Microsoft Excel and Minitab software. Dose- and time-response regression lines were generated at a 95% confidence level through probit analysis, which was employed to calculate the lethal concentrations (LC_{25}, LC_{50} and LC_{95}), and assess the relationship between venom dose and mosquito mortality over time to evaluate the insecticidal efficacy of the venom. A similar analysis was conducted to evaluate venom repellency.

Graphical representations, including dose-response and time-mortality plots, were constructed to illustrate the dose- and time-dependent mortality patterns.

RESULTS

1-The HPLC Analysis of the *Vespa orientalis* Venom:

The HPLC chromatogram of the hornet venom sample (Fig. 1), showed three prominent peaks, signifying the presence of the major bioactive peptides and proteins in vespid hornet venom. Peak P1, with a retention time of 12.3 minutes and the highest peak intensity of 2218.4 mAU, was likely the primary active compound in the venom. Peaks P2 and P3, with retention times of 29.6 and 32.1 minutes respectively, also represented significant components of the venom.

The peaks were tentatively identified based on their retention times and comparison with known literature values.

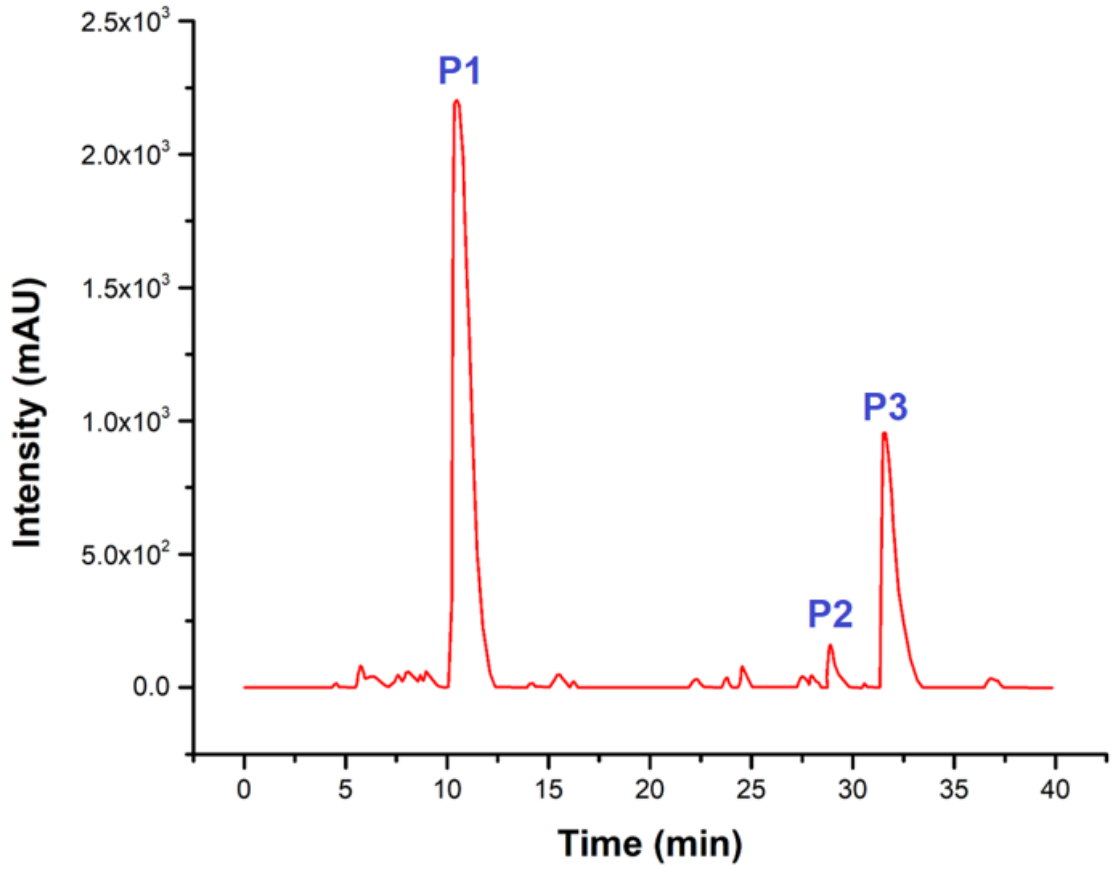

Fig. 1: HPLC profile for the *Vespa orientalis* venom at 215 nm.

2-The Larvicidal Activity of the *Vespa orientalis* Venom:

The data represented in Figure (2), showed a direct larvicidal activity of the *V. orientalis* venom against the 3rd instar *C. pipiens* larva, where for all the applied concentrations (0.2, 0.4, 0.8, 1 and 1.5 mg/ml) at the different exposure periods (12, 24, 36, 48, 60 and 72 hours), there was a noticeable strong dose- and time-responsive relationship with the observed mortality of the larva.

At the lowest applied concentration (0.2 mg/ml), the mortality started at 17.8% at 24 hours and increased gradually to 43.8% at 72 hours. This suggested limited immediate efficacy but noticeable cumulative lethality over time; at 0.4 mg/ml, the mortality increased from 30% at 24 hours to 52.8% at 72 hours. The higher initial mortality indicated increased potency at this concentration; at 0.8 mg/ml, the mortality began at 35.6% at 24 hours and reached 65.2% at 72 hours, showing a significant jump, especially noticeable after 36 hours; at 1 mg/ml, the mortality rate started at 50% at 24 hours, peaking at 78.7% at 72 hours, demonstrating a high level of initial and sustained lethality; finally, at the highest applied concentration (1.5 mg/ml), a 64.4% mortality was observed at 24 hours, increasing to 91% at 72 hours, indicating near-complete lethality over time.

These findings were further supported by the data in Table (1), representing the LC values (LC_{25}, LC_{50} and LC_{95}) at 95% C.I. of the *V. orientalis* venom against 3rd instar *C. pipiens* larvae from 12 hours of exposure up to 72 hours. The venom showed potent larvicidal effect, progressing in a time- and dose-dependent trend. At 12 hours of exposure, the LC_{25} was 0.33, then gradually decreased to 0.27, 0.16, 0.14, 0.12 and 0.11, after 24, 36, 48, 60 and 72 hours, respectively. While, at 12 hours of exposure, the LC_{50} was 1.02, then it also gradually decreased to 0.75, 0.54, 0.45, 0.39 and 0.31, after 24, 36, 48, 60 and 72 hours,

respectively. While for the LC_{95}, at 12 hours of exposure, it was 15.47, later decreasing to 8.96, 9.96, 7.65, 7.51 and 3.77, after 24, 36, 48, 60 and 72 hours, respectively.

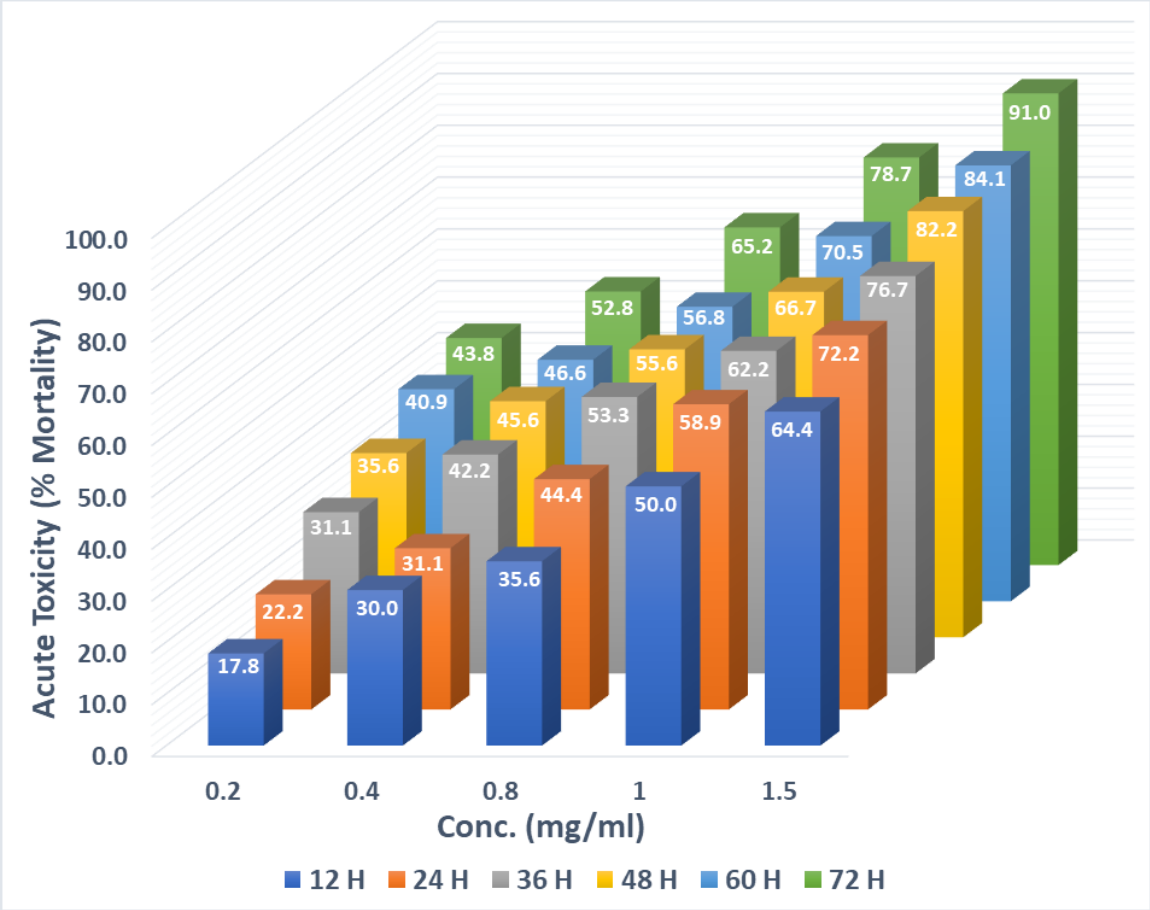

Fig. 2: The larvicidal effect of *Vespa orientalis* venom against 3rd instar *Culex pipiens*.

Table 1: Lethal concentrations of *Vespa orientalis* venom against 3rd instar *Culex pipiens*.

Exposure Duration:	LC Values (mg/ml) (95% C.I.)			Slope	χ^2
	LC_{25}	LC_{50}	LC_{95}		
12 H	0.33 (0.23 - 0.43)	1.02 (0.83 - 1.36)	15.47 (7.4 - 59.13)	1.4	3.98
24 H	0.27 (0.19 - 0.35)	0.75 (0.63 - 0.92)	8.96 (5.05 - 23.67)	1.53	3.7
36 H	0.16 (0.09 - 0.24)	0.54 (0.43 - 0.67)	9.96 (5.11 - 33.84)	1.3	2.52
48 H	0.14 (0.07 - 0.21)	0.45 (0.34 - 0.55)	7.65 (4.19 - 22.56)	1.34	4.97
60 H	0.12 (0.05 - 0.18)	0.39 (0.28 - 0.49)	7.51 (4.03 - 23.67)	1.28	7.1
72 H	0.11 (0.06 - 0.16)	0.31 (0.23 - 0.38)	3.77 (2.45 - 7.76)	1.51	7.23

3-The pupicidal activity of the *Vespa orientalis* Venom:

Even though there was a similar dose- and time-responsive effect of the applied venom, the pupicidal assay results, represented in Figure (3), showed lower overall mortality

compared to the larvicidal assay, indicating differences in susceptibility between larvae and pupae, where at 0.2 mg/ml, the mortality remained low, increasing slightly from 1.6% at 24 hours to 3.3% at 48 hours, suggesting minimal efficacy at this concentration; at 0.4 mg/ml, the mortality increased from 1.7% at 24 hours to 8.3% at 48 hours, showing some dose-dependent increase; at 0.8 mg/ml: the mortality started at 15% at 24 hours, reaching 21.7% at 48 hours, indicating moderate effectiveness; at 1 mg/ml, the mortality increased from 21.3% at 24 hours to 25% at 48 hours, showing a higher initial impact but limited incremental increase; at 1.5 mg/ml, the mortality started at 32.4% at 24 hours and increased to 26.7% at 48 hours, showing an initial high impact but with a slight decrease over time, possibly due to pupae reaching a lethal threshold earlier.

These findings were further supported by the data shown in Table (2), representing the LC values (LC_{25}, LC_{50} and LC_{95}) at 95% C.I. of the *V. orientalis* venom against the *C. pipiens* pupa from 12 hours of exposure up to 36 hours. At 12 hours: LC_{25} was 1.18 mg/ml, LC_{50} was 2.34 mg/ml, and LC_{95} was 12.22 mg/ml, reflecting a higher tolerance for lethality compared to larvae; at 24 hours: LC_{25} was 1.37 mg/ml, LC_{50} was 3.58 mg/ml, and LC_{95} was 37.06 mg/ml,, suggesting some variability or increased resistance over time; at 36 hours: LC_{25} was 1.14 mg/ml, LC_{50} was 3.37 mg/ml, and LC_{95} was 47.23 mg/ml, indicating sustained but not significantly increasing lethality over this period. The relatively high LC_{95} values, particularly at 24 and 36 hours (37.06 mg/ml and 47.23 mg/ml, respectively), indicated that very high concentrations were required for near-complete mortality, and the effectiveness did not increase as significantly over time as it did with larva, highlighting the greater resistance of pupae.

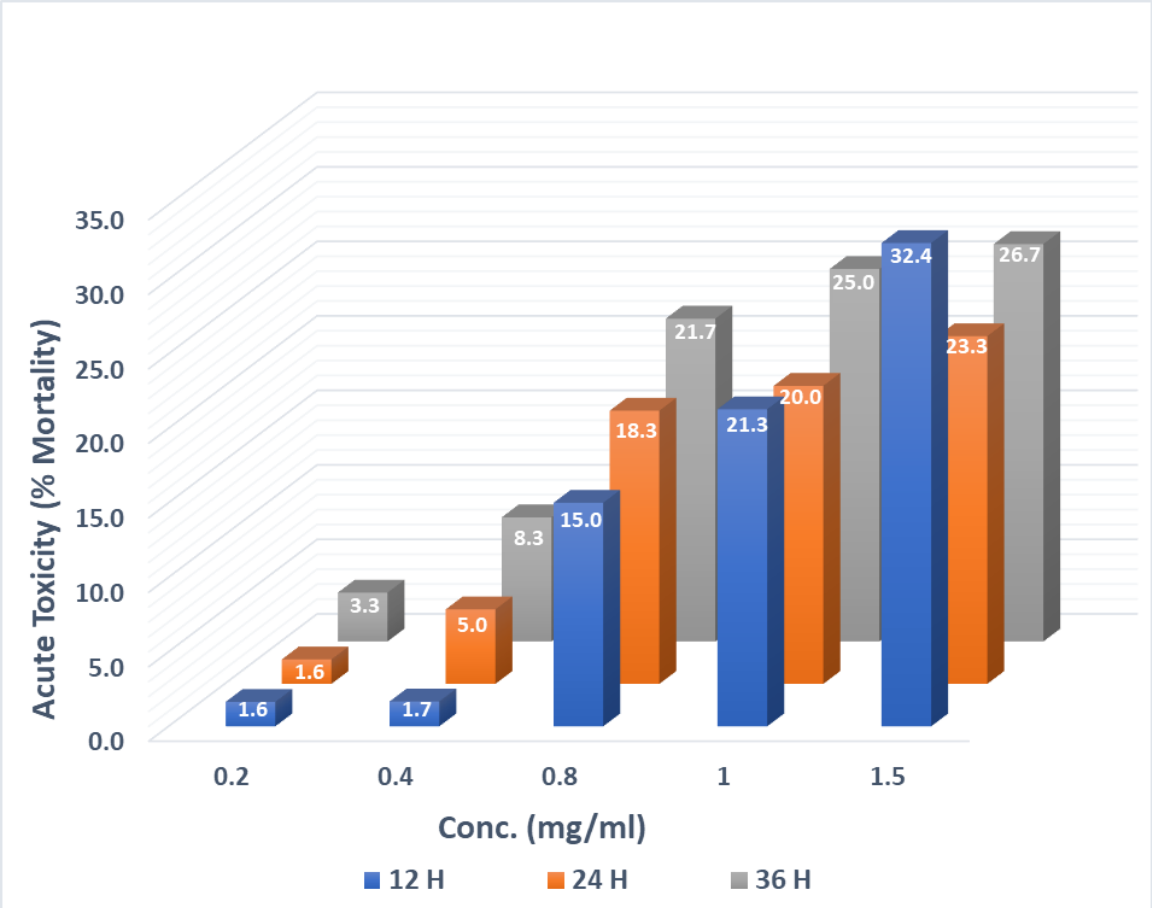

Fig. 3: The pupicidal effect of *Vespa orientalis* venom against *Culex pipiens*.

Table 2: Lethal concentrations of *Vespa orientalis* venom against *Culex pipiens* pupae.

Exposure Duration:	LC Values (mg/ml) (95% C.I.)			Slope	χ^2
	LC_{25}	LC_{50}	LC_{95}		
12 H	1.18 (0.95 - 1.7)	2.34 (1.65 - 5.14)	12.22 (5.43 - 88.43)	2.29	1.64
24 H	1.37 (1.04 - 2.37)	3.58 (2.15 - 13.06)	37.06 (10.98 - 980.76)	1.62	1.4
36 H	1.14 (0.86 - 1.83)	3.37 (2.02 - 12.11)	47.23 (12.81 - 1528.94)	1.43	1.2

4-The Repellent Effect of the *Vespa orientalis* Venom Against Adult *C. pipiens* Females:

The repellency assay of the *V. orientalis* venom, shown in Table (3), showed a clear, yet moderate, dose-dependent increase in repellency against adult *C. pipiens* females. At 0.1 mg/ml, 26.7% repellency suggested limited effectiveness at the lowest concentration; At 0.2 mg/ml, 43.3% repellency indicated moderate effectiveness; At 0.4 mg/ml, 50% repellency shows significant repellent properties at mid-range concentration; At 0.8 mg/ml, 53.3% repellency demonstrated a plateauing effect, with only a slight increase over 0.4 mg/ml; At 1 mg/ml, 63.3% repellency showed the highest effectiveness, though still below 100% repellency.

Compared to DEET (100% repellency) and distilled water (0% repellency), the venom showed significant repellent properties. However, it did not reach the effectiveness of traditional repellents like DEET.

Table 3: Repellent effect of *Vespa orientalis* venom against adult female *Culex pipiens* mosquitoes.

Conc. (mg/ml)	Fed Females		Unfed Females		Repellency (%)
	M ± SD	%	M ± SD	%	
0.1	7.33 ± 1.15	73.3	2.67 ± 1.15	26.7	26.7
0.2	5.67 ± 0.58	56.7	4.33 ± 0.58	43.3	43.3
0.4	5 ± 1	50	5 ± 1	50	50
0.8	4.67 ± 0.58	46.7	5.33 ± 0.58	53.3	53.3
1	3.67 ± 0.58	36.7	6.33 ± 0.58	63.3	63.3
Positive Control (DEET)	0 ± 0	0	10 ± 0	100	100
Neg. Control (Dist. H2O)	10 ± 0	100	0 ± 0	0	0

DISCUSSION

The search for effective, environmentally sustainable insect control strategies has gained significant momentum in recent years due to the escalating resistance of insect vectors to conventional chemical insecticides and their adverse ecological effects (Barathi *et al.*, 2024). Among these vectors, mosquitoes, particularly *Culex* sp., infer a substantial public health threat as primary carriers of various arboviruses and parasitic diseases. In this context, the use of naturally derived bioinsecticides has emerged as a promising alternative (Şengül Demirak & Canpolat, 2022). One such underexplored avenue is the insecticidal potential of vespid venoms, particularly from *Vespa orientalis* (the Oriental hornet), which remains largely uninvestigated.

While vespid venoms have been extensively studied for their biochemical composition and pharmacological effects on vertebrates, their potential toxicity against

insect pests and vectors has not been comprehensively assessed (Guido-Patiño & Plisson, 2022)**.** The present study presents one of the first investigations into the larvicidal, pupicidal, and repellent effects of *V. orientalis* venom against *Culex* sp. mosquitoes.

1-The HPLC Analysis of the *Vespa orientalis* Venom:

The chromatographic analysis of the collected vespid venom revealed distinct bioactive compounds, including serotonin and mastoparan-like peptides, aligning with findings by Quistad *et al.* (1988) and Wu *et al.* (2022). These compounds are known for their pharmacological activities, including antimicrobial, anticancer, and antioxidant effects (Herrera *et al.*, 2020). The identification of hyaluronidases corroborates with the enzymatic activities reported by Monsalve *et al.* (2020), indicating their role in venom's bioactivity. This combination of bioactive components suggests that *V. orientalis* venom possesses a multi-faceted mode of action, making it an excellent candidate for pest control applications.

2-The Larvicidal Activity of the *Vespa orientalis* Venom:

The larvicidal effects of *V. orientalis* venom demonstrated a strong dose- and time-dependent increase in mortality, with LC values declining significantly over time. The steep decline in LC_{95} from 15.47 mg/ml at 12 hours to 3.77 mg/ml at 72 hours highlights the venom's sustained efficacy over extended periods and suggests the venom's increasing effectiveness, potentially due to either the progressive cumulative toxic effect of the venom components (the synergistic action of the mastoparan-like peptides and hyaluronidases most likely disrupted the cellular and enzymatic processes essential for larval survival), or the delayed physiological effects, leading to larval mortality. This finding aligns partially with El-Naggar & Hasaballah (2018), who reported dose-dependent larvicidal effects of *Octopus cyanea* extracts. However, *V. orientalis* venom exhibited lower LC values, suggesting higher toxicity. This discrepancy may stem from differences in venom composition and extraction methods.

Contrasting these findings, Abou El Ela *et al.* (2023) reported limited larvicidal activity for bee venom and propolis, possibly due to the commercial processing of these products. In comparison, raw *V. orientalis* venom may retain its bioactivity more effectively, underscoring the importance of purity in evaluating venom efficacy. Additionally, the unique composition of hornet venom, distinct from bee venom despite some shared components, likely contributes to the observed differences in toxicity as reported by Habermann (1972) and Schmidt (1982). These results underline the need for standardization in venom extraction and application protocols to ensure consistent outcomes.

3-The Pupicidal Activity of the *Vespa orientalis* Venom:

Compared to larvae, the venom's pupicidal activity was markedly lower, with higher LC values indicating reduced susceptibility. This trend is consistent with findings by Subarani *et al.* (2013) and Yagoo *et al.* (2023), who noted significant variability in larvicidal and pupicidal effects of botanical extracts. The reduced efficacy against pupae may reflect their non-feeding nature, lower metabolic activity, and protective cuticular structures that limit venom penetration.

This could also theorize that the possible mechanism of action of the venom is through ingestion, even though cuticular permeability is another theorized possibility, affecting the cuticular lining of the alimentary canal of the larva, a missing mechanism in pupa since it is a non-feeding stage.

The venom's limited pupicidal activity raises questions about its broader applicability. While larvicidal efficacy supports its potential as a biocontrol agent, its reduced impact on pupae could necessitate complementary strategies, such as integrating the venom with other bioactive agents or employing mechanical interventions to disrupt pupal stages. Further physiological studies comparing venom uptake and metabolic processing in larvae versus pupae could elucidate the underlying reasons for this discrepancy.

Interestingly, these findings partially contrast with Jayakumar *et al.* (2016), who observed comparable larvicidal and pupicidal effects for plant oils after 24 hours but noted a decline in larvicidal efficacy over prolonged exposure. This highlights the possible potent properties of vespid venom-derived bioactives, which maintain their potency over time.

4-The Repellent Effect of the *Vespa orientalis* Venom Against Adult *C. pipiens* Females:

The moderate repellent activity of *V. orientalis* venom against adult *C. pipiens* females highlights its potential as a deterrent. The dose-dependent increase in repellency aligns with El-Naggar & Hasaballah (2018),who reported similar trends with *O. cyanea* extracts. However, the venom's inability to achieve complete repellency, as observed with DEET, underscores its limitations. Factors such as environmental degradation of venom components, limited volatility, or insufficient binding to olfactory receptors in mosquitoes could contribute to its reduced efficacy.

To enhance its repellent properties, formulation improvements should be explored, such as encapsulation techniques or synergistic combinations with other natural repellents. Additionally, behavioral studies on mosquito olfactory responses to venom-derived compounds could provide insights into optimizing the venom's deterrent effects making it a more viable alternative to synthetic repellents.

CONCLUSION:

The findings of the present study disclosed a significant bioactive potential of *Vespa orientalis* venom against *Culex pipiens* mosquitoes. The larvicidal assays demonstrated strong dose- and time-dependent mortality rates, with the venom showing potent cumulative effects. Mortality increased significantly with both higher venom concentrations and extended exposure periods, with near-complete lethality (91%) observed at 1.5 mg/ml after 72 hours. The decreasing LC values over time further underscore the venom's progressive effectiveness in larval control.

In contrast, the pupicidal assays indicated lower susceptibility of pupae to the venom. While a dose- and time-dependent response was observed, the overall mortality rates and LC values suggested that pupae exhibit greater resistance compared to larvae. This highlights the varying vulnerability of mosquito life stages to the venom.

The repellent activity of *V. orientalis* venom against adult *C. pipiens* females was moderate and dose-dependent, with the highest observed repellency of 63.3% at 1 mg/ml. While effective, the venom did not match the complete repellency observed with DEET, indicating a potential but limited application in adult mosquito control.

Broader Implications and Limitations:

These findings highlight the promise of *V. orientalis* venom as a sustainable alternative to synthetic pesticides. Its potent efficacy in disrupting mosquito development, as evidenced by the larvicidal activity, coupled with moderate repellency, positions it as a viable candidate for integrated pest management (IPM). By targeting multiple life stages of the mosquito, *V. orientalis* venom offers a multifaceted approach that could reduce adult emergence. Moreover, the venom's eco-friendly and biodegradable nature could reduce reliance on chemical insecticides, mitigating their environmental and health impacts. Additionally, the identification of bioactive peptides opens avenues for the development of synthetic analogues, potentially enhancing specificity and safety profiles for large-scale applications.

From an ecological perspective, the use of venom-based biocontrol agents aligns with the growing emphasis on biodiversity conservation and environmental sustainability. However, the potential impact on non-target species, such as beneficial insects or aquatic organisms, warrants careful assessment to ensure ecological balance.

Despite its potential, the current study has limitations that warrant consideration. Laboratory conditions may not fully capture field complexities. The venom's efficacy across

different mosquito species and ecological contexts remains unexplored. Furthermore, the precise mechanisms underlying the venom's bioactivity are not fully understood, necessitating molecular and biochemical studies to elucidate these pathways.

Recommendations and Future Directions:

Future research should focus on scaling up the production of *V. orientalis* venom and refining delivery methods to enhance its practical application. Exploring its interactions with microbial communities in breeding habitats could uncover additional ecological benefits. Comparative studies with other hymenopteran venoms may provide broader insights into bioactivity patterns. Advanced analytical techniques, such as mass spectrometry and molecular docking, should be employed to identify and optimize active compounds, bridging laboratory findings with field applications. Given the venom's potent larvicidal activity, efforts should prioritize optimizing formulations and isolating key bioactive components for eco-friendly use. However, its lower efficacy against pupae suggests a need for higher doses, combined treatments, or deeper investigations into pupal resistance mechanisms. Enhancing its moderate repellency could involve combining it with other repellents or chemical modifications. Additionally, assessing the venom's environmental impact, effects on non-target species, and persistence is essential for sustainable use. Ensuring human and animal safety, including allergenicity evaluations, is crucial before scaling up for broader application.

Table of Abbreviations:

Abbreviation	Meaning
×g	Relative Centrifugal Force
◦C	Degrees Celsius
cm	Centimeter
DEET	*N*,*N*-Diethyl-*meta*-toluamide
HPLC	High Pressure Liquid Chromatography
kHz	Kilohertz
LC	Lethal Concentration
M	Molar
mg	Milligram
min	Minute
ml	Milliliter
nm	Nanometer
pH	Potential of Hydrogen
rpm	Revolutions per Minute
UV	Ultraviolet
v/v	Volume per Volume

Declarations:

Ethical Approval: All experiments in this research were approved by the Ethics Committee of the Faculty of Science, Ain Shams University, Cairo, Egypt (Approval code: ASU-SCI/ENTO/2024/5/1).

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REFERENCES

Abou El Ela, A., Mostafa, A., Ahmed, E., & Khalil, A. E. (2023). Larvicidal and Biochemical Effects of Some Essential Oils and Bee Products Against *Culex pipiens* Larvae (Diptera: Culicidae). *Egyptian Academic Journal of Biological Sciences. A, Entomology*, *16*(3), 1–12. https://doi.org/10.21608/ eajbsa. 2023. 311828

Alto, B. W., Muturi, E. J., & Lampman, R. L. (2012). Effects of nutrition and density in *Culex pipiens*. *Medical and Veterinary Entomology*, *26*(4), 396–406. https:// doi. org/10.1111/j.1365-2915.2012.01010.x

Anoopkumar, A. N., & Aneesh, E. M. (2022). A critical assessment of mosquito control and the influence of climate change on mosquito-borne disease epidemics. *Environment, Development and Sustainability*, *24*(6), 8900–8929. https://doi. org/10.1007/s10668-021-01792-4

Arias-Castro, J. H., Martinez-Romero, H. J., & Vasilieva, O. (2020). Biological and Chemical Control of Mosquito Population by Optimal Control Approach. *Games*, *11*(4), 62. https://doi.org/10.3390/g11040062

Barathi, S., Sabapathi, N., Kandasamy, S., & Lee, J. (2024). Present status of insecticide impacts and eco-friendly approaches for remediation-a review. *Environmental Research*, *240*(1), 117432. https://doi.org/10.1016/j.envres.2023.117432

Dias, N. B., de Souza, B. M., Gomes, P. C., & Palma, M. S. (2014). Peptide diversity in the venom of the social wasp *Polybia paulista* (Hymenoptera): A comparison of the intra- and inter-colony compositions. *Peptides*, *51*, 122–130. https://doi. org/10. 1016/j.peptides.2013.10.029

El-Naggar, H., & Hasaballah, A. (2018). Acute Larvicidal Toxicity and Repellency Effect of *Octopus cyanea* Crude Extracts Against the Filariasis Vector, *Culex pipiens*. *Journal of the Egyptian Society of Parasitology*, *48*(3), 721–728. https://doi. org/ 10.21608/jesp.2018.76592

Elhawary, N., Sultan, K., Rehan, I., & Bazh, E. (2020). Larvicidal efficacy of some natural products against *Culex pipiens* larvae. *Egyptian Veterinary Medical Society of Parasitology Journal (EVMSPJ)*, *16*(1), 102–113. https://doi.org/10.21608/ evmspj.2020.125129

Feás, X., Vidal, C., Vázquez-Tato, M. P., & Seijas, J. A. (2021). Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae), Venom Obtention Based on an Electric Stimulation Protocol. *Molecules*, *27*(1), 138. https://doi.org/10.3390/molecules 27010138

Guido-Patiño, J. C., & Plisson, F. (2022). Profiling hymenopteran venom toxins: Protein families, structural landscape, biological activities, and pharmacological benefits. *Toxicon: X*, *14*, 100119. https://doi.org/10.1016/j.toxcx.2022.100119

Habermann, E. (1972). Bee and wasp venoms. *Science*, *177*(4046), 314–322. https:// doi.org/10.1126/science.177.4046.314

Hanif, K., Zubair, M., Hussain, D., Ali, S., Saleem, M., Khan, H. A. A., Nazir, T., & ul Hassan, M. W. (2022). Biopesticides and insect pest management. *International Journal of Tropical Insect Science*, *42*(6), 3631–3637. https://doi.org/10. 1007/s42690-022-00898-0

Herrera, C., Leza, M., & Martínez-López, E. (2020). Diversity of compounds in *Vespa* spp. venom and the epidemiology of its sting: a global appraisal. *Archives of Toxicology*, *94*(11), 3609–3627. https://doi.org/10.1007/s00204-020-02859-3

Hoffman, D. R. (1985). Allergens in Hymenoptera venom XIII: Isolation and purification of protein components from three species of vespid venoms. *The Journal of Allergy and Clinical Immunology*, *75*(5), 599–605. https://doi.org/10.1016/0091-6749 (85) 90036-3

Hoffman, D. R. (2006). Hymenoptera venom allergens. *Clinical Reviews in Allergy and Immunology*, *30*(2), 109–128. https://doi.org/10.1385/CRIAI:30:2:109

Irsad, Shahid, M., Haq, E., Mohamed, A., Rizvi, P. Q., & Kolanthasamy, E. (2023). Entomopathogen-based biopesticides: insights into unraveling their potential in insect pest management. *Frontiers in Microbiology*, *14*. https://doi.org/ 10. 3389/ fmicb.2023.1208237

Jayakumar, M., Arivoli, S., Raveen, R., & Tennyson, S. (2016). Larvicidal and pupicidal efficacy of plant oils against *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae). *Journal of Entomology and Zoology Studies*, *4*(5), 449–456.

Karunaratne, S. H. P. P., & Surendran, S. N. (2022). Mosquito control: A review on the past, present and future strategies. *Journal of the National Science Foundation of Sri Lanka*, *50*, 277–292. https://doi.org/10.4038/jnsfsr.v50i0.11244

Khan, H. R., Sultana, N., & Sharmin, P. (2018). Larvicidal efficacy of the crude leaf extracts of *Eucalyptus camaldulensis* Dehn (Myrtales: Myrtaceae) against the mosquito larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Dhaka University Journal of Biological Sciences*, *27*(2), 135–144. https://doi.org/10. 3329/ dujbs. v27i2.46462

Konno, K., Kazuma, K., & Nihei, K. (2016). Peptide Toxins in Solitary Wasp Venoms. *Toxins*, *8*(4), 114. https://doi.org/10.3390/toxins8040114

Manikandan, S., Mathivanan, A., Bora, B., Hemaladkshmi, P., Abhisubesh, V., & Poopathi, S. (2023). A Review on Vector Borne Disease Transmission: Current Strategies of Mosquito Vector Control. *Indian Journal of Entomology*, *85*(2), 503–513. https://doi.org/10.55446/IJE.2022.593

Meuti, M. E., Siperstein, A., & Wolkoff, M. (2023). Rearing and Maintaining a *Culex* Colony in the Laboratory. *Cold Spring Harbor Protocols*, *2023*(8), pdb. Prot 108080. https://doi.org/10.1101/pdb.prot108080

Monsalve, R. I., Gutiérrez, R., Hoof, I., & Lombardero, M. (2020). Purification and molecular characterization of phospholipase, antigen 5 and hyaluronidases from the venom of the Asian hornet (*Vespa velutina*). *PLoS ONE*, *15*(1), 1–12. https://doi.org/10.1371/journal.pone.0225672

Moreau, S. J. M. (2013). "It stings a bit but it cleans well": Venoms of Hymenoptera and their antimicrobial potential. *Journal of Insect Physiology*, *59*(2), 186–204. https://doi.org/10.1016/j.jinsphys.2012.10.005

Mueller, U., Reisman, R., Wypych, J., Elliott, W., Steger, R., Walsh, S., & Arbesman, C. (1981). Comparison of vespid venoms collected by electrostimulation and by venom sac extraction. *Journal of Allergy and Clinical Immunology*, *68*(4), 254–261. https://doi.org/10.1016/0091-6749(81)90148-2

Mukund, H., & Manjunath, P. M. (2017). Comparative Enzyme Activity of *Vespa orientalis* Venom and its Photooxidized Venom Products. *Journal of Biochemistry and Biophysics*, *1*(1). https://doi.org/10.15744/2576-7623.1.104

Nosrat, C., Altamirano, J., Anyamba, A., Caldwell, J. M., Damoah, R., Mutuku, F., Ndenga, B., & LaBeaud, A. D. (2021). Impact of recent climate extremes on mosquito-borne disease transmission in Kenya. *PLOS Neglected Tropical Diseases*, *15*(3), e0009182. https://doi.org/10.1371/journal.pntd.0009182

Oguh, C., Okpaka, C., Ubani, C., Okekeaji, U., Joseph, P., & Amadi, E. (2019). Natural Pesticides (Biopesticides) and Uses in Pest Management- A Critical Review. *Asian Journal of Biotechnology and Genetic Engineering*, *2*(2 SE-Review Article), 126–143. https://journalajbge.com/index.php/AJBGE/article/view/37

Quistad, G. B., Skinner, W. S., & Schooley, D. A. (1988). Venoms of social hymenoptera-toxicity to the lepidopteran, *Manduca sexta*. *Insect Biochemistry*, *18*(6), 511–514. https://doi.org/10.1016/0020-1790(88)90001-7

Rádis-Baptista, G., & Konno, K. (2024). Spider and Wasp Acylpolyamines: Venom Components and Versatile Pharmacological Leads, Probes, and Insecticidal Agents. *Toxins*, *16*(6), 234. https://doi.org/10.3390/toxins16060234

Sadek, R. Z., El Banna, S. M., Semida, F. M., & Zalat, S. M. (2022). Potential Insecticidal Effect of a Wasp (*Polistes dominulus*) and a Bee (*Apis mellifera*) Venoms in Controlling *Spodoptera littoralis*. *Advances in Entomology*, *10*(04), 300–319. https://doi.org/10.4236/ae.2022.104022

Schmidt, J. O. (1982). Biochemistry of Insect Venoms. *Annual Review of Entomology*, *27*(1), 339–368. https://doi.org/10.1146/annurev.en.27.010182.002011

Schmidt, J. O., Blum, M. S., & Overal, W. L. (1983). Hemolytic activities of stinging insect venoms. *Archives of Insect Biochemistry and Physiology*, *1*(2), 155–160. https://doi.org/10.1002/arch.940010205

Şengül Demirak, M. Ş., & Canpolat, E. (2022). Plant-Based Bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects*, *13*(2), 162. https://doi.org/10.3390/insects13020162

Smith, J. J., Herzig, V., King, G. F., & Alewood, P. F. (2013). The insecticidal potential of venom peptides. *Cellular and Molecular Life Sciences*, *70*(19), 3665–3693. https://doi.org/10.1007/s00018-013-1315-3

Subarani, S., Sabhanayakam, S., Kamaraj, C., Elango, G., & Kadir, M. A. (2013). Efficacy of larvicidal and pupicidal activity of *Catharanthus roseus* aqueous and solvent extracts against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, *6*(8), 625–630. https://doi.org/10.1016/S1995-7645(13)60107-8

Tang, X., & Hou, T. (2008). Development of chamaejasmin microemulsion and its biological activity against *Aphis craccivora* and *Culex pipiens pallens*. *Flavour and Fragrance Journal*, *23*(4), 258–262. https://doi.org/10.1002/ffj.1879

Turillazzi, F., Pieraccini, G., Turillazzi, S., Orsi Battaglini, N., & Severino, M. (2022). Venom Collection by Electrical Stimulation in the Invasive Species *Polistes dominula* Reared Using a Vespiculture Regime. *Molecules*, *27*(24), 1–8. https://doi.org/10.3390/molecules27248821

Wu, Y. H., Zhang, Y., Fang, D. Q., Chen, J., Wang, J. A., Jiang, L., & Lv, Z. F. (2022). Characterization of the Composition and Biological Activity of the Venom from *Vespa bicolor* Fabricius, a Wasp from South China. *Toxins*, *14*(1). https://doi.org/10.3390/toxins14010059

Xu, X., Li, J., Lu, Q., Yang, H., Zhang, Y., & Lai, R. (2006). Two families of antimicrobial peptides from wasp *(Vespa magnifica)* venom. *Toxicon*, *47*(2), 249–253. https://doi.org/ 10. 1016/j.toxicon.2005.10.015

Yagoo, A., Milton, M. C. J., & Vilvest, J. (2023). Mosquito larvicidal, pupicidal and ovicidal effects of the extracts of *Peltophorum pterocarpum* flowers on *Aedes aegypti* and *Culex quinquefasciatus*. *Veterinary Parasitology: Regional Studies and Reports*, *46*, 100929. https://doi.org/10.1016/j.vprsr.2023.100929

Zhou, S. T., Luan, K., Ni, L. L., Wang, Y., Yuan, S. M., Che, Y. H., Yang, Z. Z., Zhang, C. G., & Yang, Z. Bin. (2019). A strategy for quality control of *Vespa magnifica* (Smith) venom based on HPLC fingerprint analysis and multi-component separation combined with quantitative analysis. *Molecules*, *24*(16), 1–9. https://doi.org/10.3390/molecules24162920