

Synergizing the deltamethrin larvicidal activity against *Aedes albopictus* larvae using cinnamaldehyde in Diwaniyah, Iraq

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Abstract

The current work on mosquito larvae was performed to evaluate the resistance status of larvae to deltamethrin (DM) and to detect if the larvicidal activity (LA) of this chemical could be synergized after exposing the larvae to cinnamaldehyde (CD). Here, 200 *Aedes albopictus* larvae were employed for the experiment and were divided randomly into 2 groups (100/each group and placed in petri-dishes (PD), 10 larvae/PD), and they are the DM group (1ml of 0.04 mg/l in 99ml of distilled water (DW) was placed in each PD) and the DM+CD group (1ml of 0.04 mg/l and 1ml of 0.9mg/l respectively were placed with 98ml DW in each PD). The experiment was lasted for 24hrs. Larvae were detected to have resistance against DM as 45% to 60% of the larvae were killed by the DM, 40% to 55% resistance rate. However, when evaluating DM activity with the use of CD, the LA was synergized showing mortality in 87% to 92% of the larvae in which a significant increase in the mortality in DM+CD group was noticed more than that in the DM group. Furthermore, RT-qPCR was run to identify the expression status of the P540 monooxygenase gene, *Cyp6p15*, and found that the gene expression was significantly inhibited in the DM+CD group when comparing that in the DM group that showed overexpression of this gene. This work results provide viable information about the potential activity of the cinnamaldehyde in synergizing the larvicidal activity of deltamethrin.

Keywords: cinnamaldehyde, deltamethrin, larvicide, resistance

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التأثير التازري لنشاط المبيد الدلتامثرين المضاد ليرقات بعوض *Aedes albopictus* باستخدام السينامالدهايد في الديوانية، في العراق

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الخلاصة

اجري البحث على يرقات البعوض لتقييم مقاومة يرقاتها للدلتاميثرين، وهل تزداد فعالية المادة الاخيريه على يرقات البعوض بعد تعريض اليرقات للسينامالديهيد. استخدم ٢٠٠ يرقة بعوض من جنس *Aedes albopictus* للتجربه، قسمت بشكل عشوائي الى مجموعتين وبواقع ١٠٠ يرقة في كل طبق بتري. استخدمت ١ مل من الديلتاميثرين بتركيز ٠,٠٤ ملغ / لتر في ٩٩ مل من الماء المقطر لكلا المجموعتين في التجربه الاولى، اضيف ١ مل من السينامالديهيد بتركيز ٠,٩ ملغ / لتر في ٩٩ مل من الماء المقطر بالاضافه الى ماده الاولى في كلا الطبقتان في التجربه الثانيه. استمرت التجربه ٢٤ ساعه. في المرحله الاولى اضيف للدلتاميثرين فقط لكلا المجموعتين، بلغت نسبة الهلاكات في اليرقات ٤٥% و ٦٠% للطبق الاول والثاني على التوالي، ومعدل مقاومه ٥٥% و ٤٠% للمرحله الاولى من التجربه في الطبقتان على التوالي. في المرحله الثانيه من التجربه اضيف الديلتاميثرين والسينامالديهيد معا حيث كانت نسبة الهلاكات في اليرقات ٨٧% و ٩٢% للطبق الاول والثاني. فضلا على ذلك، استخدم فحص ال RT-qPCR لتحديد الوصف لجين P540 احادي

الاوكسجين، Cyp6p15، لوحظ تأثير جيني كبير عند استخدام الديلتاميثرين والسينامالدهيد مع مقارنه مع التأثير الجيني عند استخدام الديلتاميثرين فقط. ان نتائج هذا البحث اظهرت تاثير تعاضدي للسينامالدهيد مع الديلتاميثرين في استخدام الاخير كمبيد ليرقات البعوض.

Introduction

Resistance to insecticide is an important global subject that affects people life in different health and economic subjects. Mosquitoes are considered as one of the most important disease transmitting vectors (1-3). Mosquitoes are a well-known vector to transmit different pathogens such as plasmodium (4), dengue virus (5), the west Nile virus (6), the yellow fever virus (7), Zika virus (8). Those pathogens cause severe health conditions that sometimes result in high mortalities in human (9). Controlling of these diseases depends highly on controlling those vectors; however, the process has become not easy as insecticide resistance emerges every day to different insecticides, the old or the newly invented insecticides (10). Mosquitoes are being understood to develop insecticide resistance via different mechanisms such as the P450 monooxygenase system present in mosquitoes that acts to generate detoxification of insecticides by P450-dependent degradation processes (11). Interestingly, these detoxification techniques also were suggested via the presence of bacterial P450 of the mosquito microbiota (12-14).

Using insecticide without efficient and successful results introduces more complication into the environment leading to catastrophic pollution that adds more impacts on the health and economy of the world (15,16). The current work on mosquito larvae was performed to evaluate the resistance status of larvae to deltamethrin (DM) and to detect if the larvicidal activity (LA) of this chemical could be synergized after exposing the larvae to cinnamaldehyde (CD).

Materials and methods

The protocol of larval rearing and conditions was followed from (14,17,18). Here, 200 *Aedes albopictus* larvae (third instar) were employed for the experiment and were divided randomly into 2 groups (100/each group and placed in petri-dishes (PD), 10 larvae/PD), and they are the DM group (1ml of 0.04 mg/l in 99ml of distilled water (DW) was placed to each PD) and the DM+CD group (1ml of 0.04 mg/l and 1ml of 0.9mg/l respectively were placed with 98ml DW in each PD). These PDs were incubated under the conditions of $27\pm 2^\circ\text{C}$ and 85% of humidity. The experiment was lasted for 24hrs. A triplicate for each group was performed.

RT-qPCR

For this part of this study, the experiment was re-run to collect the live larvae after 20hrs. The total RNA was

extracted using the hot phenol extraction method (19). The gene expression of the P450 monooxygenase, *Cyp6p15*, was tested. The cDNA was synthesized using 100ng of the RNA and following the use of RevertAid Premium Kit (Fermentas, USA). The RT-qPCR was done using KAPA SYBR FAST qRT-PCR Kit (Biosystem, USA) in the Bio-Rad system (Hercules, USA) using the primers F: CGG ATA TTC AGG AGA GG and R: ATA ACC AGG TCG TAT GT. The normalization gene used was *rpL8* (GenBank M99055.1). The conditions and methodology used for the RT-qPCR were from (20).

Statistical analyses

Mean \pm SE were employed to process and display the data at a significant probability at $P < 0.05$. Graphpad Prism software V7.0 (USA) was recruited to do the analyses.

Results

Larval mortality

Larvae were detected to have resistance against DM as 45% to 60% of the larvae were killed by the DM, 40% to 55% resistance rate. However, when evaluating DM activity with the use of CD, the LA was synergized showing mortality in 87% to 92% of the larvae in which a significant increase in the mortality in DM+CD group was noticed more than that in the DM group (Table 1) (Figure 1).

Table 1: Shows the mortality rates of the larvae in each group of the experiment

Time point	Triplicates	Mortalities	
		DM+CD	DM
After 24hrs	1	92 (92%)	54 (54%)
	2	89 (89%)	60 (60%)
	3	87 (87%)	45 (45%)

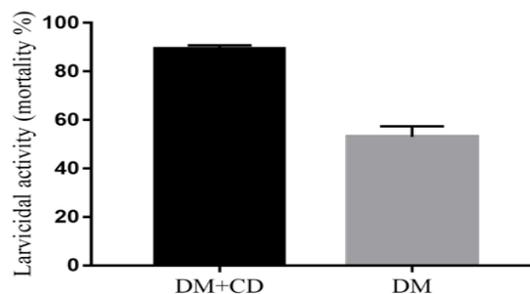


Figure 1: The larvicidal activity comparison between the two experimental groups.

Cyp6p15 expression

Cyp6p15 was significantly inhibited in the DM+CD group when comparing that in the DM group that showed overexpression of this gene (Figure 2).

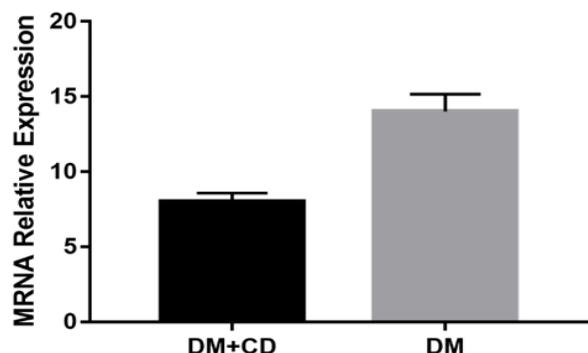


Figure 2: The MRNA relative expression in the two experimental groups.

Discussion

Resistance to insecticide is an important global subject that affects people life in different health and economic subjects. Mosquitoes are a well-known vector to transmit different pathogens such as plasmodium (4), dengue virus (5), the west Nile virus (6), the yellow fever virus (7), Zika virus (8). Controlling of these diseases depends highly on controlling those vectors; however, the process has become not easy as insecticide resistance emerges every day to different insecticides, the old or the newly invented insecticides (10). Mosquitoes are being understood to develop insecticide resistance via different mechanisms such as the P450 monooxygenase system present in mosquitoes that acts to generate detoxification of insecticides by P450-dependent degradation processes (11). According to this information, the current work was important to be performed to place more successful procedures to control mosquitoes.

The results, here, presented interesting data that *Aedes albopictus* larvae had resistance against deltamethrin. According to Li *et al.* (18) who found resistance in larvae of *Aedes albopictus* against deltamethrin in China, the current results completely agree with that work. The latest workers suggested that this resistance was initiated due to activities of certain enzymes such as P450 monooxygenase especially in adult mosquitoes (18). Difficulty in controlling larval mosquitos or developing resistance against pyrethroid by larvae belong to this mosquito species could end up with spreading and emerging of different diseases that this mosquito transmits such as Dengue and Chikungunya (21). *Aedes albopictus*, Asian tiger mosquito, is a mosquito species that actively feed on blood by biting during day time (22,23) and was also found to transmit viruses such as

Zika virus (24). Controlling this mosquito via inventing different procedures to overcome the problem of insecticide resistance is important to stop spreading of these pathogens via this mosquito.

Interestingly, the current work results identified that cinnamaldehyde improved the activity of deltamethrin against the larvae employed for this work. *Cinnamomum osmophloeum* leaf essential oil was found to act as a larvicide against *Anopheles gambiae s.s* larvae (17,25), and these co-workers predicted that this activity might have been due to cinnamaldehyde (26). The current work used cinnamaldehyde with deltamethrin to synergize the activity of each other resulting in high rates of mortalities in these larvae. This work results provide viable information about the potential activity of the cinnamaldehyde in synergizing the larvicidal activity of deltamethrin.

Conclusion

To evaluate the resistance status of larvae of mosquito to deltamethrin. We use cinnamaldehyde to study there larvicidal activity of deltamethrin against larvae of mosquito (*Aedes albopictus*). Larvicidal activity was of deltamethrin synergized and showing mortality reach to 92% when use cinnamaldehyde.

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Conflict of interests

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