

Bioefficacy of Skeeter Abate and Spintor on *Anopheles gambiae* and *Aedes aegypti* Mosquitoes from insecticide resistance areas in Lagos and Oyo States, Nigeria

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ABSTRACT

Introduction: The development of resistance in mosquitoes to the insecticide used against them as larvicides and adulticides is an important issue to be considered as difficulty with control would be encountered due to the emergence of insecticide-resistant mosquitoes. Resistance to Organophosphates and Pyrethroids, widely used to control Mosquitoes is widespread in Nigeria. **Objectives:** This study therefore evaluates the effect of insecticide resistance on the larvicidal activity of Skeeter Abate and Spintor a biopesticide, on *Anopheles gambiae* and *Aedes aegypti*. **Methods:** Mosquito larvae were collected from five populations where insecticide resistance have been reported in Lagos and Oyo States in Nigeria. They were evaluated at a temperature of $27 \pm 2^\circ\text{C}$ and R.H. of $70 \pm 10\%$ and a photoperiod of 12:12 (LD). Insecticide resistance was confirmed in the areas by adult susceptibility test using World Health Organization standard procedures. The larval bioassay was conducted at different concentrations of Skeeter Abate and Spintor insecticides. **Results:** There is a positive correlation between resistance to permethrin in adult mosquitoes and the concentration of Spintor and Skeeter Abate required to effect 99% mortality in the larval samples. All *Aedes aegypti* species collected from the study sites were 100% susceptible to permethrin. Resistance to permethrin in *Anopheles gambiae* were low in three sites (Yaba, Ikorodu and Iwo Road) while resistance is suspected in two sites (Lekki and Ojoo). Spintor and Skeeter Abate were effective in the control of the *Anopheles gambiae* and *Aedes aegypti* larvae. **Conclusion:** Insecticide susceptibility tests and resistance surveillance should be conducted before embarking on mosquito control programmes.

Keywords: Insecticide resistance Mosquitoes, *Aedes aegypti*, *Anopheles gambiae*, biopesticide, Spintor and Skeeter Abate.

INTRODUCTION

Vector-borne diseases are an increasing cause of death and suffering worldwide. Of all the disease-spreading arthropods, mosquitoes, the greatest public health problem (WHO, 2005), cause physical, mental and health effects to man and animals. WHO declared the mosquito "public enemy number one" because they are responsible for the transmission of various dreadful diseases such as malaria, filariasis, yellow fever, dengue fever and several types of encephalitis (WHO, 1996, Sachs & Malaney, 2002; Boutayeb, 2006).

Malaria pathogens are transmitted to humans by the bite of female *Anopheles* mosquitoes. About 50% of Nigerians suffer from at least one episode of malaria each year. Yellow fever is a viral disease which is mainly transmitted to humans by *Aedes aegypti*. The WHO estimates that the disease affects an estimated 200,000 persons, causing an estimated 30,000 deaths (WHO, 2006) yearly.

The emergence of mosquito species resistant to insecticides widely used in malaria and dengue control has the potential to impact severely on the control of these disease vectors. The Insecticide Resistance Action Committee (IRAC) suggests the use of Insecticide Resistance Management (IRM) to help reduce the negative effects of resistance. The objective of IRM is to prevent or delay the evolution of resistance to insecticides or to help regain susceptibility in already resistant insect populations (IRAC, 2005). A National Research Council report (NRC, 1986) on strategies and tactics for pesticide resistance management described insecticide susceptibility as a resource in resistance management.

Skeeter Abate is an organo-phosphorus insecticide highly effective against mosquito larvae and other aquatic insects, while its toxicity to fish, birds, mammals and humans is very low. Its low toxicity to non-target organisms and low effective dosage make Skeeter Abate an appropriate larvicide in many situations (WHO, 1997). Presently, Skeeter Abate is being used in larvicidal programmes in Lagos State, Nigeria.

Spintor is a naturally derived biorational insecticide which is highly toxic to Lepidoptera, Diptera and some Coleoptera and has a unique mode of action involving the postsynaptic nicotinic acetylcholine and GABA receptors resulting in tremors, paralysis, and death (Salgado, 1998). It has a very low mammalian toxicity and a favourable environmental profile with low persistence and low toxicity to a number of predatory insects (Miles & Dutton, 2000).

Insecticide resistance in adult populations of *An. gambiae* and *Ae. aegypti* has been reported in many studies (Shousha, 1948, Oduola *et al.*, 2010, Ranson *et al.*, 2010, Kamgang *et al.*, 2011, Oduola *et al.*, 2012). However, the effects of this resistance on the success of larviciding as a part of IVM have not been well documented. This study therefore evaluates the effect of insecticide resistance on the larvicidal activity of Skeeter Abate and Spintor on *An. gambiae* and *Ae. aegypti* mosquitoes. Such knowledge is essential in defining future control strategies against this medically important mosquito. There has been no report on the susceptibility status of anopheles mosquitoes from Lekki, Lagos. Our finding thus provides this information.

MATERIALS AND METHODS

Mosquito sampling

Anopheles gambiae and *Aedes aegypti* larvae were collected from six sites (Ikorodu, Yaba, Lekki, and Ojoo (in Lagos State) and Iwo road (in Oyo State) where insecticide resistance have been earlier suspected (Fig. 1). Larvae were collected from different breeding habitats ranging from shallow, well, gutters, tyres, to standing water between May and October, 2012. They were scooped into bowls, sieved, put in small buckets and washed with clean water and were taken to the insectary for rearing to adult stage.

Rearing of larvae to adult stage in the insectary

The larval samples collected in the field were reared in the insectary of the Vector Control Research Unit, National Institute of Medical Research (NIMR), Lagos, Nigeria. They were fed with mashed biscuit of low fat (12.30g) plus yeast capsules (7.59g) and maintained under a standard insectary conditions (temperatures between 27-29°C and relative humidity between 80-97%). The emerged adults were transferred into cages using an aspirator and maintained on 10% sucrose solution for 24 hours prior to further tests.

Anopheles gambiae s.s. third larval instar (Kisumu strain) were from the Molecular Entomology and Vector Control Research Laboratory, Public Health Division, Nigerian Institute of Medical Research, Lagos. The mosquito colony established in 2002 under the WHO/MIM – TDR Project A30026 and has not been previously exposed to any form of insecticide. The colony was maintained at a temperature of 27± 2°C and R.H. of 70± 10% and a photoperiod of 12/12 (LD).

Preparation of stock solutions and test concentrations.

Stock solutions were prepared by weighing 5.0mg, 10.0mg, 15.0mg, 20.0mg and 25.0 mg of the Spintor wettable powder into five Eva water bottles labelled A_{SPD} to E_{SPD} respectively, 1000ml of distilled water was poured into each bottle and then screwed. The screwed bottles were shaken vigorously to dissolve the chemical inside. Stock solutions were used as freshly prepared stock solution.

Skeeter Abate pellets were ground into powder using a mortar and pestle. Stock solutions were prepared by weighing 0.5mg, 1.0mg, 1.5mg, 2.0mg and 2.5mg of the ground Skeeter Abate powder into five Eva water bottles labelled A_{SKE} to E_{SKE} respectively, 1000ml of distilled water was poured in each bottle and then screwed. The screwed bottles were shaken vigorously to dissolve the chemical inside. Stock solutions were used as freshly prepared stock solution.

Insecticide bioassay

Bioassays were carried out on non-blood fed 2 to 3 day old female mosquitoes that emerged from the larvae collected from the field. Standard procedures were followed for susceptibility tests using WHO test kits and

insecticide-impregnated papers obtained from Vector Control Research Unit, NIMR (WHO, 1998). One test paper impregnated with permethrin at 1.0% concentration was used to assess the presence of resistance. The mosquitoes from each location were tested with 3 replicates of 20 to 25 samples per area; these were then exposed for 60 minutes to each of the insecticides or to untreated paper (control). The number of mosquitoes knocked down was recorded every ten minutes of exposure after which the insects were maintained for 24 hours on 10% sucrose solution and mortality of the insect was recorded. The results were analyzed to obtain the LC_{50} by using computerised probit analysis (Raymond, 1985). The WHO (1998) criteria for determining resistance or susceptibility were applied: 98 to 100% mortality indicates susceptibility, 80 to 97% mortality requires confirmation of resistance. While <80% mortality suggests resistance.

Larval bioassay

The susceptibility of larvae of *Anopheles gambiae* and *Aedes aegypti* to Spintor and Skeeter Abate was tested in the laboratory using a methodology adapted from the Elliot larval test (WHO, 1997). Groups of 20 larvae of the late third and early fourth instars were transferred by means of droppers to small test disposable cups. 100ml of the different concentrations of the stock solutions were measured out using a 100ml measuring cylinder and poured into the different cups containing the larvae. Three replicates of 20 larvae were assigned to each treatment. A cup containing 100ml of water was used as control. Mortality responses were recorded after 24 hours. A larva was classified as dead if it did not move when gently touched with the point of a pipette and those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed were also counted as dead larvae.

RESULTS

Insecticide susceptibility varied considerably in *Anopheles gambiae* mosquitoes collected from the five sites. The 24 hours mortality rate recorded in Lekki, Yaba, Ikorodu, Ojoo and Iwo road were 86.3%, 98%, 100%, 93.4% and 100%, respectively (Table 1). Resistance is suspected in adult *A. gambiae* in Lekki and Ojoo areas. On the other hand, 100% mortality was found in *A. Aegypti* adults in all the locations. Thus, all adult *Aedes* mosquitoes are still susceptible to permethrin.

Larvicidal efficacy of spintor on *anopheles gambiae* larvae.

Larvicidal efficacy of Spintor in the five study sites is as shown in Figure 2. Generally, the larvae mortality increased with increase in concentration of the larvicide. The 24hour mortality of wild strains of *An. gambiae* larvae collected from Ikorodu, Yaba, Lekki, Iwo road and Ojoo ranged from 53-100%, 96-100%, 18-98%, 48-96% and 8-91% respectively. Larvae collected from Yaba were the most susceptible with 100% mortality at 10mg/l concentrations and above. This was followed by larvae collected from Ikorodu, Iwo road, Lekki and Ojoo (Fig.2) areas.

Larvicidal efficacy of skeeter abate on *anopheles gambiae* larvae.

The 24hour mortality of wild strains of *An. gambiae* larvae collected from Ikorodu was 100% while for Ojoo, Yaba, Iwo and Lekki areas, it ranged from 95-100%, 70-96%, 48-96% and 5-100% respectively (Fig. 3). Larvae collected from Ikorodu were the most susceptible with 100% mortality at all the concentrations. This was followed by larvae collected from Ojoo, Yaba, Iwo road and Lekki (Fig. 3). *Aedes aegypti* larvae collected from the study sites were 100% susceptible to Skeeter Abate.

The regression analyses (Figs 4 -13) show positive correlation between larval mortality and the concentration of Spintor and Skeeter Abate respectively. The larvae mortality increased with increase in concentration of both insecticides.

A comparison of the efficacies of the two larvicides in the selected sites is shown in Table 2. The relative performance in terms of 50% and 95% mortality across all samples were comparatively equal. However, Skeeter abate performed non significantly in Iwo road as compared with Spintor from the same site.

Evaluation of the correlation between insecticide resistance and larvicide resistance

Effect of insecticide resistance was compared across all populations. *Anopheles* mosquitoes collected from Lekki and Ojoo sites showed higher LC_{99} values as compared with the rest of the populations and, these were sites where resistance to permethrin were detected. Susceptible sites showed lower LC_{99} values (Table 3).

DISCUSSION

The success of malaria vector control programs could be daunted by the menace of insecticide resistance. Resistance of mosquitoes to insecticides in Nigeria has been extensively studied (Awolola *et al.*, 2003; 2005; Djouaka *et al.*, 2008; Oduola *et al.*, 2010; 2012). There have been few reports in Lagos and Oyo States on the susceptibility status of *Aedes* mosquitoes to Permethrin impregnated papers using WHO standard methods. The 24 hour mortality results of this study shows that Spintor and Skeeter Abate were effective against *Aedes aegypti* larvae and adults producing 100% mortality.

In Lagos State, resistance of anopheles mosquitoes to various classes of insecticides is becoming more alarming (Oduola *et al.*, 2010). Awolola *et al.*, 2003 reported the resistance of anopheles mosquitoes to pyrethroid insecticides while Djouaka *et al.*, (2008) reported the resistance to permethrin in Ojo in Oyo State. From our studies, resistance is suspected in some parts of Lagos State (Lekki) and not found in the other parts studied. There has been no report on the susceptibility status of *Anopheles* mosquitoes from Lekki thus, our finding could be said to provide this information.

There has been a few studies on the effectiveness of Spintor and Skeeter Abate on *Anopheles gambiae* larvae. However, a lot of works have studied the effect of Spintor and Skeeter Abate on *Aedes aegypti* mosquito larvae. Our results agree with the study carried out by Darriet *et al.*, (2005) which showed that Spintor had a lethal action on susceptible and resistant specimens of *An. gambiae*. The result of the study carried out by Marina *et al.* (2011) showed that Skeeter Abate granules and a suspension concentrate formulation of Spintor were both highly effective larvicides against *Ae. aegypti* and *Ae. albopictus*. Moderate adult insecticide resistance values were recorded from two populations out of five (Ojo in Oyo State and Lekki in Lagos State). There have been a few reports on larval and adult resistance to insecticides which has varied significantly. Tikar *et al.*, (2011) reported some level of susceptibility to Skeeter Abate and malathion in the larvae of *Anopheles* mosquitoes, Rodriguez *et al.*, (2007) on the other hand evaluated levels of resistance in *Aedes aegypti* and reported that in larval bioassays, Skeeter Abate resistance was high with high resistance of the adults to pyrethroids.

In conclusion, resistance to insecticides could pose a major challenge to vector control programs and completely render control programs ineffective thus insecticide susceptibility tests and resistance surveillance is an essential step in resistance management and should be conducted before undertaking mosquito control programmes. Spintor and Skeeter Abate are very effective in the control of mosquito larvae in areas where insecticide resistance is suspected.

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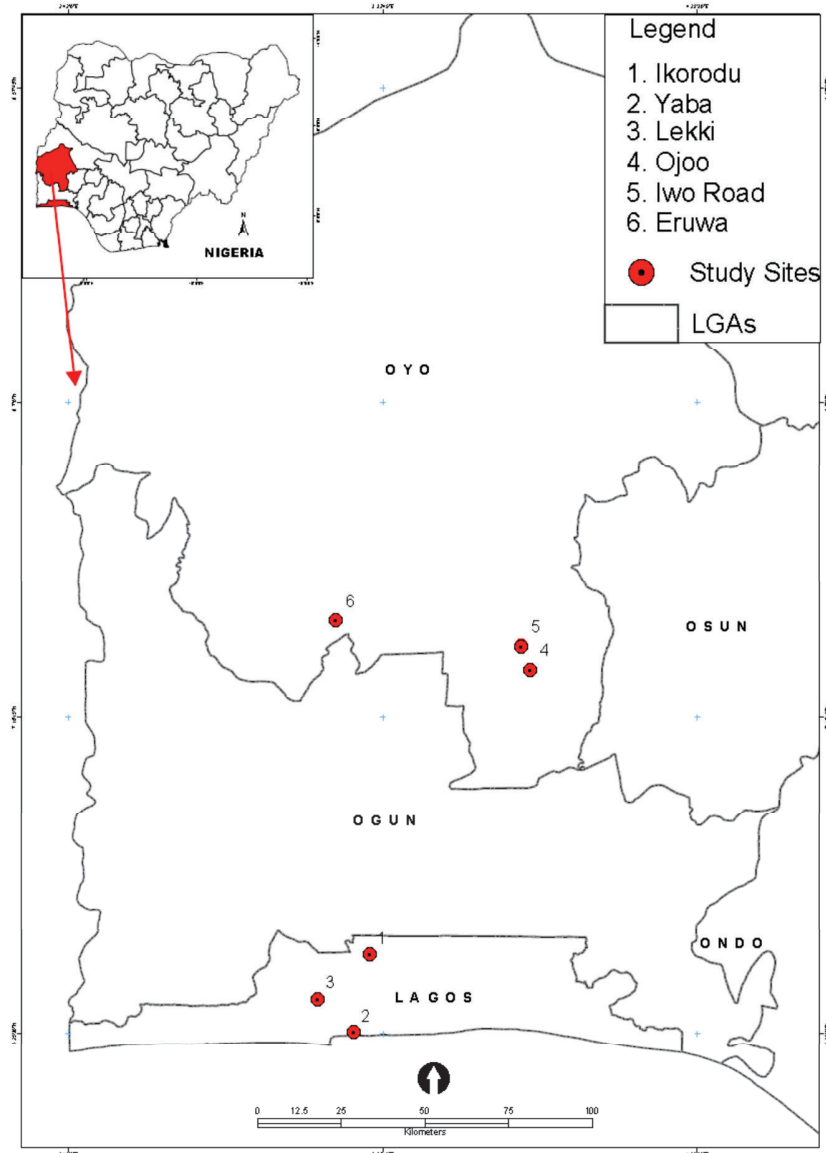


Figure 1: Map showing the larval collection sites

Table 1: Resistance Status of Anopheles and Aedes adult mosquitoes from the selected sites

Site	GPS coordinate		24hours mortality value	Susceptibility status (WHO, 1998)	24hours mortality value	Susceptibility status (WHO, 1998)
	Longitude	Latitude	Aedes		Anopheles	
Lekki	3 ^o 27.983'	6 ^o 25.746'	100%	Susceptible	86.3	Resistance suspected
Yaba	3 ^o 22.275'	6 ^o 30.987'	100%	Susceptible	98%	Susceptible
Ikorodu	3 ^o 30.644'	6 ^o 38.013'	100%	Susceptible	100%	Susceptible
Ojoo	3 ^o 55.017'	7 ^o 27.812'	100%	Susceptible	93.4%	Resistance suspected
Iwo road	3 ^o 56.497'	7 ^o 24.042'	100%	Susceptible	100%	Susceptible

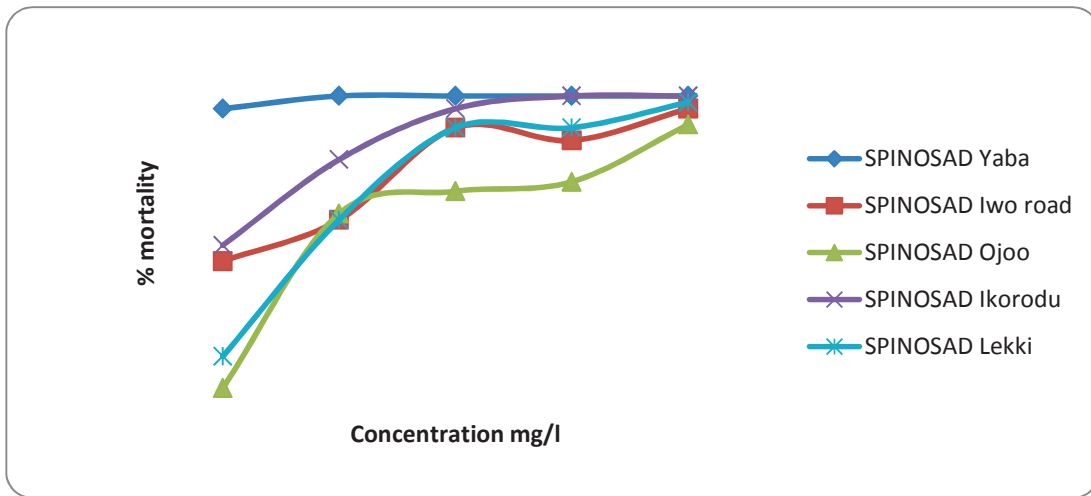


Figure 2: Larvicidal efficacy of Spintor in the five study sites after 24 hours.

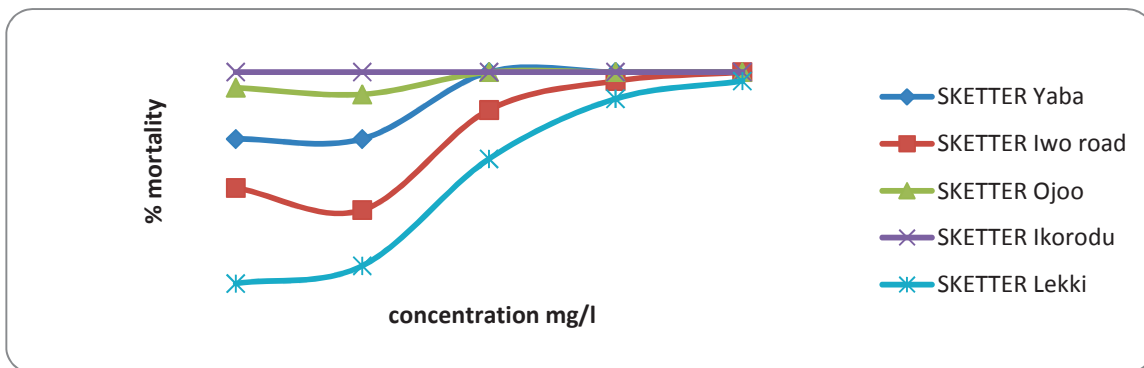


Figure 3: Larvicidal efficacy of Skeeter Abate (Skeeter Abate) in the five study sites after 24 hours

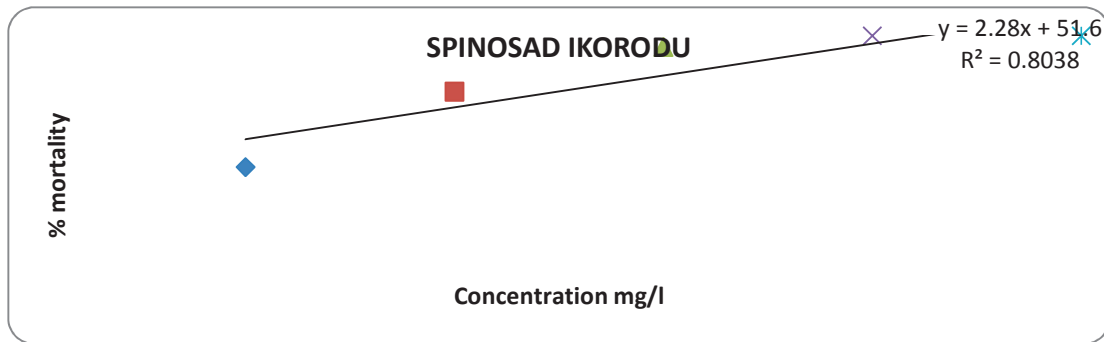


Figure 4: Regressions analysis for Spintor using Anopheles mosquitoes collected from Ikorodu

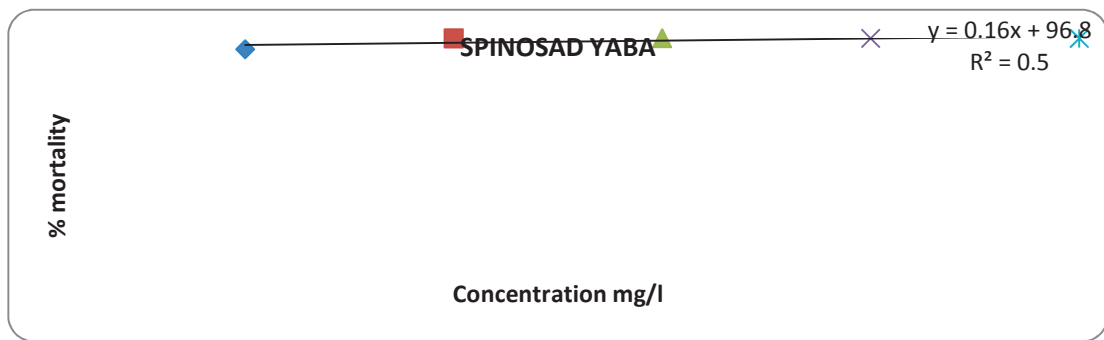


Figure 5: Regressions analysis for Spintor using Anopheles mosquitoes collected from Yaba

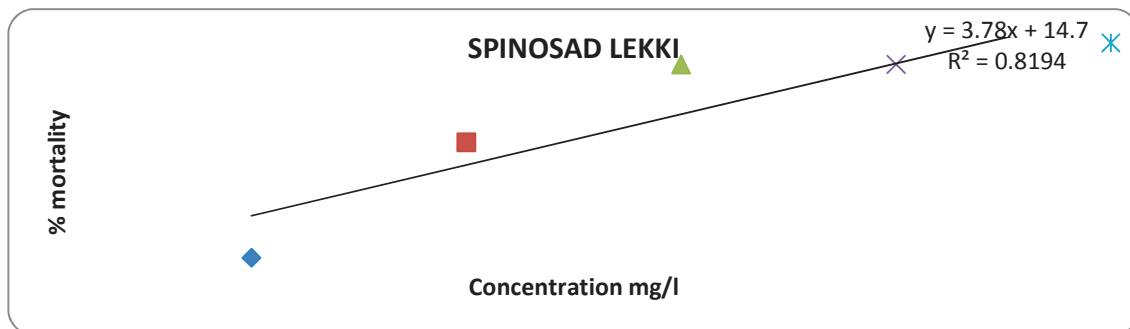


Figure 6: Regressions analysis for Spintor using Anopheles mosquitoes collected from Lekki

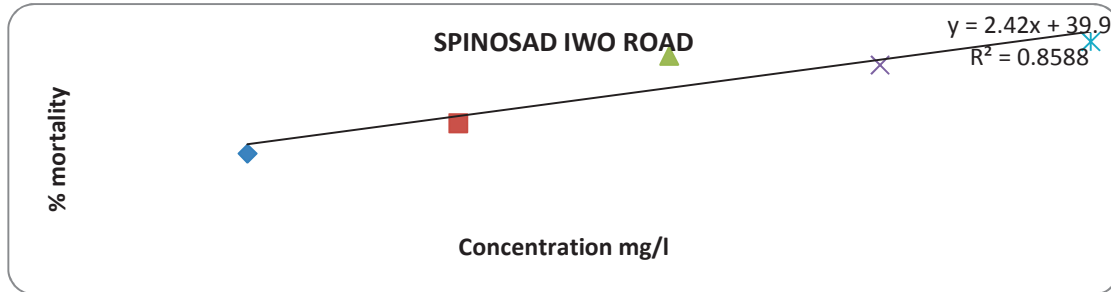


Figure 7: Regressions analysis for Spintor using Anopheles mosquitoes collected from Iwo Road

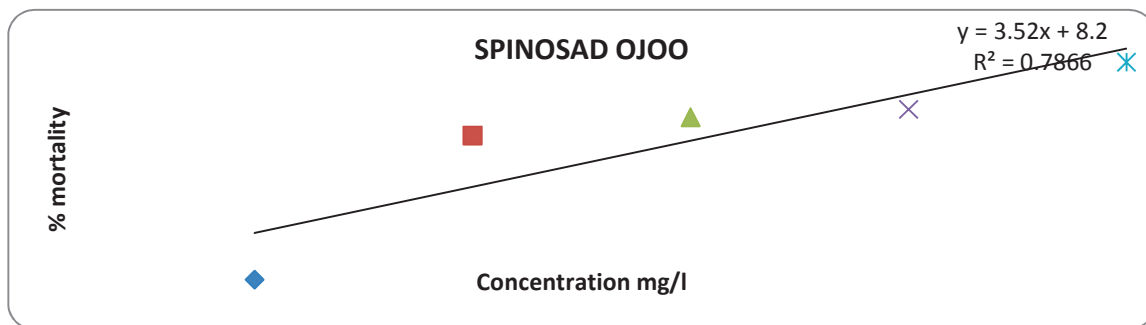


Figure 8: Regressions analysis for Spintor using Anopheles mosquitoes collected from Ojoo

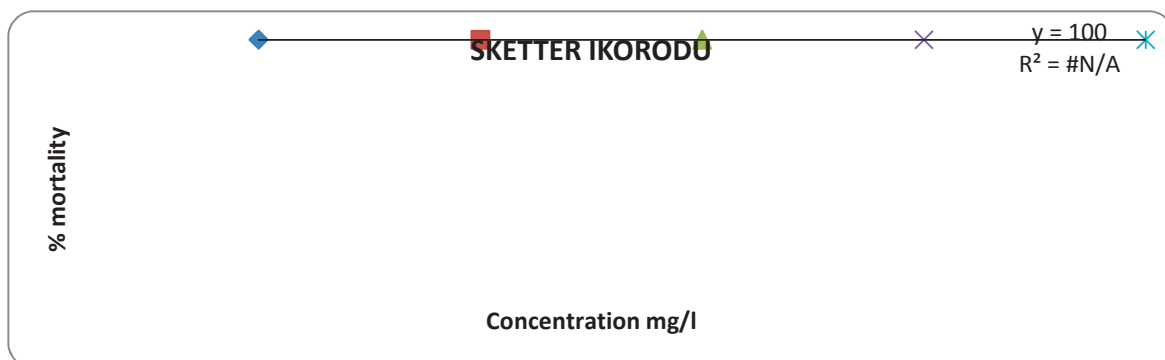


Figure 9: Regressions analysis for Skeeter abate using Anopheles mosquitoes collected from Ikorodu

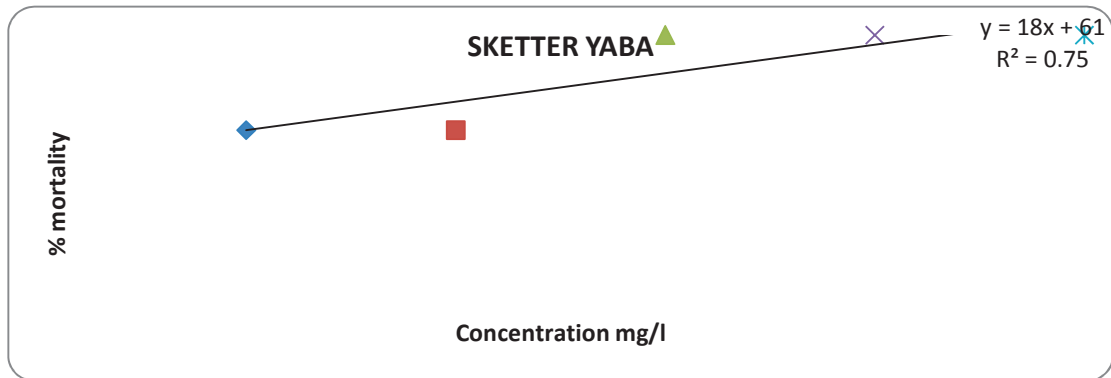


Figure 10: Regressions analysis for Skeeter abate using *Anopheles* mosquitoes collected from Yaba

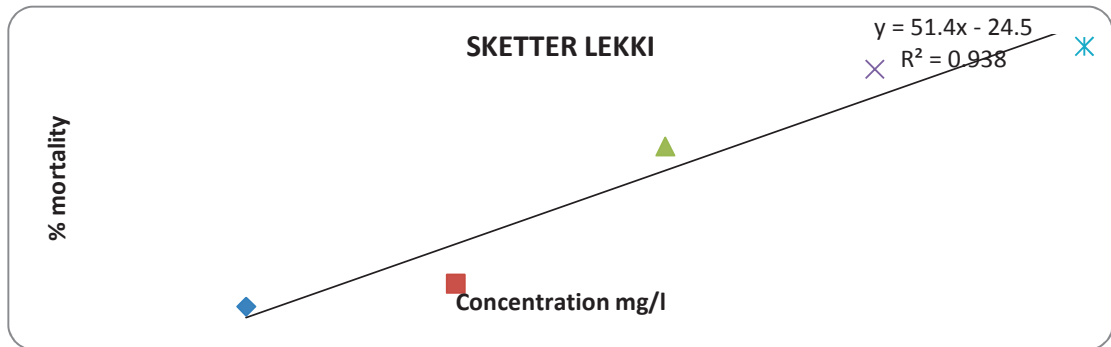


Figure 11: Regressions analysis for Skeeter abate using *Anopheles* mosquitoes collected from Lekki

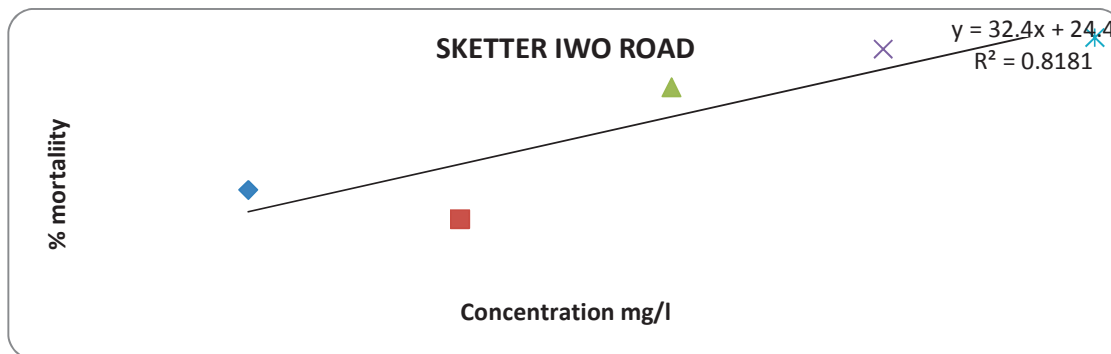


Figure 12: Regressions analysis for Skeeter abate using *Anopheles* mosquitoes collected from Iwo road

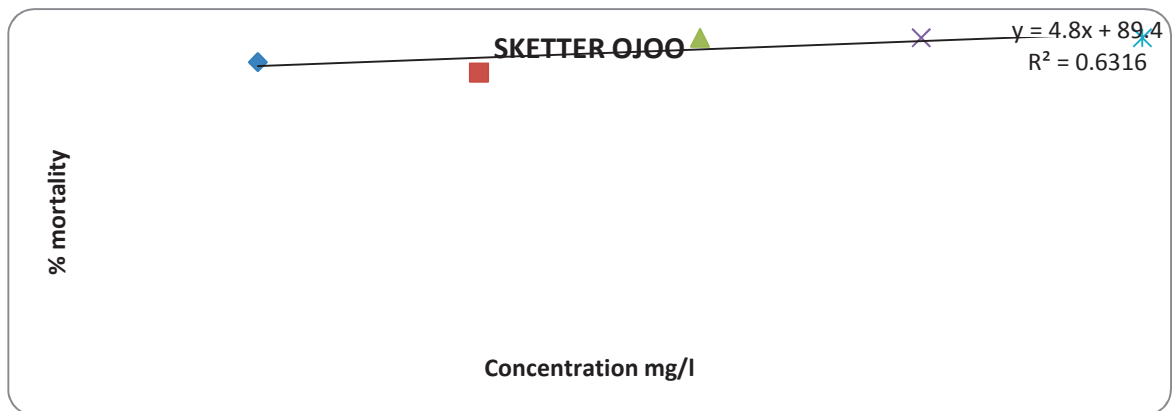


Figure 13: Regressions analysis for Skeeter abate using Anopheles mosquitoes collected from Ojoo

Table 2: Performance of Spintor and Skeeter Abate on Anopheles mosquitoes larval samples from the selected sites in Lagos and Oyo States

LARVICIDE	SAMPLE SITE	TOTAL NUMBER EXPOSED (N)	MORTALITY MT ₅₀ (95% CI)	MORTALITY MT ₉₅ (95% CI)	X ²
SPINOSAD	YABA	(60)	65.88(38.61-91.99)	81.14(44.97-123.37)	3.73*
SKETTER	YABA	(60)	52.27(32.50-85.42)	67.76(56.33-79.21)	7.98*
SPINOSAD	IWO - ROAD	(60)	48.21(30.58-67.25)	57.73(35.01-86.51)	14.68*
SKETTER	IWO - ROAD	(60)	25.46(13.92-35.43)	47.65(34.52-100.28)	2.01 ^{ns}
SPINOSAD	OJOO	(60)	57.44(45.51-88.46)	66.96(58.66-98.11)	43.6*
SKETTER	OJOO	(60)	62.41(45.24-80.81)	88.90(83.68-95.20)	7.88*
SPINOSAD	IKORODU	(60)	49.89(46.44-5432)	69.96(63.66-79.45)	38.2*
SKETTER	IKORODU	(60)	67.76(56.33-80.21)	95.1(88.89-102.83)	9.11*
SPINOSAD	LEKKI	(60)	46.87(39.14-63.12)	62.51(56.94-70.45)	5.02*
SKETTER	LEKKI	(60)	45.00(29.02-70.20)	75.88(48.61-101.99)	21.01*

X² values are for the test of fit of the long time probit model used to estimate the MT₅₀ and

MT₉₅; ns=deviations not significant, *deviations significant, P<0.05

Table 3: Table showing the Susceptibility status of Anopheles mosquitoes and there effect on Spinosad and Temephos.

LARVICIDE	LC ₉₉ (mg/l)	% SUSCEPTIBILITY VALUE (Adults)	SUSCEPTIBILITY STATUS (Using W.H.O criteria)	SAMPLE LOCATION
SPINOSAD	0.9 mg/l	98%	Susceptible	Yaba
SKEETER	2.0 mg/l			
SPINOSAD	20 mg/l	100%	Susceptible	Ikorodu
SKEETER	1.0 mg/l			
SPINOSAD	25 mg/l	86.3%	Resistance suspected	Lekki
SKEETER	2.5 mg/l			
SPINOSAD	25mg/l	93.4%	Resistance	Ojoo
SKEETER	2.0 mg/l		Suspected	
SPINOSAD	23.6 mg/l	100%	Susceptible	Iwo road
SKEETER	2.25 mg/l			

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