EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 1
147-150
Year: 2018

SUSCEPTIBILITY OF *ANOPHELES* MOSQUITOES TO AGRICULTURAL PESTICIDES (MALATHION, CARBARYL)

[1]*Dr. Mohammed Ismail Humaida, [2]Abdulgadir Almubark Eltyeb and [1]Dr. Ahmed Subahi Ahmed

[1]Department of Public and Environmental Health, Faculty of Medicine and Health Sciences, University of Kordofan, Sudan.
[2]Central Darfur State Minstiry of Health.

***Corresponding Author: Dr. Mohammed Ismail Humaida**
Department of Public and Environmental Health, Faculty of Medicine and Health Sciences, University of Kordofan, Sudan.

Article Received on 15/11/2017 **Article Revised on 05/12/2017** **Article Accepted on 25/12/2017**

ABSTRACT

Background: Insecticide-tolerant strains of the mosquito vector have been identified as key factors of resurgence of Malaria. **Objective:** This study aimed to examine the susceptibility of *anopheles* mosquitoes to agricultural pesticides (Malathion and Carbaryl). **Methodology:** Larvae of *Anopheles gambiae* mosquitoes were collected from El-Mogran area, Khartoum State, Sudan. The collection process was performed using an enamel bowl to collect larvae from the edge of body of water. Within two hours after collection, all sampled larvae were transported to medical entomology laboratory. In the laboratory, the mosquitoes were divided into control group and study groups; the control group were not exposed to any pesticides except water, and the study groups were exposed to different concentrations of Malathion and Carbaryl pesticides. Tests of larval susceptibility of mosquitoes were conducted folowed the recommendations of WHO materials and methods. The results recorded after 24h. **Results:** The study revealed that there is difference in mosquitoes mortality for the same dose, in the trials of Malathion, the higher concetration that achieved 100% kill is $2.5x10^{-5}$ %, (0.25 mg/L). While in the trials of Carbaryl, the higher concetration that achieved 100% kill is $4.25x10^{-4}$ % (4.25 mg/L). The study revealed that the lethal concentration LC_{50} for Malathion and Carbaryl is 3x 10^{-6} %, (0.03mg/L) and $1x10^{-5}$ %, (0.1 mg/L) respectively. Malathion more toxic to mosquitoes as compared with Carbaryl. **Conclusion:** Both Malathion and Carbaryl have toxic effect on mosquitoes. There should be co-ordination between ministry of health and ministry of agriculture to control of malaria vectors and agricultural pests together.

KEYWORDS: Susceptibility, Mosquitoes, Malathion, Carbaryl.

INTRODUCTION

Malaria remains one of the most widespread, potentially fatal infectious diseases. It is an important public health concern both in those countries where transmission occurs regularly, as well as in areas where transmission has been largely eliminated.[1]

Each year an estimated 300–500 million clinical cases of malaria occur, making it one of the most prevalent infectious diseases.[2] According to the latest estimates, there were about 198 million (124-283 million) cases of malaria in the year 2013 and an estimated 584,000 deaths (367,000-755,000). Malaria mortality rates have fallen by 47 percent globally since year 2000 and by 54 percent in the WHO African Region. Most deaths occur among children living in Africa, where a child dies every minute from malaria.[3] The development and spread of drug-resistant strains of malaria parasites and insecticide-tolerant strains of the mosquito vector have been identified as key factors of resurgence.[4]

Malaria is transmitted by the bite of certain species of infected, female, anopheline mosquitoes. A single infected vector, during her life time, may infect several persons.[5] The big problem in control of malaria is developing of resistance to insecticides by vectors. Since 2010, resistance to at least one class of insecticides has been reported in at least one malaria vector species in 60 of the 96 malaria-endemic countries that conducted monitoring; also, 49 countries reported resistance to at least two classes of insecticide. Resistance to all four available classes of insecticide has been reported. Resistance to pyrethroids was most commonly reported, with three quarters of countries that monitored this class in 2014 reporting resistance.[6]

Malathion is a man-made organophosphate insecticide that is commonly used to control mosquitoes and a variety of insects that attack fruits, vegetables, landscaping plants, and shrubs. It can also be found in other pesticide products used indoors and on pets to control ticks and insects, such as fleas and ants.

Malathion is the active ingredient in mosquito control products including Fyfanon and Atrapa. These products contain over 95% malathion and are often applied undiluted. However, they may be diluted with a petroleum solvent similar to kerosene before application, in which case petroleum solvent will make up most of the pesticide solution.[7]

Carbaryl is the common name for a chemical known as 1-naphthyl methylcarbamate. Carbaryl belongs to a family of chemicals that kill or control insects (insecticides) known as carbamates.[8] Carbaryl is used to control a wide variety of pests, including moths, beetles, cockroaches, ants, ticks, and mosquitoes. Products with carbaryl can be formulated as dusts, wettable powders, liquid concentrates, granules, or baits. Carbaryl products are used on fruits, vegetables, rangeland, lawns, ornamental plants, trees, and building foundations.[9]

This study aimed to determine the susceptibility of *anopheles* mosquitoes to agricultural pesticides (Malathion and Carbaryl).

MATERIALS AND METHODS

Study area

El-Mogran area located in Khartoum State, which is located in North Eastern part of the centre of Sudan. The state is located between 21^o, 25-24^o, 45 East and 15^o, 9-16^o, 45 North. The state covers 20, 736km2. Khartoum State is divided into three administration governorates: Khartoum, Omdurman and Khartoum North. The governorates are bounded by North Kordofan in the west and in the north by Nile River State and in the north west by the Northern State and by the White Nile State in the South and Gazeera State in the east. The climate is Semi-desert, dry and hot in summer (maximum temperature of 47.1oC and minimum temperature of 22.7oC. The range of rainfall is 150 mm per year.[10]

Collection of mosquito's larvae

The larvae of *Anopheles gambiae arabiensis* mosquitoes were collected from stagnant water due to rain and leakage of piped water supply system in Al-Mogran area, Khartoum State. The collection process was performed using an enamel bowl to collect larvae from the edge of body of water. Many dips were taken. The collected larvae were pooled in plastic containers, supplied with water from their collecting site. Within two hours after collection, all sampled larvae were transported to medical entomology laboratory, Faculty of Public and Environmental Health, University of Khartoum, in order to carry the experiments.

Test procedures

In the laboratory, the larvae were let to spend 72 hours to avoid any external effect on the experiments, and the larva of mosquito were maintained in a room temperature, in a good ventilation.

Insecticides

Susceptibility tests with larvae were conducted with two standard solutions of Malathion and Carbaryl. Test procedures conducted according to World Health Organization (WHO), methods and materials.[11]

Intervention and mortality calculation

About 25 larvae were placed in each exposure container that contain solution (water and insecticides), the larva leaved of 24h and then we calculate mortality.

The mortality of the test sample is calculated by summing the number of dead mosquito larva across all exposure replicates and then expressing this as a percentage of the total number of exposed larvae of mosquitoes:[12]

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$$

A similar calculation should be made in order to obtain a value for the control mortality. If the control mortality is ≥20%, the tests must be discarded. When control mortality is <20%, then the observed mortality must be corrected using Abbott's formula, as follows:[13,14]

$$\text{Corrected mortality} = \frac{(\%\text{observed mortality} - \%\text{ control mortality})}{(100 - \%\text{ control mortality})} \times 100$$

If the control mortality is <5%, no correction of test results is necessary, whereas mortality of ≥5% requires correction.

Data analysis

The bioassay with larvae of mosquitoes was analyzed for the 24h. LC_{50} for each test was determined by probit analysis.[15] This was calculated by finding the probit value of the percentage mortality from the probit table and plotting it against the logarithm of different concentrations. A horizontal line was drawn from the 50% (5% probit value) to meet the line graph. The intersection point on the abscissa corresponded to the 24h LC_{50}.[16]

RESULTS AND DISCUSSION

In this study, we conducted four trails for each concentration for both Malathion and Carbaryl in the same conditions. The mortality of mosquitos larvae appear in real form because there is no mortality in control group.

Mortality was determined after 24h from exposure to insecticides (Malathion, Carbaryl), the average of four trails was taken. The study revealed that there is difference in larvae mortality for the same dose, and this may be due to variations of some morpho-physiological characteristics, such as lager size, thicker cuticle and increase fat content or may be due to cross resistance, which is produced by insecticides belonging to the same group of studied pesticides.[13]

In This study, the trials conducted with Malathion found that the higher concetration that achieved 100% kill is $2.5x10^{-5}$ %, (0.25 mg/L), (**table.1)**, which is less than that found in similar study conducted in Salamanca Province, Spain, which revealed that the concentrations of Malathion resulting in 99.9% kill of mosquitoes were 1.3640 ppm.[17] While in trials conducted with Carbaryl the study found that the higher concetration that achieved 100% kill is $4.25x10^{-4}$ % (4.25 mg/L), (**table.2)**.

The trails excuted with Malathion and Carbaryl revealed that the lethal concentration LC_{50} for Malathion is $3x10^{-6}$ **%, (**0.03 mg/L)**,** and for Carbaryl is $1x10^{-5}$ **%, (**0.1 mg/L)**, (table.3).** The trails revealed that Malathion is more toxic for mosquitoes larvae as compared with Carbaryl, because the LC_{50} of Malathion is lower than LC_{50} of Carbaryl, this relationship proved by (University of Minnesota) which said that ″the lower the LC_{50} the more toxic the chemical″.[18] Thus the possibility of developing resistance is more in Carbaryl than Malathion, because Carbaryl is more used in agricultural pests control, thereby mosquitoes may be exposed to small doses of Carbaryl frequantly, which contribute in developing of resistance.

Table 1: The average of four trials of Malathion toxicity on mosquito's larva for 24 hours.

Concentration %	Log of concentration	Log of Concentration +7	% of corrected mortality in tests (experiments)				Average of corrected mortality of 4 trails	Probit
			Trail 1	Trail 2	Trail 3	Trail 4		
$2.5x10^{-5}$	-4.6021	2.3979	100%	100%	100%	100%	100%	7.33 ≈
$2x10^{-5}$	-4.6990	2.3010	88%	92%	100%	100%	95%	6.64
$1.5x10^{-5}$	-4.8239	2.1761	80%	96%	92%	100%	92%	6.41
$2.5x10^{-6}$	-5.6021	1.3979	48%	36%	32%	48%	41%	4.77
$2x10^{-6}$	-5.6990	1.3010	40%	32%	24%	28%	31%	4.50
$1.5x10^{-6}$	-5.8239	1.1761	32%	28%	20%	16%	24%	4.29
$1x10^{-6}$	-6.0000	1.0000	32%	24%	16%	48%	30%	4.48
$2.5x10^{-7}$	-6.6021	0.3980	36%	24%	12%	16%	22%	4.23
$2x10^{-7}$	-6.6990	0.3010	28%	20%	8%	20%	19%	4.12

Table 2: The average of four trials of Carbaryl toxicity on mosquito's larva for 24 hours.

Concentration %	Log of concentration	Log of Concentration +7	% of corrected mortality in tests (experiments)				Average of corrected mortality of 4 trails	Probit
			Trail 1	Trail 2	Trail 3	Trail 4		
$4.25x10^{-4}$	-3.3716	3.6284	100%	100%	100%	100%	100%	7.33 ≈
$2.55x10^{-4}$	-3.5934	3.4066	100%	100%	100%	100%	100%	7.33 ≈
$4.25x10^{-5}$	-4.3761	2.6239	68%	92%	100%	100%	90%	6.28
$3.4x10^{-5}$	-4.4685	2.5315	62%	84%	100%	100%	86.5%	6.08
$2.55x10^{-5}$	-4.5935	2.4065	44%	56%	96%	96%	73%	5.61
$1.7x10^{-5}$	-4.7696	2.2304	36%	48%	60%	56%	50%	5.00
$4.25x10^{-6}$	-5.3716	1.6284	32%	20%	20%	16%	22%	4.23
$2.55x10^{-6}$	-5.5935	1.4065	20%	16%	16%	66%	24%	4.29
$4.25x10^{-7}$	-6.3716	0.6284	16%	12%	12%	24%	18%	4.08

Table 3: The lethal concentration LC_{50} of Malathion and Carbaryl for mosquitoes.

Insecticides	Lethal Concentration LC_{50}	
	%	mg/L
Malathion	$3x 10^{-6}$	0.03
Carbaryl	$1x10^{-5}$	0.1

CONCLUSION

Both Malathion and Carbaryl have toxic effect on larvae of *Anopheles* mosquitoes, but the Malathion is more toxic than Carbaryl. There should be co-ordination between ministry of health and ministry of agriculture to control of schistosomiasis snails and agricultural pests together.

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