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Breeding habitats, identification and susceptibility of *Anopheles arabiensis* Patton (Diptera: Culicidae) larvae towards some insecticides in Gash Barka Zone, Eritrea

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Abstract
Gash Barka Zones (GBZ; western Eritrea) is one of the malaria endemic areas of the country. The present study aims to monitor and investigate the susceptibility of the larvae *Anopheles arabiensis* Patton (Diptera: Culicidae) to insecticides in three administrative localities in the GBZ, following WHO standard protocols. These localities were Kolentebay village (west), Selam suburb (Barentu town; center) and Molqui town (East). The study was conducted from Dec. 2017 –May 2018. The larvicide tested, at different concentrations, were temephos (the only larvicide used in the country), bendiocarb (used for indoor residual spraying, IRS) and permethrin (used for insecticide treated nets, ITNs). After mapping the breeding habitats in the three localities, larvae were collected, taken to the laboratory; the 3rd and 4th instars (L3 and L4) were separated from the others. A total of 1,350 larvae were used in the bioassay. Results were analyzed by SPSS Programs (V.23). For the populations collected from Kolentebay (K), Selam (S) and Molqui (M), the minimum and maximum ranges of LC50 and LC90 were for temephos from 0.0001-0.0103mg/L, and 0.005-0.91mg/L. For bendiocarb from 0.00007-0.0062mg/L, and 0.0059-0.061mg/L, respectively. For permethrin from 0.0002-0.0023mg/L and 0.00126-0.373mg/L), following the same order. The slopes of the log-dose probability lines (LD-P lines) for temephos were 0.743, 0(NA) and 0.658 for S, M and K larval populations, respectively. For bendiocarb were 1.073, 1.316 and 0.515, respectively for the localities, and for permethrin were 0.743, 0.579 and 0.77, following the same order. For knock-down Time (KDT), the highest mortality after 10min was 20%) for temephos, and bendiocarb, and 10% for permethrin. The maximum mortality after 1hr (i.e. 80%) was registered for temephos. It can be concluded that the population from M locality was more susceptible to temephos than the populations collected from S and K; the KdT of temephos for the population collected from K was higher than that of S and M in all tested concentrations.

Keywords: *Anopheles arabiensis* larvae, mapping, susceptibility, temephos, bendiocarb, permethrin, Eritrea, LC50, LC90

1. Introduction
Malaria is one of the most common infectious disease and enormous public health problem in Sub-Saharan Africa (SSA). The disease is caused by five species of parasites that affect humans, and all these species belong to the genus *plasmodium* of these *P. falciparum*, *P. vivax*, are the most important. Malaria due to *P. falciparum* is the most deadly form, and it predominates in Africa. *P. vivax* has a wide distribution than *P. falciparum*, because it is able to develop in the *Anopheles* mosquito vector at lower temperature and survive at higher altitude and in cooler climate. Malaria parasites are spread to human through the bites of infected female *Anopheles* mosquitoes, called "malaria vectors". There are about 400 different species of *Anopheles* mosquito, but only 30 of these are vectors major importance [1]. Mosquitoes are responsible for transmitting the most important vector-borne diseases (VBDs), above all malaria, but also lymphatic filariasis, Japanese encephalitis, dengue fever, Yellow Fever, Rift Valley Fever, West Nile fever and other forms of encephalitis [2]. The control of malaria in Africa faces major difficulties, due to the problem of the resistance of the parasite to the most commonly used drugs and the vector to the insecticide [3]. Several vector species have developed resistance to different classes of the insecticide throughout the African countries

Eritrea, malaria a major health problem. However, only few reports on malaria cases in DBZ have been published, where 4.49% of the general infectious diseases are for malaria infections [4]. From the total national malaria cases, 80% of the cases are reported in GBZ. The semiarid climatic conditions, the seasonal incidence of malaria and the isolation of towns and villages in Eritrea make it an ideal country to implement larval control as one of the principal interventions to reduce malaria. In most regions of the country, there is a very short malaria transmission season that coincides with the short rainy –season. However, in some areas, mosquitoes and associated malaria transmission persist throughout the year, even during the long dry -season. At key locations throughout Eritrea, larval control using the organophosphate temephos has proven to be a feasible strategy under these dry and semiarid climatic conditions, since mosquito larval habitats sites are discrete and easily targeted by field teams. In Eritrea insecticide resistance is well- established with *An. arabiensis* being resistant to DDT, permethrin and *lambda*-cyhalothrin. However, no enough evidence for the susceptibility of *An. arabiensis* for the chemicals used for larviciding.

In Eritrea, there are convincing indications that malaria incidence is sharply declining from 53.5 cases/1000 population at risk in 1998 to 8.4 cases/1000 population in 2015. However, small epidemics could be missed, hence the increase of sentinel sites from 26 in 2008 to 32 in 2014 help the National Malaria Control Program (NMCP) to detect early the presence of an outbreak [4]. These achievements make the NMCP confident enough to subtitle their program "Moving towards Malaria Elimination".

The NMCP in Eritrea currently uses temephos for larval control, in conjunction with routine vector control operations that include insecticide treated bed nets (ITNs) and bendiocarb spraying for control of adult mosquitoes, *i.e.* indoor residual spraying (IRS). One eminent concern is that the mosquitoes might develop resistance to organophosphate (OP) temephos in the future, as the NMCP intensifies operations to meet expectations of the WHO Roll Back Malaria (RBM) Program. Drawbacks, *e.g*. vector resistance to these compounds, cost and environmental pollution provide a basis for redefining long-term larval control strategies for the country. The NMCP has considered the use of the biocides *Bacillus thuringiensis var. israeliensis* (Bti) and *B. sphaericus* (Bs). Both biocides have proven to be useful for control of mosquito species in a variety of breeding habitats in other countries [5, 6], and showed very high environmental safety. However, experimental evaluation of both agents specifically as control agents against malaria vectors is still limited.

2. Materials and methods

2.1 Study area

Eritrea is located in the horn of Africa, having borders with Djibouti in the east, Ethiopia in the south, Sudan, in the west, and shares maritime with KSA and Yemen across the Red Sea; the area is 124, 127 km^2. Eritrea is divided in six Administrative Zones. Gash Barka Zone (GBZ) area is 33,450 km^{2,}. the largest zone of the country in rich savanna region. Population is *ca*. 912,026; not evenly distributed. GBZ is and the main economic area of the nation having several macro dams used for agricultural project, as well as mining companies. The population mainly depends on agriculture, trading and animal rearing. GBZ is divided into 14 administrative subzones and known as the most malarious zone of the country

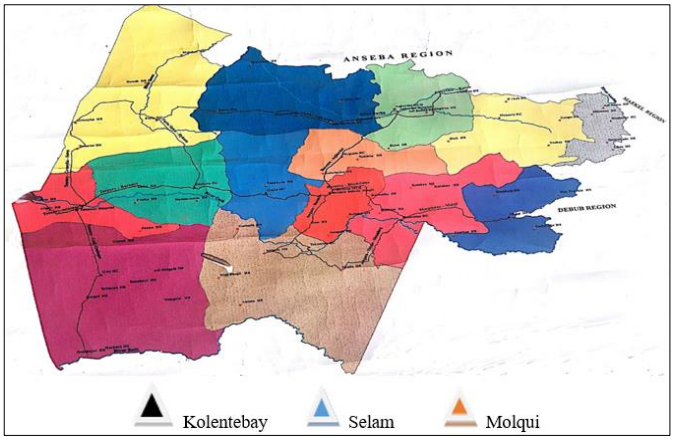


Fig 1: Map of Gash Barka zone

The rainy-season starts in late June and ends in October; average annual rain fall is 260.3mm distributed over these months. Minimum temperature is 16.9^{0}C and maximum of 37.80^{0}C, average relative humidity (RH) of 38- 68% [7]. The study villages (localities) were comparable in terms of population size, malaria prevalence, and vector density. The main water sources are shallow wells.

2.2 Study localities

2.2.1 Mulki (M): It is the largest village in its sub-zone. The village lies in the eastern part of the GBZ and is the center of the sub-zone with a population of 4,437 o farmers, herders and traders. Mulki is situated along a small Jehan River and is surrounded by mountains; latitude 14^{0}53'22.5" N longitude 38^{0}13'37.2" E and elevation of 1176m above sea level (Asl). All the breeding site are usually treated by the larvicides temephos and Bti.

2.2.2 Selam (S): This village lies in Barentu sub-zone, the center of the GBZ, having a small river along the village. The population is about 17, 320, depends on the same activities mentioned above. The latitude 15^{0}6'28.5" N longitude 37^{0}35'11.4 E and 1004m asl. The breeding sites are also as above.

2.2.3 Kolentebay (K): lies in the western part of the GBZ in Forto Sawa; the population is 4,469 of herders and farmers. The village lies between latitude, 15^{0}30'34.2" N, longitude' 37^{0}4'51.4" E and 787m elevation asl. The breeding site is again treated as above.

2.2.4 Study design

This study was conducted as cross-sectional from Dec. 2017 up to May 2018, to determine *Anopheles* preferred breeding sites and habitats in MS and K villages, and to determine the susceptibility in terms of LC50, LC90, the slope of the log-dose probability lines (Ld-P lines), and knock-down time (KdT) of the larvae against the OP temephos, the carbamate bendiocarb and the pyrethroid permethrin.

2.2.5 Materials

According to the WHO standard, the materials used for the mosquito larvae collections are plastic dippers, plastic buckets and forms used to record the density, pipettes to remove the non-target organisms. The materials used for the bioassay test were flasks (1L), beakers (1L), pipettes (5ml), trays (1L), pipettes (200 and 1000 µl), forms for recording KdT and

mortality, stop watch, insecticides (temephos 50% EC, permethrin 25%EC and bendiocarb 80%WP), test tubes (50ml), sterile gloves and distilled water (DW).

2.2.6 Methods

2.2.6.1 Collection of larvae

Larvae were collected from natural and man-made habitats, *e.g*. standing water bodies, rock holes, animal foot print and shallow wells. The dipping method was used. The sampling was done using a standardized dipper (15 cm dia. and 500 ml vol.). Between each dip an interval of 2-3 min was given so as to allow the 3rd and 4th larval instars (L3 and L4) and the pupae to return to the surface. For those places where the water surface was covered with floating algae, the algae were cleared and then watched for 3-5 min. so that the larvae may come to the surface. In each habitat, early (L1 and L2) and late (L3 and L4) instars, and the pupae were counted. The presence of shading, vegetation, algae and the non-target organisms (*e.g*. aquatic insects and small size fish) were reported.

2.2.6.2 Identification of *Anopheles* larvae

The collected larvae were identified as *Anopheles* species according to several published keys [8-13]. The L4 larvae were killed by 70% ethanol and mounted using the standard procedures of larval mounting for identification using a dissecting microscopes.

2.2.6.3 Preparation of concentrations

A stock solution was prepared for each larvicide.

Temephos 50% EC: 0.005, 0.025, 0.125 and 0.625 mg/L (ppm).
Permethrin 25% EC: 0.0025, 0.0125, 0.0625 and 0.3125 mg/L (ppm).
Bendiocarb 80% WP: 0.004, 0.020, 0.100 and 0.500 mg/L (ppm).

The concentrations from each insecticides were transferred to 1L trays. Ten L3 and L4 were transferred separately to each tray. The exposure period was 24 hr. Tap water only was used for the control. Three replicates were used /concentration and the control; the experiment was repeated twice. The KdT was recorded for each concentration after 10, 15, 20, 30, 40, 50 and 60 min of exposure, in addition to the acute mortality after 24 hr. Data was collected using standard WHO susceptibility tests format. Simple map (sketch) of the area was prepared to determine the mosquitoes preferred breeding sites in the 3 villages. Non- target organisms, shade, algae and vegetation were recorded every other month.

2.2.7 Data Analysis and Interpretation

Data was analyzed by SPSS (Version 23). Descriptive analysis was used for the % mortality at various concentrations of the tested larvicides. Percent corrected mortality was computed using Abbott's formula.

$$\%\ \text{Corrected mortality} = \frac{[\%\text{test Mortality} - \%\ \text{control mortality}]}{100 - \%\ \text{control mortality}} \times 100$$

KdT (50% and 95%) and LC50 and LC90, slope of the Ld-p line were computed for each chemical.

3. Results

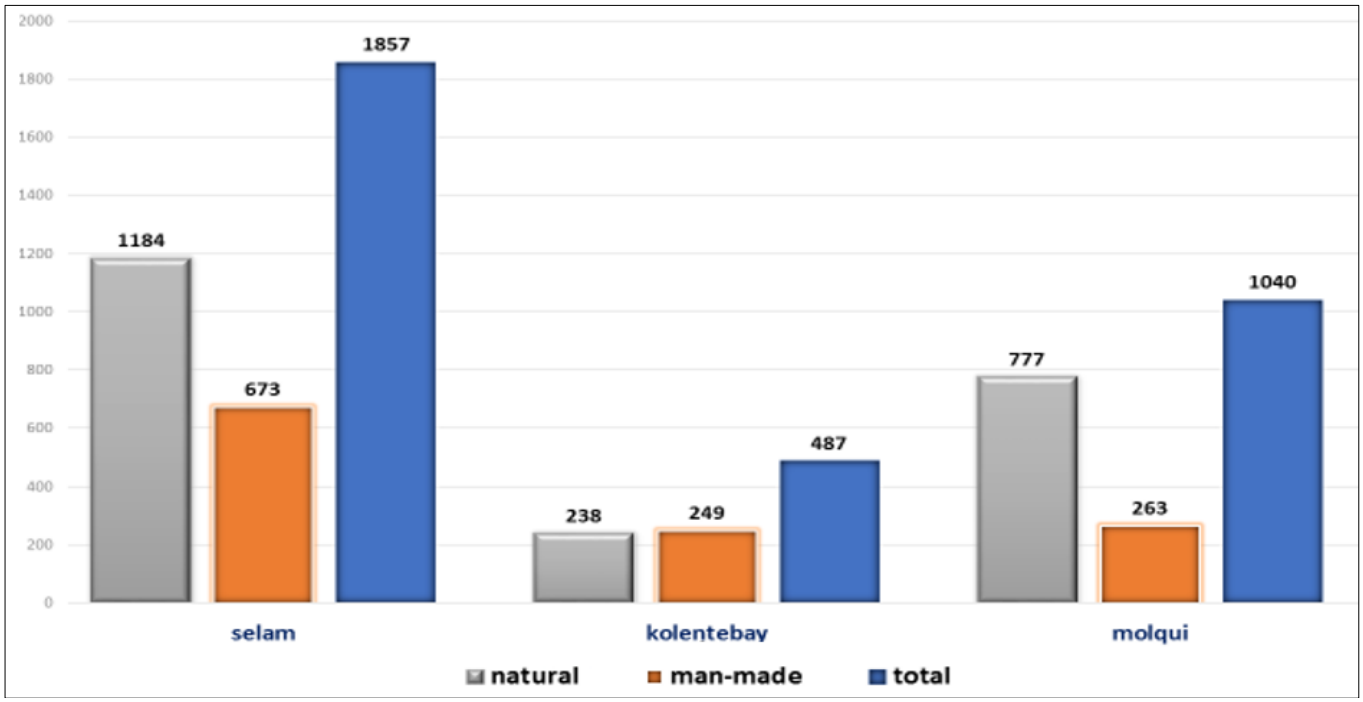
3.1 Breeding habitats

The breeding sites in the study area (55) were small water bodies close to the flowing water, open to the sunlight, mainly natural (34) and others were man-made (21), in addition to animal footprint; few were found under partial shades (8 sites). Most of the breeding sites were invested with algae (38) and non-target organisms (50%) (Table 1).

The larval distribution and density proved to be different between sites (Fig. 2); the higher numbers were collected from the natural habitats, except in Kolentebay where there were no significant differences between numbers for natural and man-made habitats (238, and 243, respectively). For Selam the numbers collected from the natural habitats were 1,184, compared to 673 from man-made habitat. However, Molqui figures were 777 and 263, following the same order of habitats. The larval density was as follows: Selam 19.1 L /dip, Molqui 13.8 L /dip and Kolentebay 9.9 L /dip. The majority of the breeding habitats in the three sites, whether natural or man-made, were not shaded [47]. Vegetation was absent from all habitats. Most of the habitats showed the presence of algae (38 out of 55 habitats). The non-target organisms were reported in 50% of the visited habitats (Table 1).

Table 1: Breeding habitats of *Anopheles* mosquito in the study sites

Village	Habitat type	No. of habitat visited	No. *Anopheles* larvae		pupa		Shade		Veget-Ation		Algae		Non-target organisms	
			L1 and L2	L3 and L4	P	A	P	A	P	A	P	A	P	A
Selam	Nat	15	638	546	5	10	0	15	0	15	11	4	5	10
	MM	7	387	286	1	6	2	5	0	7	2	5	2	5
Molqui	Nat	10	421	356	3	7	1	9	0	10	8	2	7	3
	MM	8	187	76	2	6	3	5	0	8	5	3	4	4
Kolentebay	Nat	9	131	107	0	9	1	8	0	9	7	2	6	3
	MM	6	72	171	1	5	1	5	0	6	5	1	3	2
Total		55	1836	1542	12	43	8	47	0	55	38	17	27	27



Nat = natural, MM= Man-Made, P= Present A=Absent

Fig 2: Larval distribution by type of habitats during the study period at the three study sites

4. Identification of larvae

The randomly collected populations of mosquito from all the study sites were all identified as *Anopheles arabiensis*, which is the main vector of malaria in Eritrea.

4.1 Bioassay

4.1.1 KdT

Tables (2, 3, and 4) show the KdT for each of the tested insecticides after exposing the larvae at different time intervals within the first 60 min. Temephos effect differed between the larval populations collected from the three sites. For example, Selam population when tested at 0.005 mg/L resulted in 10% mortality after 20 and 30 min, 20% after 40 min and 40% after 1hr. The effect of the same insecticides, with the same concentration, on Molqui population, behaved similar to Selam population, except after 1 hr of exposure, where only 30% was killed. Regarding Kolentebay population, under the same conditions, resulted in mortalities of 10% starting the first 10 min, but did not exceed 20% after 1 hr. When the concentration of temephos was increased to 0.025 mg/L (5x), again, the populations behaved differently. Selam population was not significantly affected within the first 60 min. The case was different for Molqui population where the results showed 10% mortality after 15 min and reached 50% after 1hr. Kolentebay population showed 10% mortality after 15 min, but did not exceed 20% after 1hr. When the concentration increased by 125x (0.625 mg/L), Selam population, for the first time showed 10% mortality after 10 min, and continued to increase by time and reached 80% after 1hr. Molqui population showed 20% mortality after 10 min, and reached to 70% after 1hr. The third population, Kolentebay, after 10 min showed 10% mortality and reached to 20% after 20 and 30 min, but did not exceed 60% after 1hr.

For permethrin (0.0025mg/L), none of the larvae died within the first 10 min in Selam and Molqui, but the population from Kolentebay registered10% mortality within the first 10min and reached 20% after 1hr., except for the highest concentration. When the concentration of permethrin increased 0.0625mg/L (25x), the population behaved differently within 15min Selam, 10% and maximum 30% after 60min; these of Molqui, mortality was 10% after 20min and did not exceed 30%after 1hr, but the population from Kolentebay showed 10% mortality after 10min and reached 50% within 1hr. When the concentration was increased to 0.3125mg/l (125x), the population from Selam showed 10% mortality after 10min, but did not exceed 40% after 1hr, whereas the population from Molqui showed 10% mortality after 10, 15 and 20min exposure and reached to 40% after 1hr. Kolentebay population mortality was 10% after 10 and 15min and 30%after 20min, but did not exceed 60%mortality after 1hr exposure.

The mortality caused by bendiocarb showed differences between the larval populations collected from the three sites. For example, Selam population when tested at 0.004 mg/L resulted in 10% mortality after 20 min of exposure, 20% after 30, 40, 50min and 1hr. The effect of this concentration on Molqui population was10% after 20, 30, 40, 50min and 1hr exposure. Regarding Kolentebay population, the mortalities were 10% starting in the first 10 min up to 1 hr o exposure. When the concentration of bendiocarb was increased, to 0.020 mg/L (5x), again, the populations behaved differently. Molqui population was not significantly affected within the first 60 min. The case was different for Kolentebay population where the results showed 10% mortality after 10 min and reached 30% after 50min and 1hr o exposure. Selam population showed 10% mortality after 20 min, but did not exceed 40% after 1hr o exposure. When the concentration increased by 125x (0.5 mg/L), Kolentebay population, for the first time showed 20% mortality after 10 min, and continued to increase by time and reached 70% after 1hr. Molqui population showed 10% mortality after 20 min, and reached to 60% after 1hr. The third population, Selam, after 15 min showed 10% mortality and reached to 20% after 20min and increased gradually, but did not exceed 70% after 1hr o exposure.

Table 2: Lethal time (LT 50% and 95%) of mosquito larvae after being exposed to temephos, Gash Barka Zone Eritrea (December 2017- May 2018).

Selam											
Knock-down time (KdT)											
Concentrations mg/L	**No. of larvae /rep**	**Time in (min).**							**Mortality after 24hr. (mean± SE)**	**KdT 50 (Min)**	**KdT 95% (Min)**
		10	**15**	**20**	**30**	**40**	**50**	**60**			
0.005	10	0	0	1	1	2	3	4	9.00±0.58	74.3	267
0.025	10	0	0	1	1	2	4	4	9.67±0.33	68.0	225
0.125	10	0	1	2	3	5	5	6	10.00±0.00	45.7	178
0.625	10	1	2	3	4	6	7	8	10.00±0.00	32.0	133
Control	10	0	0	0	0	0	0	0	1.00		
Molqui											
0.005	10	0	0	1	1	2	2	3	9.67±0.33	99.00	480.40
0.025	10	0	1	1	2	3	4	5	9.67±0.33	61.80	270.40
0.125	10	1	1	2	3	4	4	6	10.00±0.00	72.80	529.50
0.625	10	2	3	3	4	5	6	7	10.00±0.00	35.80	355.20
Control	10	0	0	0	0	0	0	0	1.00		
Kolentebay											
0.005	10	1	1	1	1	1	1	2	4.67±0.33	57,273.20	40420430.00
0.025	10	0	1	1	1	1	2	2	5.00±0.58	2,179.20	1172293.00
0.125	10	1	1	1	1	2	2	3	8.00±0.58	300.20	13399.60
0.625	10	1	1	2	2	3	4	6	10.00±0.00	62.20	452.90
Control	10	0	0	0	0	0	0	0	0.00		

Table 3: Lethal time (LT 50% and 95%) of mosquito larvae after being exposed to Permethrin, Gash Barka Zone Eritrea (December 2017 – May 2018).

Selam											
Knock-down time (KdT)											
Conc. (mg/L)	**Mean No. of larvae/rep**	**Time (min)**							**Mortality after 24hr. (mean± SE)**	**KdT 50% (min)**	**KdT 95% (min)**
		10	15	20	30	40	50	60			
0.0025	10	0	1	1	1	2	2	2	8.00±0.58	208.0	3,357.0
0.0125	10	0	1	1	2	2	3	3	9.00±0.00	105.2	870.0
0.0625	10	0	1	1	1	2	3	3	9.67±0.33	109.2	835.0
0.3125	10	1	2	2	3	3	4	4	10.00±0.00	90.3	1,969.0
Control	10	0	0	0	0	0	0	0	0.33		
Molqui											
0.0025	10	0	0	0	0	1	1	1	5.33±1.2	127.9	401.8
0.0125	10	0	0	0	1	1	1	2	7±1	121.9	480.5
0.0625	10	0	0	1	1	2	2	3	8±o.58	99.0	480.4
0.3125	10	1	1	1	2	3	3	4	10±0	101.4	1288.1
Control	10	0	0	0	0	0	0	0	0.67		
Kolentebay											
0.0025	10	1	1	1	1	1	2	2	7.67±0.88	2179.2	1,172,293
0.0125	10	1	1	1	2	3	3	4	9±0	101.4	1,288.1
0.0625	10	1	1	2	3	3	4	5	10±0	68.0	646.1
0.3125	10	1	1	3	3	4	5	6	10±0	49.1	335
Control	10	0	0	0	0	0	0	0	0		

Table 4: Lethal time (LT 50% and 95%) of *Anopheles arabiensis* larvae after being exposed to bendiocarb, Gash Barka Zone, Eritrea (Dec. 2017 – May 2018).

Selam											
Knock-down time (KdT)											
Conc. (mg/L)	**No. of larvae /rep**	**Time in (min)**							**Mortality after 24hr (Mean ±S.E)**	**Kd %**	
		10	**15**	**20**	**30**	**40**	**50**	**60**		**KdT 50% min**	**KdT 95% min**
0.004	10	0	0	1	2	2	2	2	8.67±0.88	127.9	953.0
0.020	10	0	0	1	2	2	3	4	9.67±0.33	73.7	293.0
0.100	10	0	0	1	2	3	4	6	10.00±0.00	53.9	153.0
0.500	10	0	1	2	3	4	5	7	10.00±0.00	44.9	161.0
Control	10	0	0	0	0	0	0	0	0.00		
Molqui											
0.004	10	0	0	1	1	1	1	1	4.00±0.58	465.3	9652.1
0.020	10	0	0	1	1	1	1	2	8.00±0.58	206.3	1913.2

0.100	10	0	1	1	1	3	4	5	9.33±0.33	63.8	265.3
0.500	10	0	0	1	1	2	4	6	10.00±0.00	57.10	151.0
Control	10	0	0	0	0	0	0	0	0.67		
Kolentebay											
0.004	10	1	1	1	1	1	1	1	6.67±0.67	NA	NA
0.020	10	1	1	1	2	2	3	3	8.00±1.00	171.0	4378.0
0.100	10	1	2	2	3	4	5	6	8.67±0.67	50.3	382.9
0.500	10	2	3	4	5	5	6	7	10.00±0.00	32.3	356.4
Control	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.67**		

4.2 Lethal concentration (LC50% and LC90%)

4.2.1 Temephos

The LCs proved to be different for the different populations collected from the three villages. The LC50% of Selam population was 0.0001mg/L, and for Kolentebay was 0.0103 mg/L, whereas for Molqui the concentrations tested resulted in a flat Ld-p line and the LCs cannot be calculated. The LC90s for the population from Selam was 0.006mg/L and that for Kolentebay population 0.91mg/L. The slopes were 0.743, 0 and 0.658, for Selam, Molqui and Kolentebay, respectively, and the ratio for LC90/LC50 was 60 and 88.4 (Selam and Kolentebay), respectively (Table 5; Fig 3)

4.2.2 Permethrin

As shown in table (6); fig. (4) the concentrations of permethrin used to kill 50% and 90% of the mosquito larvae differed between the three populations. The LC50 for the population from Selam was 0.0002 mg/L and the LC90% was 0.0097mg/L with LC90/LC50 ratio of 48.5; the slope was 0.743. The LC50 and LC90 for the two Molqui and Kolentebay populations were as follows: (0.0023 and 0.00027mg/L for LC50, and 0.373 and 0.00126mg/L for LC90, respectively. The ratio for the concentration LC90/LC50 were 162.2 and 4.7, following the same order of population sites. The slopes for the three population were 0.743, 0.597 and 0.77, for Selam, Molqui and Kolentebay, respectively.

4.2.3 Bendiocarb

The LCs for bendiocarb for the population collected from Selam showed that the LC50 and LC90 were 0.00035 and 0.0059mg/L, respectively, and the slope and concentration LC90/LC50 were 1.073 and 16.90, following the same order. Whereas for the populations collected form Molqui and Kolentebay the LC50s were 0.0062 and 0.00007 mg/L, the LC90 was 0.061 and0.0214mg/L, respectively. Following the same order the ratios (LC90/LC50), were 9.80 and 305.70, and the slopes of the Ld-p lines were 1.136 and 0.515 (Table 7; Fig.5)

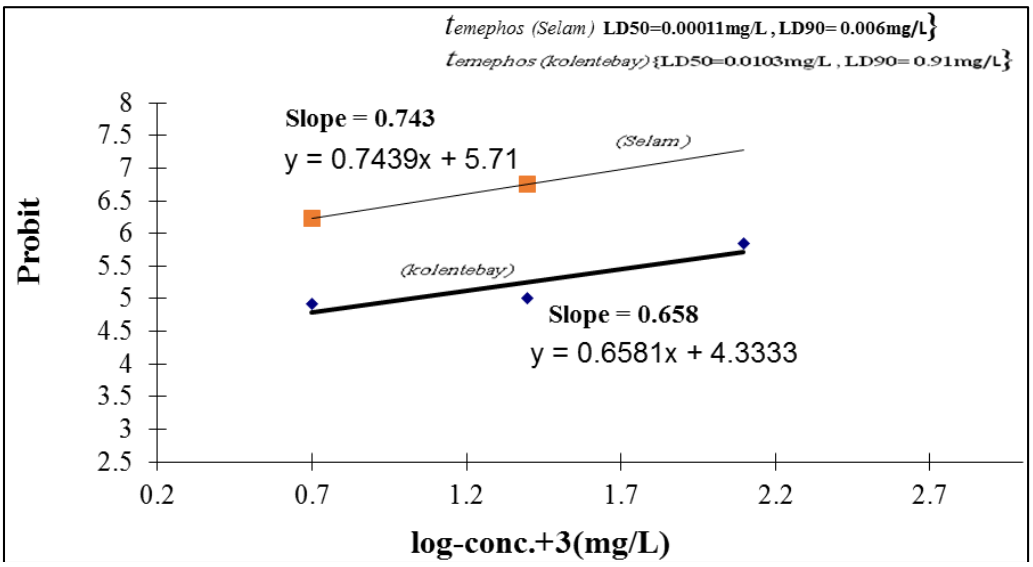


Fig 3: Lethal concentration (LC) of *A. arabiensis* to Temephos in Selam and Kolentebay GBZ Eritrea.

Table 5: Dose –response relationship of Temephos against *Anopheles arabiensis* larvae from Selam, Molqui and Kolentebay, Gash Barka Zone, Eritrea (December 2017 – May 2018).

Temephos 50% EC							
Conc. (mg/L)	% mortality			LC50	LC90	LC90/LC50	Slope
	Selam	Molqui	Kolentebay				
0.005	88.9	96.3	46.7	Selam* 0.0001 Mol.* 0.0001 Kol. 0.0103	Selam 0.006 Mol. 0.0165 Kolente*. 0.91	Selam 60.0 Mol. 165.0 Kol.. 88.4	Selam 0.743 Mol. 0 Kol.. 0.658
0.025	96.3	96.3	50.0				
0.125	100.0	100.0	80.0				
0.625	100.0	100.0	100.0				
Mean ±S.E	96.30±0.08	98.15±0.3	69.20±0.37				
C.V%	0.8	0.3	5.8				
Control	10.0	10.0	0.0				

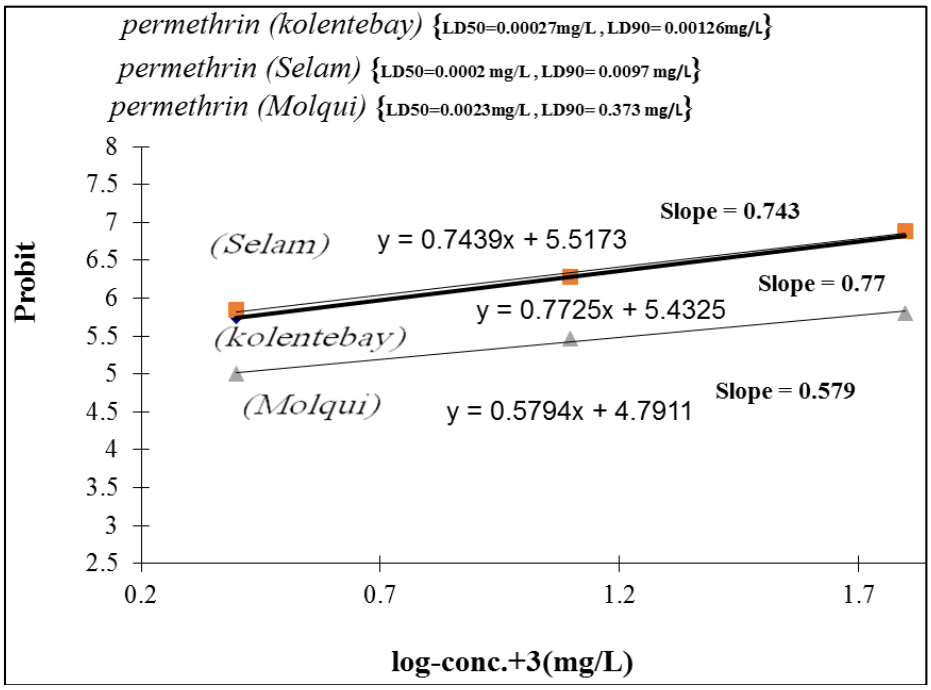


Fig 4: LC of *A. arabiensis* to permethrin in Selam, Molqui and Kolentebay, GBZ, Eritrea.

Table 6: Dose –response relationship of permethrin against *Anopheles arabiensis* larvae at Selam, Molqui and Kolentebay, Gash Barka Zone, Eritrea, (December 2017 – May 2018)

Permethrin 25% EC							
Conc. (mg/L)	% of mortality			LC50	LC90	LC90/ LC50	Slope
	Selam	Molqui	Kolentebay				
0.0025	80.0	49.9	76.7	Selam* 0.0002 Mol. 0.0023 Kol.*. 0.00027	Selam 0.0097 Mol. 0.373 Kol. 0.00126	Selam 48.5 Mol. 162.2 Kol. 4.7	Selam 0.743 Mol. 0.579 Kol.. 0.77
0.0125	90.0	67.8	90.0				
0.0625	96.7	78.5	100.0				
0.3125	100.0	100.0	100.0				
Mean ±S.E.	91.70 ± 0.13	74.10±0.3	91.7±0.16				
C.V.%	1.50	4.5	1.9				
Control	3.30	6.7	0.0				

*calculated by extrapolation

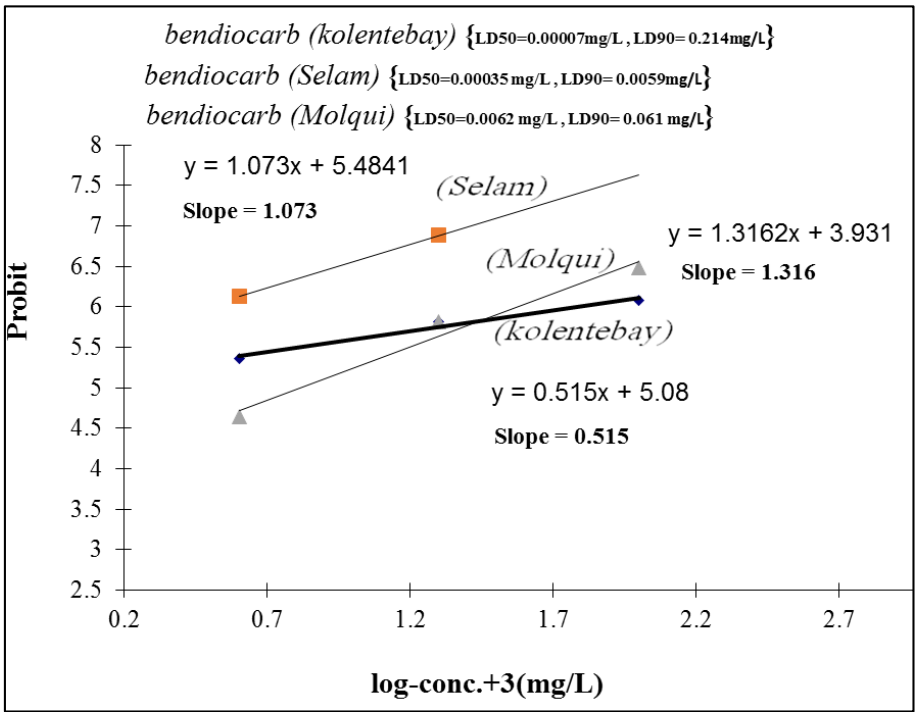


Fig 5: Lethal concentrations of *A. arabiensis* to Bendiocarb in Selam, Molqui and Kolentebay, GBZ, Eritrea.

Table 7: Dose- response relationship (LC50 and LC90) of Bendiocarb at Selam, Molqui and Kolentebay, Gash Barka Zone, Eritrea, (December 2017 – May 2018)

Bendiocarb 80% WP							
Conc. (mg/L)	% mortality			LC50	LC90	LC90/LC50	Slope
	Selam	Molqui	Kolentebay				
0.004	86.70	35.70	64.30	Selam* 0.00035 Molqui 0.0062 Kolente*. 0.00007	Selam 0.0059 Molqui 0.061 Kolente. 0.0214	Selam 16.90 Molqui 9.80 Kolente. 305.70	Selam 1.073 Molqui 1.136 Kolente. 0.515
0.02	96.70	78.60	78.60				
0.1	100.00	92.80	85.70				
0.5	100.00	100.00	100.00				
Mean ±S.E	95.90±0.09	76.80±0.42	82.20±0.22				
C.V.%	1.00	5.95	2.90				
Control	0.00	6.70	6.70				

*calculated by extrapolation

5. Discussion

The study covered the habitats, identification, KdT, and susceptibility of *Anopheles* species in three villages in GBZ of Eritrea. The only detected Anopheline larvae collected from all habitats in the three sites proved to be *An. arabiensis*. This species is known as the main malaria vector in Eritrea [14]. *An. arabiensis* larvae were collected from different natural habitats, *e.g.* River-beds, stagnant water, animal foot-prints, *etc*., and man-made habitats, *e.g*. shallow wells. These breeding habitat were the same in the three study sites. Mosquitoes prefer to breed in small water collections, clean and open to the sun- light as mentioned by Soleimani *et al*. [15]. This agreed with the present work findings in which all studied habitats proved to be un-shaded, without any vegetation, 50% of them contained algae, in addition to the majority of habitats showed the presence of non-target organisms, *e.g.* aquatic insects and small-size fish in some areas like Molqui. According to the study conducted in the Sudan by Mahgoub *et al*. [16], *Anopheles* larvae were found to breed in water bodies, which are covered with algae. Similar study by Hamza and Rayah [17] also revealed that *An. arabiensis* was found to breed in irrigation canals, seepage from water pipes, neglected wells, artificial containers, and man-made ditches.

The larval distribution and larval density differed in the study sites and between months of collection. The highest larval incidence was found at Selam village during January (756 larva from 22 habitats), followed by Molqui and Kolentebay (452 from 18 habitats and 175 from 15 habitats, respectively), and the lowest number was collected during May, *i.e*. Selam (454), Molqui (267) and Kolentebay (152), from the same previously mentioned habitats. The reduction of density during May could be due to higher temperature and fewer breeding habitats. A study which was conducted in the Sudan, Gedarif State, Eastern Sudan, bordering Eritrea from the west, revealed that the larval distribution in the dry- season was much more less than the wet- season, due to decreasing number of temporary breeding sites (*i.e*. drying), and the area was free from adults on April and May [18]. Similar findings in the former South Sudan (currently Republic of South Sudan) were reported by Ageep *et al.* [19]. The mean larval density collected from natural *vs.* man-made breeding habitat was 65.1 and 34.9%, following the same order. Similar results were reported from Ghana by Mattah *et al.* (2017) [20]. The larval density was higher in Selam (19.1 L/ dip), followed by Molqui (13.8 L/dip), the lowest was in Kolentebay (9.9 L/dip).

The KdT result showed that permethrin and bendiocarb at all concentrations tested did not cause any mortalities in the first 10 min of exposure. Whereas, temephos showed 10% mortality within 10 min in all tested concentration, except the highest concentration (0.5 mg/L) resulted in 20% mortality in Kolentebay and 60% after 1hr in the same population. In Selam and Molqui, the mortality was higher than that of Kolentebay (i.e. 80 and 70%, respectively) after 1hr of exposure, even though the Kd started late in both populations than Kolentebay population. However, the population exposed to permethrin also showed 10% mortality after 10min in Kolentebay population, which indicates the susceptibility of this population, compared to the others at all concentrations. Selam population showed 10% mortality only at the higher concentration of permethrin after 10min and reached 40% after 1hr, which is similar to bendiocarb case. for these of Molqui and Kolentebay also 40% and 60% after 1hr. However, for the highest concentration of each of the tested insecticides, temephos resulted in 80% mortality for Selam population and the lowest % mortality 40% for permethrin both Selam and Molqui populations. The three tested insecticides at their highest concentration resulted in 100% mortality. Therefore, they proved to be excellent larvicides that can be used in rotation to avoid development of resistance as result of using temephos only.

The study revealed that at 0.025, 0.125 and 0.625mg/L of the OP temephos the % mortality reviled between 96.3-100% in Selam and Molqui. Similar study was conducted in the Sudan (Khartoum state) on temephos using the same concentrations and resulted in mortalities of 96 to 100% [21]. On the other hand, the results for the pyrethroid permethrin in the three villages effected 49.9 (for Molqui population) to 100% (all populations) at 0.0025 to 0.3125 mg/L. A study conducted in the western Kenya showed that *An. arabiensis* larvae were resistant to permethrin [22]. The mortalities of the populations exposed to bendiocarb was ranging between 35.7 to 100% at 0.004 to 0.500 mg/L. The lower value belong to Molqui and Kolentebay populations, whereas at the higher concentration 100% mortality resulted was for the three populations.

The population from Molqui was more susceptible to temephos (LC50= 0.0001mg/L), compared to that of Selam (0.0001mg/L) and Kolentebay (0.91mg/L), when using the same concentrations. The population from Kolentebay proved to be susceptible to bendiocarb (LC50 =0.00007mg/L), whereas those of Selam and Molqui were 0.00035mg/L and 0.0062mg/L, respectively. However, regarding permethrin the LC50s for Selam, Molqui and Kolentebay populations were 0.0002, 0.0023 and 0.00027mg/L, following the same order of populations.

With regard to homogeneity and heterogeneity of these populations to the three tested insecticides, in terms of the slope of the Ld-p lines, the response of the populations to bendiocarb were reflected in higher slope values (1.07 for

Selam and 1.136 for Molqui), which indicate that these two populations have homogeneous response to this insecticide in these two localities, whereas the Kolentebay population slope did not exceed 0.5, *i.e*. heterogeneous to bendiocarb. Therefore, care must be taken when dealing with Selam and Molqui populations to avoid development of resistance than the other two insecticides (0 to 0.743 for temephos and from 0.57 to 0.77 for permethrin). This suggest that the populations are still heterogeneously responding to these two insecticides, compared to bendiocarb. Therefore, they must be considered when adopting the rotation of insecticides concept as a practice.

The resistance ratio, in terms of LC90/LC50 showed that depending on LC50 alone as a measure is not enough. The data showed big difference between the doses causing 50% mortality and those causing 90% mortality. Therefore, the ratio, RR' was very high in almost all treatments, populations and insecticides. That is to say, any small change in the concentration of any of these insecticides might be reflected in unexpected increase in mortality. Thus, more detailed and intensive studies on the dose-response relationships between these populations to different insecticidal groups must be established.

6. Conclusions

1. *An. arabiensis* was the only Anopheles species found in all habitats of the three sites**.**
2. The breeding habitats for all the population were similar in all the study sites, and indicated that *Anopheles* mosquito prefer to breed in small and clean water bodies and open to sunlight in both natural and man-made habitats
3. Temephos was most effective against Selam and Molqui larval populations.
4. In Kolentebay population, permethrin proved to be the most effective.

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