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Susceptibility *Aedes aegypti* to Malathion and permethrin Insecticides in Kassala City, Sudan

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Abstract

Insecticidal measures, especially in the outbreak-risk areas, are the most important for the control of Aedes aegypti, the main vector of dengue fever and dengue hemorrhagic fever. Mosquito larvae were collected from the 20 clusters in Kassala City, Sudan, during winter, and autumn 2015. Adults Ae. aegypti were tested for susceptibility to permethrin and malathion using WHO (1998) standard procedures and test kits for female mosquitoes. Tests were carried out from different breeding sites where larvae were reared in cages and fed on 10% sugar solution from cotton at insectary of malaria in Kassala City. Females of Ae. aegypti (non-blood fed) 24-48 hours post emergence or five days old were used. The patterns of insecticide susceptibility to malathion, and permethrin of Ae. aegypti was determined. The adult's populations were found highly susceptible to malathion 5% and permethrin 0.75% insecticides. Frequent insecticide susceptibility test for Ae. aegypti is very crucial in drawing vector control programme strategies.

Key words: Malathion, Permethrin, Susceptibility, *Aedes aegypti*, Sudan

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1. INTRODUCTION

During the late 1930s, the new synthetic insecticides were discovered and they were widely used in the control of insect pests and vectors. DDT was first introduced for mosquito control in 1949 (Patterson, 2016). Resistance to DDT was first recorded in *Aedes stritaeniorhynchus* and *Aedes solicitans* in the year after its introduction (Abu Hassan, 2014). More than 100 mosquito species have been reported to be resistant to one or more insecticides worldwide (WHO, 1992). Rapid development of insecticide resistance has been recorded in many mosquito species (Hemingway and Ranson, 2000). Insecticide resistance occurs when a population of insects is exposed to insecticide for a period of time and at a frequent rate (Lee *et al*., 2003). The level or degree of insecticide resistance depends on the volume and frequency of insecticide application and other factors such as frequency of resistance gene(s) that with any mutation modifies the behaviour or the physiology of the insect vectors in a way that impairs the function of molecular targets (Nazni *et al*., 2005). Mosquitoes exhibit rapid insecticide resistance development because of their short life cycles and abundant number of progeny (Hemingway and Ranson, 2000).

Resistance of DDT has been reported and pyrethroid resistance is widespread in *Ae. aegypti* (Fonseca-Gonzalez *et al*., 2011) while development of resistance to organophosphates and carbamates was recorded in this species (Tikar *et al*., 2009). Permethrin resistance was noted in both *Ae. aegypti* and *Ae. albopictus* (Ponlawat and Harrinton, 2005) and resistance to pyrethroid was also reported in both species (Somboon *et al*., 2003). Mohsin *et al*. (2016) also reported *Ae. aegypti* was observed susceptible against malathion at all localities including Samanabad, Nishtar, Ravi and Iqbal towns with 100% mortality.

Resistance development rate is measured by LC_{50} and LT_{50} values (Paul *et al*., 2006). All 32 populations of *Ae. aegypti*

were observed to have proof of developing resistance (62.5%) or levels of survival deemed resistant (37.5%) to permethrin. Four populations of *Ae. albopictus* were noticed with incipient resistance (80-97% mortality) and one with resistance to permethrin (< 80%). 68.7% of the *Ae. aegypti* populations was susceptible (> 98% mortality) to deltamethrin. The KDT_{50} and KDT_{95} values indicated that malathion is more toxic than permethrin (Tiwari *et al*., 2010). Karen *et al*. (2012) examined the effect of rising larval rearing temperatures on the resistance of Trinidadian populations of *Ae. aegypti* to organophosphatic insecticides. The majority of larval populations reared at 28±2°C were susceptible (≥ 98% mortality) to fenthion but resistant (< 80% mortality) to malathion and temephos. Positive correlation was observed between resistance to organophosphates and enhanced activities of larval populations reared at 28±2°C. Although the larvae reared at increased temperatures showed differences in resistance levels against organophosphates, a general increase in susceptibility was noticed (Husham *et al*., 2010). The aim of this study was to determine susceptibility of *Ae. aegypti* to malathion 5% and permethrin 0.75% in Kassala City of eastern Sudan.

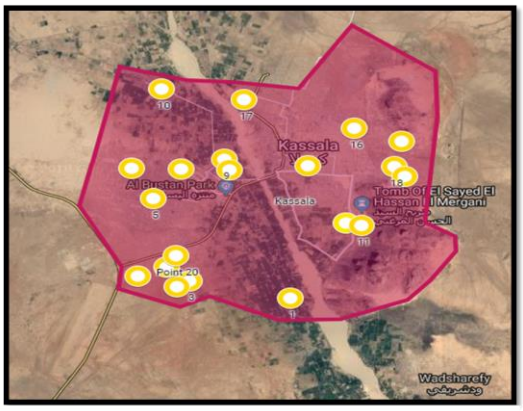
2. MATERIALS AND METHODS

2.1. Study design: entomological study was performed during the two seasons; dry (winter) and wet (autumn) for two consecutive years (2014 and 2015).

2.2. Study sites and mosquito surveillance

Kassala City is the capital of Kassala State at the eastern parts of the Sudan. The state has a total area of 55,374 km^2, lies between longitudes 34º 12' and 36º 57' E, and latitudes 15º 12' and 17o 12' N. (Himatt *et al*., 2015) (Map 1). Mean maximum temperature occurs in summer months with an average of 40ºC

in May and mean minimum temperature 15°C in January. The State falls within the arid and semi-arid region where rainfall is unreliable for domestic and economic uses. The average total annual rainfall is about 225 mm occurring dominantly between May to October while evaporation amounts to 2- 2.5 mm. Entomological surveys were carried out at 0600 hrs until at 1800 hrs and conducted in twenty households every season on both sides of Kassala city (East Algash (Alengaz East, Alengazsouth, Biriai, Mukram, Khatmia block 3and 4, Khatmia block 7and 8, Alnorab, Altora) and west Algash (Alkormota, BantAlmasna, BantNorth, BantSouth, GarbAlgash 2, GarbAlgash 3, Mastora a, Mastora b, Mastora d, Alsawagi South, Alshahid, Alsog Alshabi) in each of the 20 clusters in 2015. *Aedes* species larvae were collected from the natural breeding habitats (clay-pots, jerrycans, flower-vase, Tyres, Barrels, Cement containers basins, Water tanks) using a dipper. All water holding containers inside the households and around the households were inspected for immature stages of *Ae. aegypti*. Larvae was kept in plastic cups with 250 ml of water and labeled indicating location, date, time, house number, type of container, number of sample, indoor or outdoor collection, then, transported to the laboratory for identification according to the methods described by Rueda (2004), Cutwa and O'Meara, (2006), and Tun-Lin *et al.* (2009) and then the 4th larval instars of *Ae. aegypti* were reared in cages and fed on 10% sugar solution from soaked cotton at insectary of malaria in Kassala City.



No.	CLUSTER
1	Alsawagi South
2	Mastora d
3	Mastora a
4	Mastora b
5	BantSouth
6	BantNorth
7	BantAlmasna
8	GarbAlgash3
9	GarbAlgash2
10	Alkormota

No.	CLUSTER
11	Khatmia 7, 8
12	Khatmia 3, 4
13	Alengaz East
14	AL-Tora
15	Mukram
16	Alnorab
17	Biriai
18	Alengazsouth
19	Alshahid
20	Alsog Alshabi

1:70,000
Image source: Sentinal-2 LIC
Spatial resolution: 10 m
Spectral bands: RGB
Acquistion date: 21 Nov 2017
Geographic coordnate system: GCS_WGS_1984

Kassala City

Map of Kassala City where sites (o) of sampling are numbered
Source: National Center for Research

2.3. WHO procedures of insecticide susceptibility tests:
Insecticide susceptibility tests were performed using WHO (1998) standard procedures and test kits for female *Ae. aegypt* (non-blood fed) 24-48 hours post emergence or five days old were used. For each test, five replicates of 25 females were exposed to malathion 5% and permethrin 0.75% insecticide-impregnated test papers in test tubes for one hour. Each test included a control of (1x25) mosquitoes using impregnated papers in olive oil for malathion, and impregnated by silicone oil for permethrin test was conducted at temperature (25±2°C) and relative humidity (39.0±1.5%) while malathion test was at (29.1± 0.9°C) and relative humidity (36.7± 3.7%) During exposure time, the number of mosquitoes died was recorded on the susceptibility test forms after 5, 10, 15, 20, 25, 30, 40, 50, and 60 minutes of exposure. The females were, then, transferred into holding tubes supplied with a 10% sugar solution and kept at 27–28°C and 70–80% RH to verify the result of death or knockdown from exposure to insecticide.

Controls were also set up by exposing 25 mosquitoes to untreated papers. To avoid contamination among control papers, insecticide impregnated and clean papers, the control papers were handled first, followed insecticide-treated papers. For each insecticide type, different tubes were used which were separately kept during the test. Tests were carried out at 25±2°C and 70-80% RH. Different sets of pairs of forceps were used for handling each type of paper (Husham *et al*., 2010). The resistance/susceptibility status was evaluated using WHO (1998) standards. Mortality was recorded on the susceptibility test forms 24 hours after exposure and knockdown times (KDT_{50} and KDT_{95}) were calculated.

2.4. Data analysis:

Probit analysis was counted to calculate Knockdown time (minutes) to knockdown 50 and 95% (KDT_{50} and KDT_{95}) of exposed female *Ae. aegypti* population. WHO (2012) criteria for evaluating resistance or susceptibility in mosquito populations were used in which mortality rates of less than 80% indicated resistance while those ranged between 98%-100% indicated susceptibility. Mortality rates between 80–97% suggested the possibility of resistance (tolerance) which needed to be clarified.

3. RESULTS:

The results of susceptibility tests of *Ae. aegypti* to malathion 5% and permethrin 0.75% were according to WHO (2013) criteria in winter 2015 and autumn 2015 (Table1). Mortality after 24 hours was 100% for each test. Negative control using both olive oil for organophosphate group and silicon oil for pyrethroid showed no mortality. There were no significant differences ($P > 0.05$) between the insecticides tested and temperature ($p=0.895$), and no significant differences ($P > 0.05$) was observed between insecticides tested and relative humidity ($p = 0.840$) (Table 2). KDT_{50} during winter against malathion was

43.3 min and during autumn 32.7 min (Table 3). The KDT_{50} in winter was greater 1.3 folds than autumn 2015. KDT_{95} against malathion 5% during winter was 76.6 and in autumn 62.6. KDT_{95} in winter was greater than autumn by 1.2 folds. KDT_{50} against permethrin 0.75% was 25.4 min compared to 33.9 in autumn 2015, which decreased 0.7 times. The lowest KDT_{50} time indicated highly susceptibility, while in autumn the KDT_{95} increased in autumn versus winter by 1.5 times (61.3 min. vs. 40.1 min). Significant differences ($P \leq 0.05$) were observed between the two insecticides tested during different months (Table 3).

Table 1: Mean (±SE) percent mortality of female *Ae. aegypti* exposed to malathion 5% and permethrin 0.75% at temperature and relative humidity in Kassala City 2015

Insecticide	No. exposed	No. dead	mortality after24 hrs%	Temperature (oC)	Relative humidity%
Malathion 5%					
Winter	100.0±0.0	100.0±0.0	100.0±0.0	28.3±0.0	37.5±0.0
Autumn	100.0±0.0	100.0±0.0	100.0±0.0	31.5±0.0	40.5±0.0
Permethrin 0.75%					
Winter	100.0±0.0	100.0±0.0	100.0±0.0	28.2±±0.0	33.0±±0.0
Autumn	100.0±0.0	100.0±0.0	100.0±0.0	30.0±0.0	40.5±0.0
Control					
Winter	50.0±0.0	0.0±0.0	0.0±0.0	28.2±.05	35.2±2.2
Autumn	50.0±0.0	0.0±0.0	0.0±0.0	30.7±.7	40.5±0.0

Table 2: Analysis of variance (ANOVA) among insecticides tested at ambient temperature and relative humidity

Variables	Sum of Squares	Df	Mean Square	F	Significance Level	interpretation
Temperature						
Malathion	0.640	2	0.320	0.113	0.895	Not significant
Permethrin	14.120	5	2.824			
Total	14.760	7				
Relative humidity%						
Malathion	5.063	2	2.531	0.180	0.840	Not significant
Permethrin	70.313	5	14.063			
Total	75.376	7				

Table 3: Knockdown time 50% and 95% of female adult *Ae. aegypti* exposed to malathion 5% and permethrin 0.75%during winter and autumn 2015

Insecticide tested	Probability	95% Confidence Limits for Time (minutes)			Test of significance
		Time estimate (minutes)	Lower Bound	Upper Bound	
Malathion					
Winter	0.500	43.3	32.2	65.8	$\chi2$= 69.7,
	0.950	76.6	55.7	576.1	P-value =0.001
Autumn	0.500	32.7	16.8	56.9	$\chi2$= 125.5,
	0.950	62.6	42.6	1879.4	P-value =0.001
Permethrin					
Winter	0.500	25.4	20.7	29.5	$\chi2$= 19.6,
	0.950	40.1	33.5	62.6	P-value =0.001
Autumn	0.500	33.9	14.3	57.1	$\chi2$= 77.9,
	0.950	61.3	43.2	5971.3	P-value =0.001

KDT_{50} for permethrin was lower (25.4) min compared to (43.3) min for malathion in winter 2015. KDT_{95} permethrin had the lowest time (40.1) min compared to (76.6) min for malathion in winter 2015. In autumn 2015, the KDT_{50} for permethrin had higher time (33.9) min compared to (32.7) min for malathion, but the KDT_{95} for permethrin had the lowest time (61.3) min compared to (62.6) min for malathion (Fig. 1). A significant difference ($p < 0.05$) was observed among different months (Table 3).

The correlation coefficient ($r^2 = 0.734$), the strength of the straight-line or linear relationship, between the two variables was at the range of0.7-1.0 indicating a strong positive linear relationship via a firm linear rule. This relationship between the time and exposure response showed rapid increase in probability of insect knockdown as the time increased beyond 1.4 min. The shape of the $KDT_{50,\ 95}$ time-response curve is frequently linear and symmetric across different time of exposure (Fig. 2). The correlation coefficient (r^2=0.595), the strength of the straight-line or linear relationship, between the two variables was at the range of 0.3-0.7 indicating a moderate positive linear relationship via a fuzzy-firm linear rule. This

relationship between time and exposure response and showed rapid increase in probability of insect knockdown as the time increased beyond 1.3 min. The shape of the knockdown time-response curve is frequently linear and symmetric across different time of exposure (Fig. 3). The correlation coefficient (r^2=1.0) the strength of the straight-line or linear relationship between two variables was at the range of 0.7-1.0 indicating a strong positive linear relationship via a fuzzy-firm linear rule. The graph of the relationship between time and exposure response, showed rapid increases in probability of insect $KDT_{50, 95}$ as the time increases beyond 1.35 minutes. The shape of the knockdown time-response curves frequently linear and symmetric across different time of exposure (Fig. 4). The correlation coefficient (r^2=0.953) the strength of the straight-line or linear relationship between two variables was at the range of 0.7-1.0 indicating a strong positive linear relationship via a firm linear rule. The graph of the relationship between time and exposure response, shows rapid increases in probability of insect $KDT_{50, 95}$ as the time increases beyond 1.5 min. The shape of the $KDT_{50, 95}$ time response curve is frequently linear and symmetric across different times of exposure (Fig. 5).

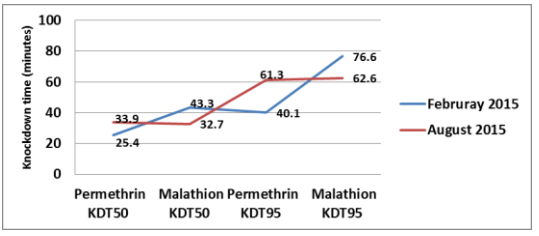
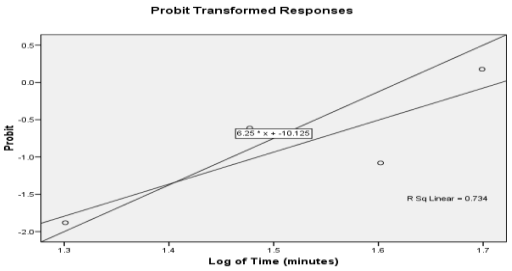
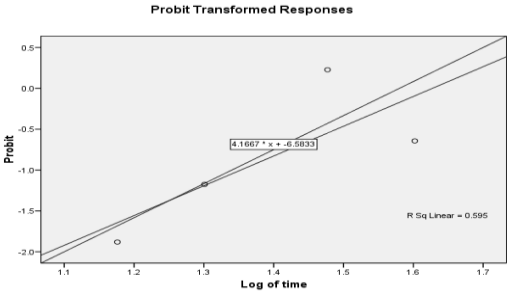


Fig. 1: Knockdown time 50% and 95% of female adult *Ae. aegypti* exposed to malathion 5% and permethrin 0.75% during winter and autumn 2015 in Kassala City.



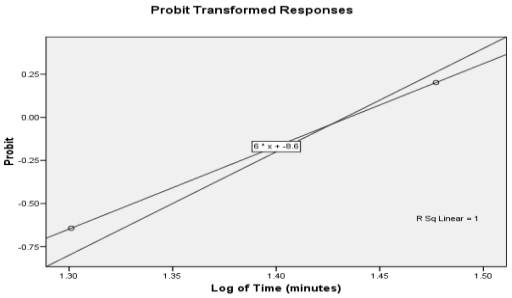
r^2=0.734 (0.7-.1.0) = strong positive linear

Fig 2: Probit transformed responses of female adult *Ae. aegypti* exposed to malathion 5% during winter 2015 in Kassala City



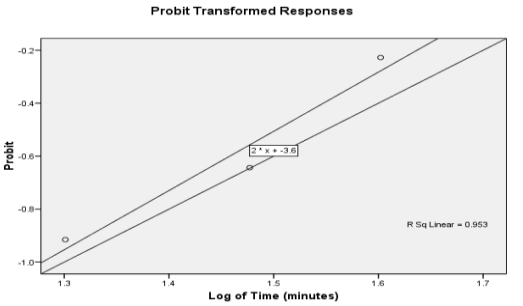
r^2=0.595 (0.3-.0.7) = moderate positive linear

Fig. 3: Probit transformed responses of female adult *Ae. aegypti* exposed to malathion 5% during autumn 2015 in Kassala City



r^2=1.0 (0.7-.1.0) = strong positive linear

Fig. 4: Probit transformed responses of female adult *Ae. aegypti* exposed to permethrin0.75% during winter 2015in Kassala City



r^2=0.953 (0.7-.1.0) = strong positive linear

Fig. 5: Probit transformed responses of female adult *Ae. aegypti* exposed to permethrin0.75% during autumn 2015in Kassala City

4. DISCUSSION:

Insecticide susceptibility of *Ae. aegypti* in Sudan especially in Kassala State is poorly documented. In this study, susceptibility tests applied on *Ae. aegypti* in different clusters of Kassala in two seasons (winter and autumn) excluding summer when authorities carried out a large scale insecticide spray in Kassala reducing the number of immature and mature stages of *Ae. aegypti*. WHO (2013) reported that mosquitoes prepared for susceptibility test should not be exposed to insecticides before such indoor residual spraying. In the current study, *Ae. aegypti* was found 100% susceptible to malathion 5% and permethrin 0.75%. The findings indicated that the population were virgin and had not been exposed to these insecticides. The finding agrees with that of Ponlawat and Harrington (2005) who found in Thailand that *Ae. aegypti* from all the sites were susceptible to malathion 5% and permethrin 0.75%. This finding is in contrast to Ponlawat and Harrington (2005b) who found that permethrin resistance was noted in both *Ae. aegypti* and *Ae. albopictus*. It also disagrees with that of Smith *et al*. (2016) who reported that pyrethroid resistance is widespread in *Ae. aegypti.* Other studies reported resistance of populations of *Ae.*

aegypti to permethrin in other parts of the world (Ayorinde *et al*., 2015).

The study showed that there was no significant difference (p=0.895) between insecticides tested and ambient temperature and ambient relative humidity. This may be because the relative humidity and temperature were adjusted in the laboratory where the test was conducted. The results are not in accordance to WHO (2013) which stated that the ambient temperature can influence the toxicity of insecticides; therefore, it is recommended to control temperature and humidity during the test and holding periods. Highly significant differences were observed between insecticides tested during different months for both malathion 5% and permethrin 0.75% insecticides in different seasons.

This may be due to seasonal variations in addition to rearing circumstances and feeding. The finding matched with the result obtained by Owusu *et al*. (2017). However, the KDT_{50} of *Ae. aegypti* against permethrin 5% was the lowest compared to that of autumn 2015. The finding indicated that the lowest knockdown time was highly susceptible, while in autumn the KDT_{95} increased in autumn compared to winter. The susceptibility of both insecticides used in the study might be returned to lack of used of such insecticides by agricultural site in Kassala City where the control only restricted to the municipality authority with rationale used to combat *Aedes* population. A similar finding was reported by Mohsin *et al*. (2016). The KDT_{50} and KDT_{95} of malathion 5% in the two seasons of *Ae. aegypti* was found to be greater than in permethrin 0.75%. Other study conducted by Tiwari *et al*. (2010) shared the same findings.

5. Conclusions

The results show that insecticide susceptible to malathion and permethrin occur in Kassala City. The results are very

important for the management of *Ae. aegypti* population in Kassala and to control the spread of its diseases.

6. Recommendations

Frequent insecticide susceptibility test for *Ae. aegypti* is very crucial in drawing vector control programme strategies.

Conflict of Interest

The authors declare no conflict of interest in relation to this work.

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