GSC Biological and Pharmaceutical Sciences, 2020, 13(01), 148-155

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Toxicity of two groups of pesticides against the mosquito *Aedes aegypti*

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Publication history**:** Received on 06 October 2020; revised on 15 October 2020; accepted on 20 October 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.13.1.0334

Abstract

The mosquito *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) is a vector for several pathogens that affect human health worldwide. Therefore, mosquito control is the best approach to prevent disease outbreaks. In this milieu, it is preferable to evaluate the effectiveness of chemical pesticides at regular intervals to identify the most effective ones and use them during the outbreaks of diseases and spread of pests. Here, we aimed to study the toxicity of six pesticides, which are classified under two groups, namely pyrethroids and organophosphates, against *A. aegypti* mosquitoes to improve disease control in Saudi Arabia. Hortak was the most effective in larval mosquito control (LC50 = 0.0031 ppm), followed by Aquapal Super 20 EW (LC50 = 0.0389 ppm), whereas Solfac was the least effective (LC50 = 0.1119 ppm). In addition, the sensitivity of the tested larvae to Safrotin and Keen 600 EC was 8.1 and 58.9 times higher than that to Resfin-5, which was the least effective, respectively. Hortak and Safrotin exhibited the highest toxicity against the larvae of *A. aegypti*. Our findings confirm that the tested pesticides can be used in mosquito-control programs during epidemic outbreaks and emergency.

Keywords: Bioassay; Larva; LC50; Organophosphate; Pyrethroid**.**

1. Introduction

Mosquitoes are distributed worldwide and inhabit diverse environmental conditions [1]. They are an annoyance to human beings owing to their painful bites [2]. Moreover, mosquitoes transmit several pathogens. *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) mosquitoes can grow indoors on small quantities of clean or polluted water and transmit several pathogens such as dengue, yellow fever, chikungunya, and Zika viruses, thus threatening human life [3, 4, 5] . In 2019, the World Health Organization (WHO) announced that approximately 50 million people a year develop dengue fever and 2.5 billion people live in endemic areas. In 2013, the infection rate increased to 390 million people [6]. It has been estimated that the number of people at risk of dengue fever is 3.9 million in 128 countries [7]. An estimated 500 000 people suffer from severe dengue fever each year, and approximately 2.5% of them die of it [8]. Furthermore, there are expectations of increase in the number of people infected with mosquito-borne diseases, especially dengue fever, which is one of the most important viral diseases in several countries [9]. In the western and southern regions of Saudi Arabia, dengue has become endemic [10, 11, 12]. In 2011, there was a pandemic, with 4411 reported cases, of which eight died [13, 14]. In 2009, dengue fever was reported in other areas such as Medina, Asir, and Jazan 2013 [15]. During outbreaks, rapid intervention is needed to eliminate mosquitoes and reduce their spread. According to the WHO [16], mosquito control relies on reducing the population density of mosquitoes in the environment to the extent that they do not cause any health problems, as an unpleasant pest or a disease vector. Therefore, the WHO recommends that control

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programs should be continued, while further developing the traditional methods. Although many types of pesticides are available, which are still highly effective against mosquitoes, their continued use has led to the emergence of resistance in mosquitoes against some pesticides [17]. The problem of mosquito resistance to chemical pesticides is increasing worldwide [18, 19, 20, 21, 22]. Therefore, the effectiveness of pesticides used in the control programs should be evaluated on a regular basis to ensure that appropriate decisions are made when urgent interventions are needed [23], especially, considering the limited studies on pesticides used in mosquito control programs in the western and southern regions of Saudi Arabia. Biological assessment of several pesticides against mosquito species has been conducted [24, 25, 26, 27]. In Colombian–Caribbean Region, Maestre-Serrano et al. [28] determined the susceptibility and resistance of *A. aegypti* mosquitoes to numerous organophosphorus pesticides, pyrethroids, and DDT.

In the present study, we aimed to assess the effectiveness of two groups of pesticides, namely pyrethroids and organophosphates, in order to identify the most efficient ones for effective use under emergency situations. The toxicity of three pyrethroid insecticides, namely, Aquapal Super 20EW, Solfac EC 050, and Hortak 50 EC, and organophosphate pesticides, namely, Safrotin, Keen 600 EC, and Resfin, against *A. aegypti* larvae was measured after 24 h of pesticide treatment. The insecticidal effect of these pesticides on the larvae of *A. aegypti* mosquitoes was evaluated by calculating the lethal concentration of 50% and 90% (LC50 and LC90, respectively).

2. Material and methods

2.1. Specimen collection

A strain of *A. aegypti* was collected from Taif Governorate (western region of Saudi Arabia). A plastic dipper equipped with aluminum arm (160 cm length) was used to collect samples from water in mosquito-positive sites such as watersheds of swamps and valleys. The larvae were placed in 300-mL plastic jars, and then transferred to the laboratory at 27 ± 1 °C and 70% ± 5% relative humidity, with 14:10 h (L:D) photoperiod.

2.2. Larvae rearing

The larvae were reared in glass trays (20 cm L × 30 cm W × 6 cm D) filled with water and fed dried bread powder, instant yeast, and dried milk in the ratio of 1:1. The newly formed pupae were removed from the trays to a plastic cup containing water and placed into screened cages (30 cm × 30 cm × 30 cm), where the adult emerged later. The adults were fed animal blood (pigeon) for 2 h a day for three days. The following generations were also reared to obtain abundant larvae for experimental purpose [29].

2.3. Material used and tested insecticides

The sensitivity of *A. aegypti* larvae against conventional pesticides was evaluated to determine the effectiveness of the pesticides by calculating the percentage of mortality.

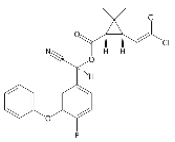
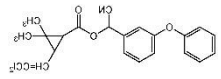
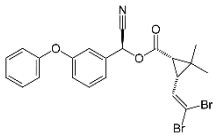
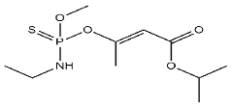
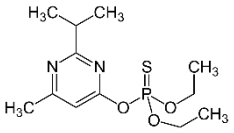
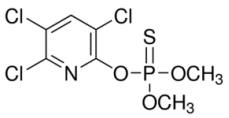
Conventional pesticides tested against *A. aegypti*. The pyrethroids and organophosphates presented in Table 1 were used against *A. aegypti* larvae.

The standard stock solution of the selected pesticides was prepared by adding 0.1 mL of pesticide into 100 mL of distilled water in a standard flask (100 mL).

2.4. Bioassay

The larval sensitivity test, following the WHO standard method [30], with pyrethroids and organophosphates, was carried out by taking into account the LC50 and LC90 for the treated larvae. Probability regression lines were drawn for the tested insecticides and statistical parameters were also calculated using the method of [31]. A series of standard solutions was prepared at different concentrations selected according to the method used by the WHO (Table 1).

Table 1 Conventional pesticides tested against *A. aegypti* larvae.

	Pesticide	Active ingredient	Chemical structure	Group
1	Solfac EC 50	Cyfluthrin 50%		Pyrethroid
2	Hortak EC 50	Cypermethrin 10%EC+Tetramethrinl1.5%)(		Pyrethroid
3	Aquapal Super 20EW	Deltamethrin 2%		Pyrethroid
4	Safrotin 20 MC	Propetamphos 20%		Organophosphate
5	Keen 600 EC	Diazinon 60%		Organophosphate
6	Resfin	Chlorpyrifos methyl 30%		Organophosphate

The experiments were conducted using white plastic plates (11 cm diameter, 4 cm depth, 250 mL volume) filled with 100 mL of water for each pesticide. There were five replicates per concentration. Twenty larvae (at the end of the third and beginning of the fourth instar stages) were placed in each dish. In addition to the treated samples, five control replicates were used; the larvae in the control plates were provided food during the test. After 24 h of treatments, the dead larvae, which did not move when touched by an autopsy needle in the siphon or neck area, were counted. The moribund larvae were also counted, which could not rise to the surface of the water or sink into the water when the water was shaken.

Abbott's equation [32] was used to adjust the death rate in case of death of more than 5% and less than 20% of the control larvae. LdP-Line software (Ehab Bakr) was used to draw toxicity lines and to infer other statistical parameters such as LC50, IC90, and Slop value, and $\chi 2$ data were obtained according to the method of [33].

3. Results

3.1. Pyrethroid insecticides

The results presented in Table 2 and Figure 1 show a direct correlation between increasing concentrations of pesticides and percentage of larval death. However, Solfac EC 50 was found to be the least effective pesticide against *A. aegypti* mosquito. The effective concentrations of pesticides ranged from 0.05 to 0.5 ppm and larval mortality ranged from 21.429% to 89.796%, respectively. On the contrary, the toxicity lines in the graphs of the three tested pesticides and their statistical constants (see Tables 2 and 3) showed a difference between the concentrations required to kill 50% and

90% of the treated larvae (LC50 and LC90). For Hortak, the LC50 was 0.0031 ppm (95% confidence interval 0.0027–0.0036), whereas the LC90 was 0.0139 ppm (95% CI 0.0111–0.019). With respect to Solfac, higher concentrations were required to kill 50% of the larvae (LC50 = 0.1119 and 95% CI = 0.0958–0.1289), and the lethal concentration, that is, LC90, was 0.04943 ppm (95% CI 0.3908–0.6814).

The resistance ratio (RR) and toxicity line values showed that the activity of Hortak was 12.6 and 36.1 times higher than that of Aquapal Super and Solfac, respectively. The values of Hortak, Aquapal Super, and Solfac were 7.4072, 1.0415, and 2.1924, which were less than the χ2 value (7.8) at a significance level of 0.05 and degree of freedom (n) of 3 (Table 2 and Figure 1).

Table 2 Sensitivity of the fourth instar larvae of *A. aegypti* after exposure to different concentrations of the pyrethroid pesticides for 24 h.

	Hortak		**Aquapal Super 20 EW**		**Solfac**	
	Concentration (ppm)	Mortality %	Concentration (ppm)	Mortality %	Concentration (ppm)	Mortality %
	0.001	16	0.02	15.306	0.05	21.429
	0.003	32	0.03	31.633	0.08	39.796
	0.005	62	0.05	63.265	0.15	65.306
	0.008	76	0.06	77.551	0.3	77.551
	0.01	90	0.08	89.796	0.5	89.796
Control	3		3		3	
LC_{50} (L. limit- U. limit)	0.0031 0.0027–0.0036		0.0389 0.0359–0.0419		0.1119 0.0958–0.1289	
LC_{90} (L. limit- U. limit)	0.0139 0.0111–0.019		0.0842 0.0748–0.0983		0.4943 0.3908–0.6814	
Slope	1.9876 ± 0.1856		3.818 ± 0.3167		1.9866 ± 0.1825	
Tabulated χ^2	7.8		7.8		7.8	
Calculated χ^2	7.4072		1.0415		2.1924	

*The calculated χ2 value was less than the tabular value for significant difference and homogeneous strain and the line is a good representative of the results.

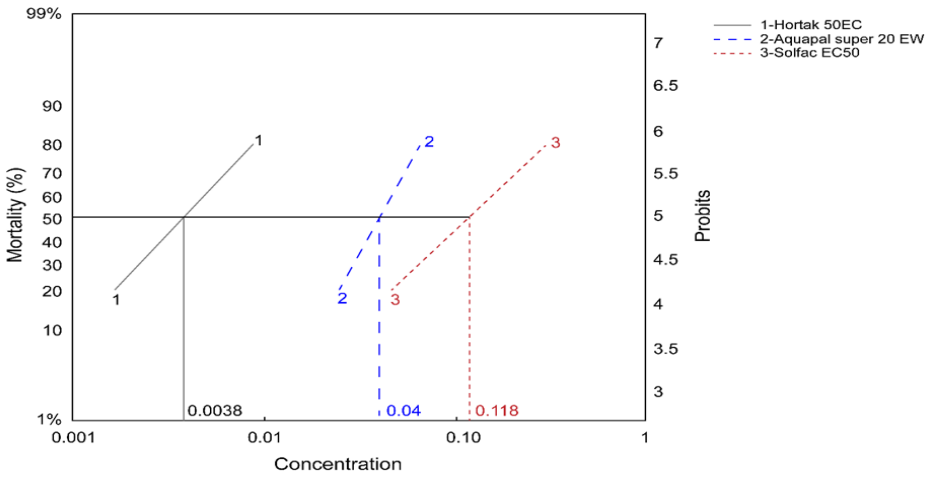


Figure 1 Toxicity line of pyrethroid insecticides against *A. aegypti* larvae after 24 h of treatment.

3.2. Organophosphate pesticides

The results presented in Table 3 and Figure 2 show that the effective concentrations of Safrotin ranged from 0.01 to 0.2 ppm and the percentage of larval death at these concentrations ranged from 16% to 90%, respectively, whereas the effective concentrations of Keen 600 EC ranged between 0.1 and 1 ppm and the death percentage of the treated larvae was 13%–94%. For Resfin-5, the effective concentrations ranged between 0.5 and 15 ppm. The percentage of larval deaths corresponding to these concentrations ranged from 14% to 90%.

Table 3 Sensitivity level of the fourth instar larvae of *A. aegypti* after exposure to different concentrations of organophosphate pesticides for 24 h.

	Safrotin		keen 600 EC		Resfin-5	
	Concentration (ppm)	Mortality %	Concentration (ppm)	Mortality %	Concentration (ppm)	Mortality %
	0.01	16	0.1	13	0.5	14
	0.03	32	0.3	35	1	33
	0.06	62	0.6	68	5	68
	0.09	76	0.9	81	10	78
	0.2	90	1	94	15	90
Control	3		0.0		3	
LC_{50} (L. limit- U. limit)	0.0444 0.038–0.0514		0.3556 0.214–0.5038		2.5899 2.1148–3.1397	
LC_{90} (L. limit- U. limit)	0.2042 0.1603–0.2817		1.199 0.9474–2.7332		18.3821 13.6955–26.8322	
Slope	1.9329 ± 0.1646		2.4282 ± 0.1979		1.5058 ± 0.1176	
Tabulated χ^2	7.8		7.8		7.8	
Calculated χ^2	3.5614		10.5029		2.9717	

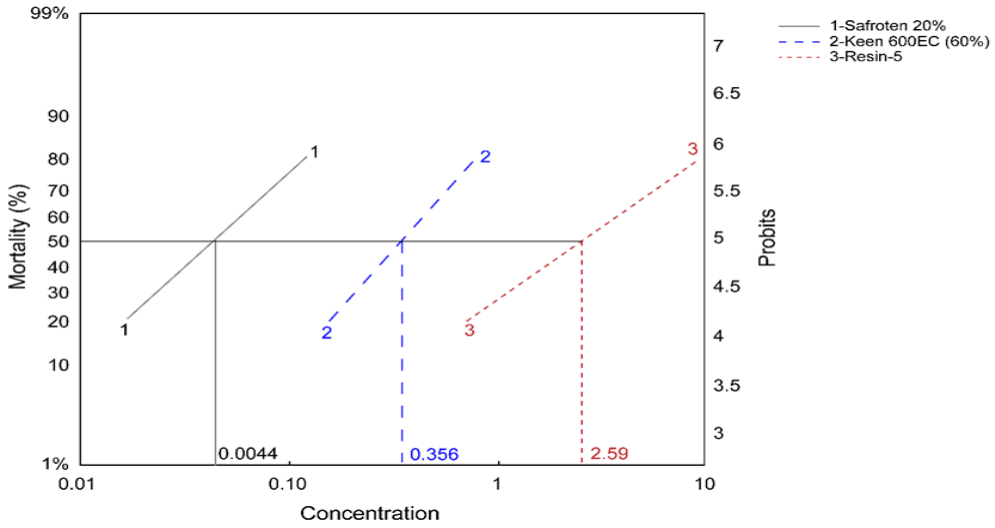


Figure 2 Toxicity line of organophosphate insecticides used against *A. aegypti* larvae after 24 h of treatment.

There was a difference in the LC50 and LC90 when used against *A. aegypti* larvae. The LC50 of Safrotin was 0.0444 (95% CI 0.038–0.0514) and LC90 was 0.2042 ppm (95% CI 0.1603–0.2817). The LC50 of Keen 600 EC was 0.3556 ppm (95% CI 0.214–0.05038), whereas the LC90 was 1.199 ppm (95% CI 0.9474–2.7332). The LC50 of Resfin-5 was 2.5899 (95% CI 2.1148–3.1397) and LC90 was 18.3821 ppm (95% CI 13.6955–26.8322).

In general, the results showed that the sensitivity of the tested larvae against Safrotin and Keen 600 EC was 8.1 and 58.9 times higher than that against Resfin-5, which was the least effective, respectively. This was confirmed by the tendency of toxicity line in the y-axis; the closer the toxicity line to the y-axis, the more effective the pesticide and more sensitive the larvae, and vice versa. The $\chi 2$ value of Safrotin (3.5614) was lower than that of the tabular value (7.8); the $\chi 2$ value of Keen 600EC and Resfin-5 was 10.5029 and 2.9717, which indicate homogeneity among the tested compounds.

4. Discussion

In the present study, the toxicity of organophosphorus (Safrotin, Keen 600 EC, and Resfin) and pyrethroid pesticides (Hortak, Aquapal Super, and Solfac) was evaluated and the exterminating effect of these pesticides on the fourth instar larvae of *A. aegypti* mosquito after 24 h was determined using the LC50 and LC90 values. The results of the present study showed that the response of the fourth instar larvae of *A. aegypti* against the tested pesticides depends on the type of pesticide used, action mechanism, and effective concentrations. This was confirmed by the differences in the percentage of larval mortality, which increased steadily with increasing concentration. The results of this study are consistent with those of previous studies and bioassay experiments against different species of mosquitoes. [25, 26, 27] have reported differences in the mortality of the fourth instar larvae according to the concentration and duration of exposure to pesticides. In the present study, Hortak EC50 was found to be more effective and more toxic than other pesticides, and its effective concentration range was 0.001–0.01 ppm, and the associated mortality rate was 90%. For Aquapal Super (20 EW), the effective concentration range was 0.02–0.08 ppm and the larval death rate was 15.306%–89.796%. Consistent with our findings, Al-Ghamdi et al. [34] and Al-Ghamdi and Mahyoub [17] confirmed the efficacy of pyrethroids against *A. aegypti* mosquitoes. Pyrethroids act as modifiers of the sodium channel and as toxins on the axons by blocking the sodium channels in both peripheral nervous systems and CNS, which results in frequent neurological flows causing paralysis and death of insects [35]. On the contrary, the results showed differences in the effect of organophosphorus pesticides on mosquito larvae. Safrotin was the most effective, followed by Keen 600 EC, whereas Resfin-5 was the least effective on the fourth instar larvae of mosquitoes. The results are consistent with those of Mahyoub et al. [36], who evaluated the effectiveness of a group of pesticides against *Culex* mosquito. Organophosphate pesticides bind to the cholinesterase and prevent it from binding to acetylcholine, causing its accumulation. It interferes with neuromuscular contact causing rapid tremors in the voluntary muscles, paralysis, and death due to failure of breathing [35].

5. Conclusion

The results of present study showed variation in mosquito response to different pesticides. Identification of the most effective pesticides is useful for reducing the problems associated with mosquito resistance to frequently used pesticides, especially during the spread of diseases. Our findings can be combined with those of other studies to create a database of pesticides that can be used in control programs.

Compliance with ethical standards

Acknowledgments

We are thankful to the team of the Research Unit of Dengue Fever and Vector Control, at King Abdulaziz University, for their support and providing facilities to complete this research.

Disclosure of conflict of interest

There is no conflict of interest.

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