



LARVICIDAL EFFECTIVENESS OF SPINOSAD AND TEMEPHOS ON *Anopheles gambiae* & *Aedes aegypti*

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ABSTRACT

Study of Temephos granules and Spinosad suspension formulation on both wild and laboratory-bred *Anopheles gambiae* and *Aedes aegypti* larvae was evaluated at 27 ± 2 °C temperature, 70 ± 10 % Relative Humidity and a photoperiod of 12 :12 (L: D). Data obtained include the LC₅, LC₅₀ and LC₉₅ values. The photomicrographs of third instar larvae of the test mosquitoes exposed to the different larvicides were taken using camera microscope. The joint action effect of the two larvicides was carried out by combining the larvicides in the ratio 1:1 based on their 24hrs single action LC50 values. On the basis of the 24hr LC₅₀ values, Spinosad exerted significant levels of toxicity to all the tested larvae than Temephos though their joint action exerted a less significant toxicity to all tested larvae. On the basis of the derived toxicity factors, Spinosad was 1.261, 2.478 and 2.522 times more toxic on laboratory-bred *Anopheles gambiae* larvae than *Anopheles gambiae* from the wild, *Aedes aegypti* from the wild and laboratory-bred *Aedes aegypti*. On the basis of the derived toxicity factors, Temephos was 1.099, 1.892 and 2.190 times more toxic on laboratory bred *Aedes aegypti* than *Aedes aegypti* from the wild, laboratory-bred *Anopheles gambiae* and *Anopheles gambiae* from the wild. Morphological distortions in *A. gambiae* and *A. aegypti* exposed to the larvicides include shrunken cuticles, disproportionate and enlarged heads, detached guts, loss of fossate hairs and collapsed midguts, Spinosad and Temephos should be used singly rather than jointly in the control of the two mosquito larvae.

KEY WORDS: Mosquito, Spinosad, Temephos, LC₅₀, toxicity factor, photomicrograph.

INTRODUCTION

Malaria caused by protozoan parasites of the genus *Plasmodium* is a life-threatening disease of public health importance, especially in sub-Saharan Africa, where it presents major obstacles to social and economic development (Awolola *et al.*, 2007). There are over 300 million acute cases of malaria globally each year, resulting in more than a million deaths and around 90% of these deaths occur in Africa, mostly in young children. (WHO, 2011)). Integrated Vector Management as recommended by WHO/AFRO is the main vector control strategy in Nigeria. World Health Organization has recommended Temephos as a larvicide for global use since early 1970 for the control of *Aedes aegypti*, *Culex* and *Anopheles* (WHO, 1986). Temephos works by inhibiting the activity of cholinesterase enzymes at the neuromuscular junction, ultimately causing paralysis and death. Spinosad, a novel bioinsecticide in the same class as *Bacillus thuringiensis*, is a new chemical class of pesticides registered by the United States Environmental Protection Agency (USEPA) to control a variety of insects. The action of spinosad is characterized by the excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors and paralysis (Salgado, 1998). Spinosad has a low persistence and low toxicity to a number of predatory insects (Williams *et al.*, 2003). As a result, the USEPA has classified it as a reduced risk material. Perera *et al.*, (2008) in Mexico showed that spinosad is as effective as Temephos at both laboratories

and field trials in eliminating the immature stages of *Aedes aegypti*. Currently in Nigeria there is a dearth of studies on the efficacy of Temephos in the control of mosquitoes. Moreover Spinosad is not used as a biopesticides in Nigeria. Objectives of the study are to evaluate the relative acute and joint action toxicity of Temephos and Spinosad against wild and laboratory-bred larvae of *Anopheles gambiae* and *Aedes aegypti* through laboratory trials.

MATERIALS AND METHODS

Laboratory – bred *A. aegypti* culture

A. aegypti larvae were collected with the aid of a 0.05mm fine sieve net in Badagry, Lagos State. The larvae were taken to the laboratory and kept in five 20cm x 7cm breeding bowls filled with 800ml of distilled water. The larvae were fed with 1.30g/day of rabbit pellets. The water in the breeding bowls was changed daily to avoid bacterial growth which can deoxygenate the water and kill the larvae. The larvae were observed daily until they molted into pupae after which twenty-five pupae each were collected from the breeding bowls and transferred with the aid of Pasteur pipette into four 300mls beakers each containing 150mls of distilled water. Two beakers each per cage were then placed inside two 50cm x 50cm mosquito breeding cages. The pupae were observed daily until they molted into adults. The cages were cleaned every two days to prevent ant's infestation and waste matter build-up. The adult mosquitoes were fed with sweet oranges. In addition the females were presented with two adult white rats from which they drew blood meal for

oviposition. The four beakers initially placed in the cages with the pupae were removed and replaced with four 300ml glass beakers each containing 200ml of distilled water and the inside of the beakers were lined with Whatman No. 1 filter papers. Observations were made until eggs were laid after which they were collected on the filter papers. The eggs were dried at room temperature and stored in sealed plastic bags and thereafter introduced into a beaker of water to hatch into larvae when needed. This procedure was repeated regularly to provide sufficient eggs that will emerge into larvae for the bioassays. The culture was maintained at 27 ± 2 °C, Relative Humidity of 70 ± 10 % and a photoperiod of 12:12 (L: D).

A. gambiae s.s

Third instar larvae of *A. gambiae s.s* were obtained from the insectary of the Molecular Entomology Laboratory, Public Health Division, Nigerian Institute of Medical Research, Lagos. The mosquito colony that had not been previously exposed to any form of insecticide was established in 2002 under the WHO/MIM – TDR Project A30026 and The colony was maintained at a temperature of 27 ± 2 °C and R.H. of 70 ± 10 % and a photoperiod of 12:12 (L: D).

Field collection of *A. aegypti* and *A. gambiae s.s* from the wild

Aedes aegypti and *Anopheles gambiae s.s* larvae were collected with the aid of a 0.05mm mesh size sieve from a natural breeding site along Badagry axis, in Lagos State where an open drainage exists. The larvae were taken to the laboratory, separated and kept in 20cm x 7cm breeding bowls filled with 800ml of distilled water. The third instar larvae were selected with the aid of a dropper into a pre-experimental holding bowl filled with distilled water for one hour before carrying out the bioassay.

Sources of chemicals

Skeeter ABATE® 5% pellets, containing 5% Temephos by weight was obtained from Harvest Field, an Agro-chemical company at Ojodu, Berger, Lagos, Nigeria. Spinosad (Spintor®) 1.25g/kg dust was obtained from the Zoology Department, University of Lagos, Lagos, Nigeria.

Preparation of stock solutions and test concentrations Temephos

Stock solution was prepared by weighing 100g of chemical which was homogenized and then poured into 100ml distilled water in a vial with aluminum foil over the mouth of the vial and covered with a screw cap. The vial was shaken vigorously to dissolve the larvicide. Stock solution was kept in a refrigerator until when needed. Test concentrations were appropriately diluted to produce final concentrations of 2g/l, 6g/l, 10g/l, 14g/l, 18g/l, 22g/l, 26g/l and 30g/l

Spinosad

Stock solution was prepared by weighing 10g of the powder in a volumetric flask and 100ml distilled water added. The flask was shaken vigorously to dissolve the material and kept in a refrigerator until when needed. Test concentrations were appropriately diluted to produce final concentrations of 0.01g/l, 0.03g/l, 0.05g/l, 0.07g/l, 0.09g/l, 0.2g/l and 0.4g/l respectively of Spinosad wettable powder.

Bioassay of wild and laboratory-bred of mosquitoes

Batches of 30 third instar larvae were transferred by means of droppers to small disposable tests cups each of the 10 concentrations. There were four replicates of each concentration and the untreated control. The depth of the water in the cup was maintained at 5-8 cm to avoid stressing the larvae. The test containers were held at 25-28 °C temperature, a photoperiod of 12h light followed by 12h dark (12L : 12D). Larvae mortality was recorded after 1h, 2h, 3h, 6h, 12h and 24h exposure. The larva was classified as dead if it did not move when gently touched with the point of a toothpick or if incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed. The assay was repeated three times using different batches of larvae on different dates. From the result LC₅, LC₅₀ and LC₉₅ values slope and heterogeneity analysis were obtained. Where the control mortality was between 5% and 20% the mortalities of treated groups were corrected. Bioassay with wild larvae was carried out following the same procedure with that of laboratory-bred larvae. The photomicrographs of third instar larvae of the test mosquitoes exposed to the different larvicides were taken using camera microscope, manufactured by Labnet International Inc. The photographs were taken by placing them on slides.

Joint action toxicity

The joint action effect of the two larvicides was carried out by combining the larvicide in the ratio 1:1 based on their 24hrs single action LC₅₀ values. This procedure was carried out for both the laboratory and field trials and also replicated four times.

Data analysis

The dose response data was analyzed by probit analysis in SPSS 17 software after Finney (1971) Statistical analyses were also carried out using Graph pad Prism-5.0 for the 2-way ANOVA statistical test to compare the percentage mortality of different larvicides. The indices of toxicity measurement derived were LC₅, LC₅₀ and LC₉₅. The values of the various test chemicals were compared.

RESULTS

Relative acute toxicity of spinosad against laboratory bred and wild *Anopheles gambiae* AND *Aedes aegypti*

The LC₅₀ values for the laboratory bred and wild *A. gambiae* larvae were 0.023g/l and 0.029g/l respectively while the LC₅₀ values for the laboratory bred and wild *A. aegypti* larvae were 0.058g/l and 0.057g/l respectively (Table 1, Figure 1) On the basis of the derived toxicity factors, Spinosad was 1.261, 2.478 and 2.522 times more toxic on laboratory-bred than on wild *Anopheles gambiae*, *Aedes aegypti* wild and laboratory bred *Aedes aegypti* respectively.

Acute toxicity of temephos against laboratory bred and wild *Anopheles gambiae* & *Aedes aegypti*

The LC₅₀ values for the Laboratory bred and wild *Anopheles gambiae*, were 14.035g/l and 16.251g/l while the LC₅₀ values for the laboratory bred and wild *Aedes aegypti* larvae were 7.418g/l and 8.150g/l respectively (Table 2 Figure 2). No significant difference occurred in same species of mosquitoes (Lab bred or wild). However the concentration was significantly higher for *A. gambiae* than *A. aegypti* larvae (Lab bred or Wild). The Laboratory

bred *A. aegypti* larvae was the most sensitive (7.418g/l) followed by *A. aegypti* (wild) (8.15g/l), then lab bred *Anopheles gambiae* (14.035g/l) while the least sensitive was *Anopheles gambiae* (Wild) (16.25) larvae (Table 2 and Fig 2). On the basis of the derived toxicity factors, Temephos was 1.099, 1.892 and 2.190 times more toxic on

laboratory bred *A. aegypti* than *A. aegypti* (wild); laboratory bred *A. gambiae* and *A. gambiae* (wild) respectively. On the basis of the 24hrs LC₅₀ values, Spinosad was generally more toxic than Temephos on both laboratory-bred and wild *A. gambiae* and *A. aegypti*.

TABLE 1: Relative acute toxicity of spinosad against laboratory bred and wild cultures of *Anopheles gambiae* and *Aedes aegypti* larvae in 24hrs

TREATMENT	LC ₅ (95% C.L.)g/l	L.C. ₅₀ (95% C.L.)g/l	L.C. ₉₅ (95%C.L.)g/l
Lab- bred <i>Anopheles gambiae</i> larvae	0.001 (0.001 – 0.002)	0.023 (0.018 - 0.030)	0.447 (0.326 – 0.649)
<i>Anopheles gambiae</i> larvae (Wild)	0.002 (0.001 – 0.003)	0.029 (0.022 – 0.037)	0.493 (0.360 – 0.715)
Lab- bred <i>Aedes aegypti</i> larvae	0.006 (0.004 – 0.007)	0.058 (0.048 - 0.070)	0.612 (0.467 – 0.836)
<i>Aedes aegypti</i> larvae (Wild)	0.005 (0.004 – 0.007)	0.057 (0.047 – 0.069)	0.616 (0.468 – 0.848)

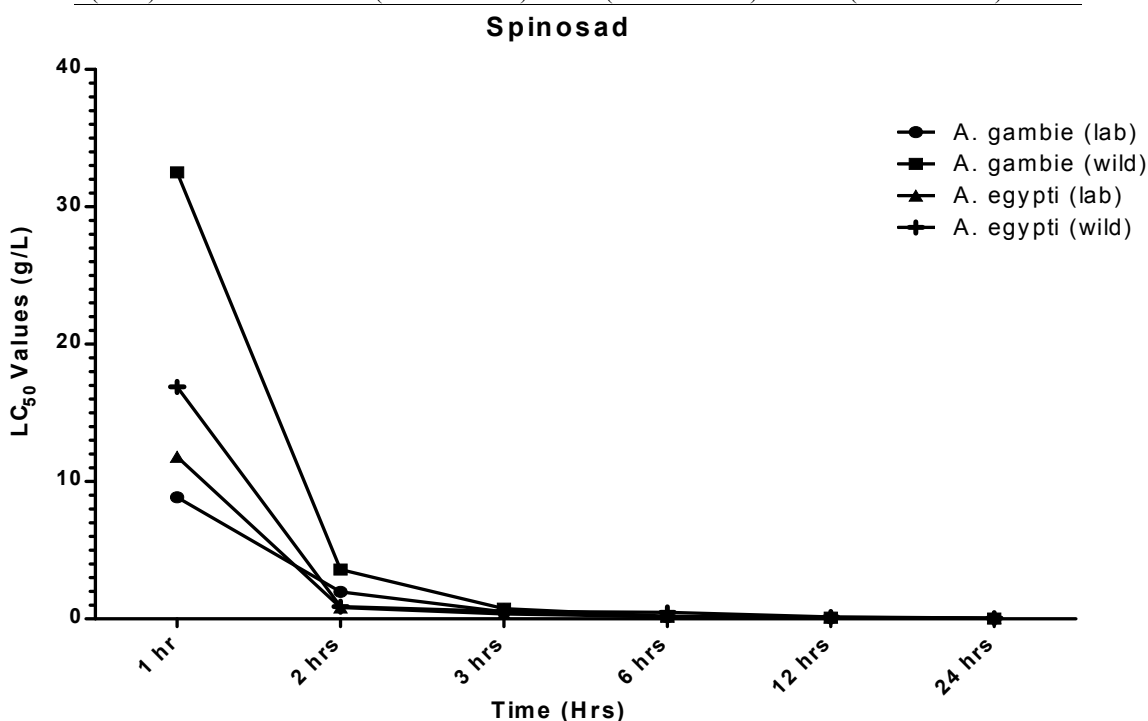


FIGURE 1: Response of *A. gambiae* and *A. aegypti* to Spinosad

TABLE 2: Relative acute toxicity of Temephos against laboratory bred and wild *Anopheles gambiae* and *Aedes aegypti* larvae in 24hrs

TREATMENT	LC ₅ (95% C.L.)g/l	L.C. ₅₀ (95% C.L.)g/l	L.C. ₉₅ (95%C.L.)g/l
Lab- bred <i>Anopheles gambiae</i> larvae	1.621 (1.098 – 2.198)	14.035 (11.917 - 16.533)	121.517 (89.533 – 179.672)
<i>Anopheles gambiae</i> larvae (Wild)	2.110 (1.305 – 2.990)	16.251 (13.331 – 19.970)	125.174 (85.661 – 212.090)
Lab- bred <i>Aedes aegypti</i> larvae	1.217 (0.877 – 1.593)	7.418 (6.300 – 8.630)	45.230 (37.264 - 56.770)
<i>Aedes aegypti</i> larvae (Wild)	1.322 (0.943 – 1.738)	8.150 (6.938 – 9.460)	50.259 (41.028 – 64.058)

TABLE 3: LC₅₀ values for Temephos on *A. gambiae* and *A. aegypti* Species

LC ₅₀ values for Temephos

Time	<i>A. gambiae</i> (lab)	<i>A. gambiae</i> (wild)	<i>A. aegypti</i> (lab)	<i>A. aegypti</i> (wild)
1 hr	583.097	389.575	183.378	169.387
2 hrs	143.430	203.831	53.193	63.314
3 hrs	66.233	80.872	32.560	40.246
6 hrs	38.620	49.371	21.478	27.106
12 hrs	20.201	20.762	12.764	14.836
24 hrs	14.035	16.251	7.418	8.150

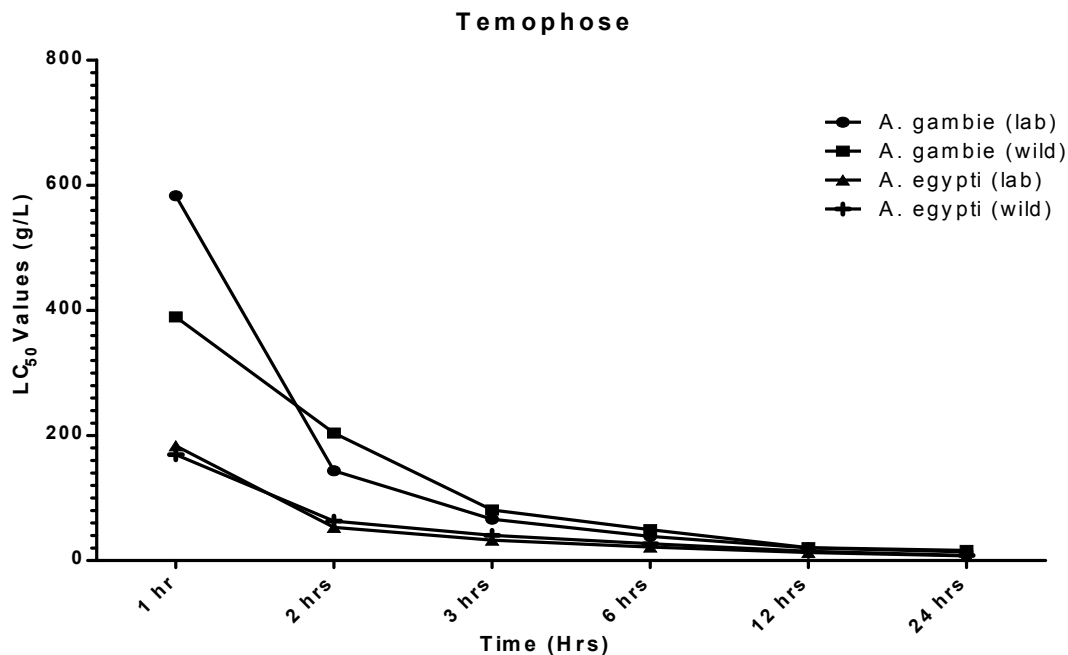


FIGURE 2: Response of *A. gambiae* and *A. aegypti* to Temephos

THE EFFECT OF SPINOSAD AT DIFFERENT CONCENTRATIONS OVER TIME

Generally, the species from the lab responded more than the ones from the wild and mortality increased with time of exposure to the test chemicals (Figure 3). At 6hrs exposures, mortality was observed at concentrations of 0.01g/l and above while no death was observed in control experiment. Lab-bred *A. gambiae* was the most sensitive with 40% mortality at 0.08g/l followed by *A. gambiae* from the wild, lab bred *A. aegypti* while *A. aegypti* from the wild was the least sensitive with 18% mortality (Figure 3b). At 12hrs exposure, 80% mortality was observed with the lab bred *A. gambiae* as the most sensitive followed by lab bred *A. aegypti*; species from the wild responded in a similar manner with *A. gambiae* being more susceptible than *A. aegypti* (Figure 3c). At 24hrs after application of the larvicide, 90% mortality was recorded for lab - bred *A. gambiae* and 80% for *A. aegypti* (Figure 3d).

The effect of temephos at different concentrations over time

Mortality was observed at concentrations of 2g /l and above and no death in the control for Temephos at 6hrs exposures (Figure 4b). Lab bred *A. aegypti* were most sensitive, recording 70% mortality while the wild species was 85%. At 12hrs exposure, *A. aegypti* species responded most but not in higher concentrations of 28g/l and 30g/l. At these higher concentrations, the *A. gambiae* species were the most sensitive. The maximum mortality recorded

was 80% with no death in control experiment (Figure 4c). 100% mortality was observed in all the species in the highest concentration of 30g/l at 24hrs after exposure to Temephos (Figure 4d).

Joint toxicity

Evaluation of the joint action toxicity of Temephos and Spinosad based on their LC₅₀ values showed that lab bred *A. aegypti* and lab bred *A. gambiae* were the most sensitive while the species from the wild were least sensitive (Figure 5). At the first 1hr of exposure, 7% mortality was observed on the Lab bred *A. gambiae*, the most sensitive followed by lab bred *A. aegypti* (5%). The species from the wild responded in a similar way with 3% mortality. Mortality followed almost the same trend increasing by the time. By 24th hour, the lab bred species recorded 100% mortality followed by *A. gambiae* from the wild with 90% mortality while *A. aegypti* from the wild was the least sensitive with 85% mortality.

On the basis of their LC₅₀ values, the test chemicals - Temephos and Spinosad acting singly were more potent than when they acted jointly (Figure 6). At 24hr after exposures single action spinosad, Temephos and joint action of the two larvicides gave 100, 100and 96% mortality respectively for lab bred *A. aegypti*; 98, 100 and 90% respectively for wild *A. aegypti*; 100, 100 and 97% respectively for lab *A. gambiae* and 97, 100 and 85% for wild *A. gambiae* (Figure 6).

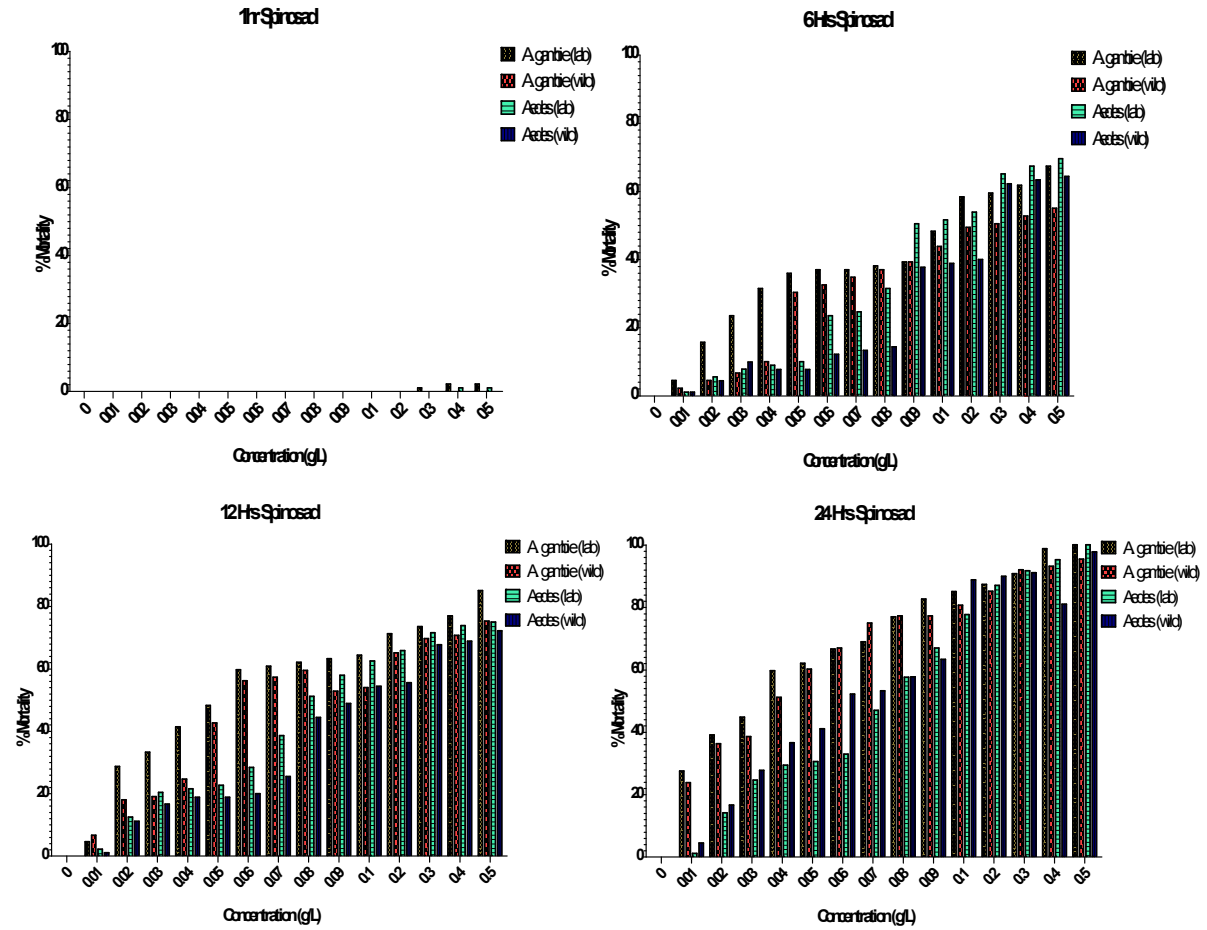
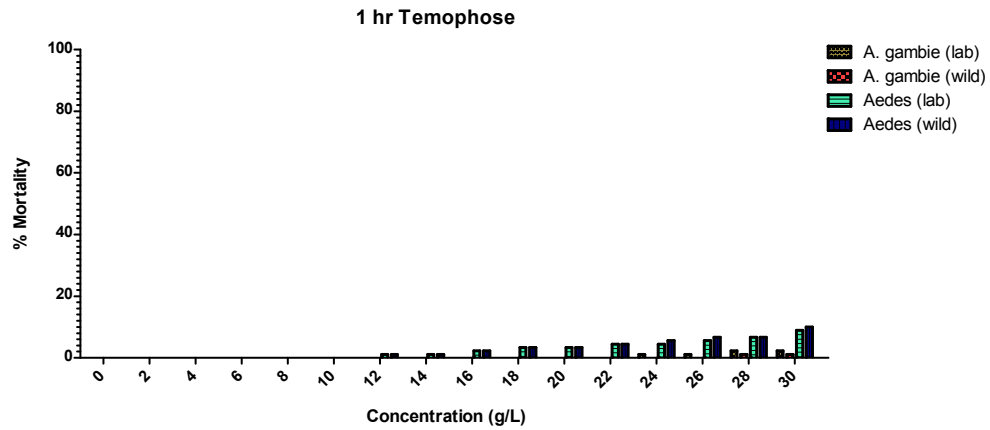


FIGURE 3A-d: Mortality of Spinosad at different concentrations over a period of 24hrs on different larval species



Spinosad and Temephos on mosquitoes

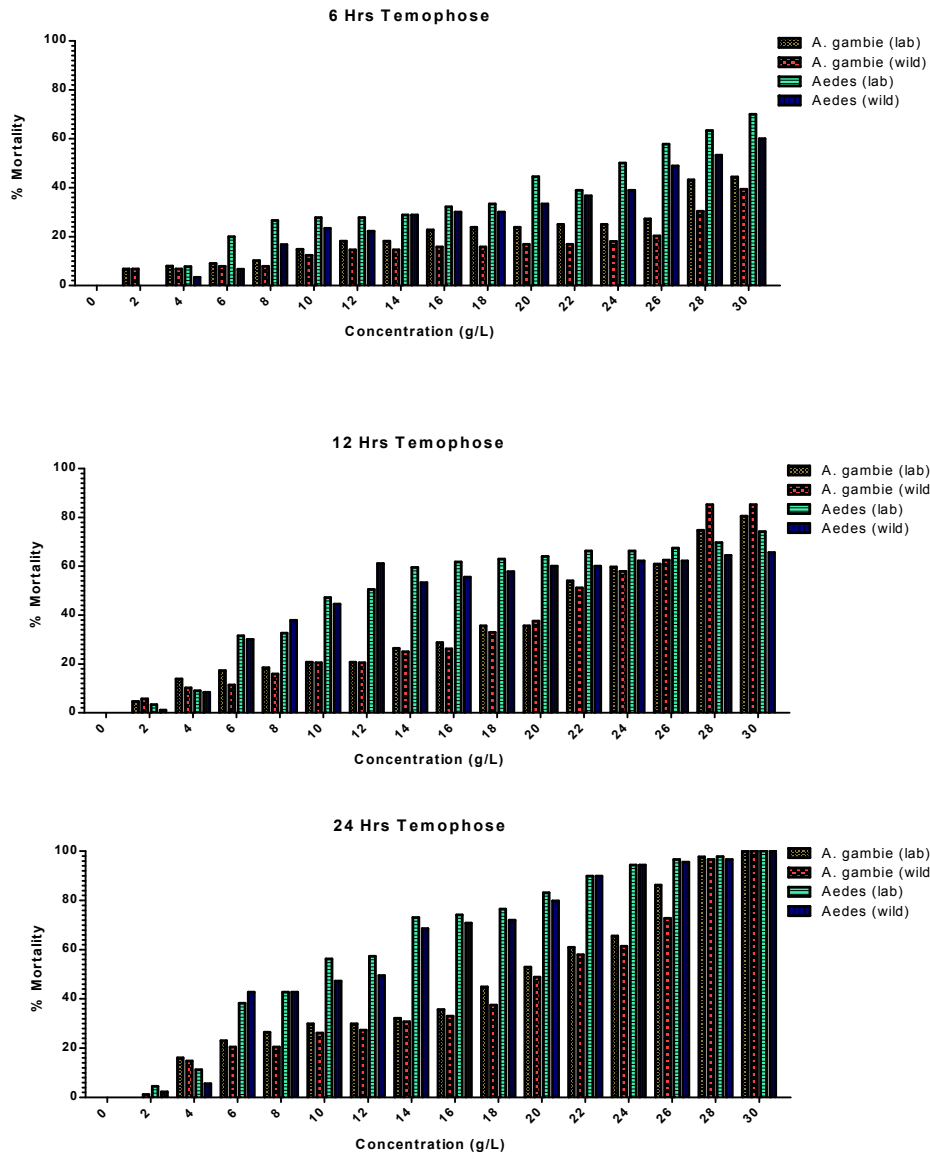


FIGURE 4A-d: The effect of Temephos at different concentrations over a period of 24hrs on the different larval species

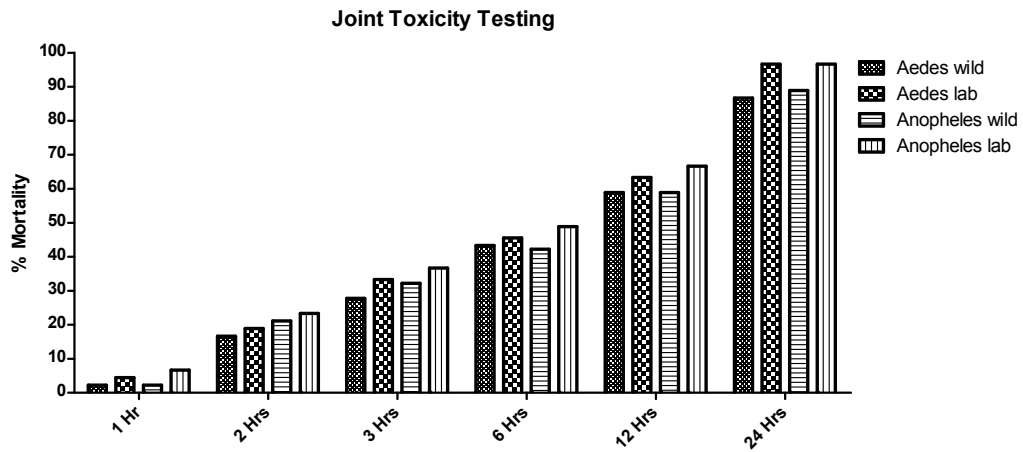


FIGURE 5: Effect of joint toxicity test over a period of 24hrs on the different larvae species

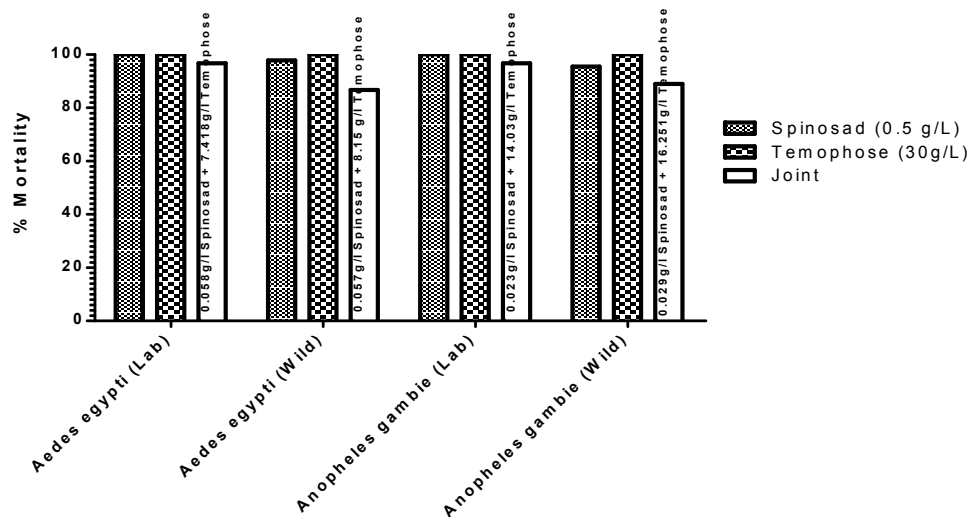


FIGURE 6: The effect of Temephos, Spinosad and Joint action on the two larvicides on the different larva species

PHOTOMICROGRAPHS SHOWING TEST ORGANISMS EXPOSED TO SPINOSAD AND TEMEPHOS

Plates 1-10 show varying degrees of morphological distortions in *A. gambiae* and *A. aegypti* exposed to the

larvicides compared to the control. These distortions include shrunken cuticles, disproportionate and enlarged heads, detached guts, loss of fossate hairs and collapsed midguts.

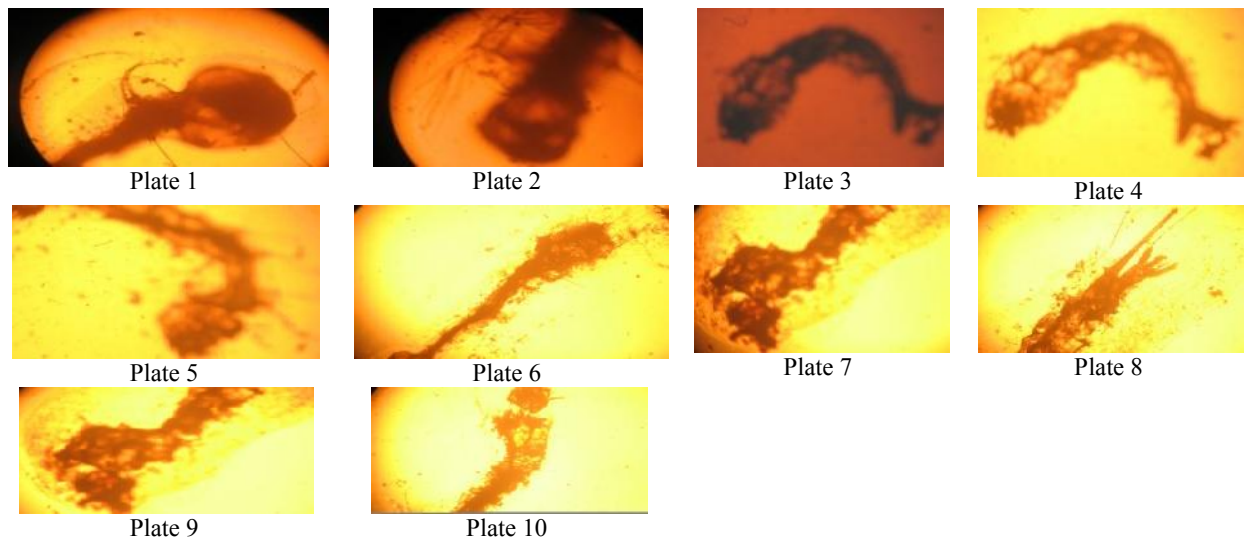


Plate 1: Laboratory-bred *A. gambiae* exposed to spinosad.

In plates 1, abdomen is transparent; most of the fossate hairs are lost. The head region is highly dense and enlarged and there is no differentiation between the thorax and abdomen.

Plate 2: *A. gambiae* (laboratory-bred) exposed to Temephos most of the fossate hairs are lost and the head is transparent

Plate 3: Laboratory-bred *A. aegypti* exposed to Temephos. There is no differentiation between the abdomen and the thorax.

Plates 4: Laboratory-bred *A. aegypti* exposed to spinosad. Thoracic and abdominal hairs are lost and there is no differentiation of the thorax from the abdomen. Abdomen is transparent.

Plate 5: The species from the wild of *A. gambiae* exposed to spinosad. The head is almost detached, all the abdominal hairs are lost and the abdomen is constricted and distorted. The mid-gut is collapsed, the cuticle shrunken and highly distorted.

Plate 6: The species from the wild of *A. gambiae* exposed to Temephos. The cuticle is shrunken and distorted, all the fossate and abdominal hairs are lost.

Plates 7 and 8: *A. aegypti* (wild) exposed to spinosad in which the cuticle is shrunken and distorted. The thoracic hairs are lost

Plates 9 and 10: The species from the wild of *A. aegypti* exposed to Temephos. The head is almost detached, midgut is constricted, abdomen is transparent and there is no differentiation between the thorax and abdomen.

DISCUSSION AND CONCLUSION

Temephos granules and spinosad suspension formulation were highly effective larvicides against both wild and laboratory bred larvae of *Anopheles gambiae s.s.* and *Aedes aegypti* mosquitoes. Spinosad showed better larvicidal activity against the two vector mosquitoes than Temephos. Spinosad was significantly ($p < 0.05$) more toxic than Temephos. Their joint action showed even less sensitivity than when they acted singly.

The findings in this study in the case of Temephos were similar to the result of Chen and Lee (2006). The 100% mortality observed in spinosad for *Aedes aegypti* is in harmony with the work of Perez *et al.*, (2007), Darriet *et al* (2009), Romi *et al.*, (2006) and Cetin *et al* (2005) for other mosquito species including *Anopheles gambiae*.

This result shows that the Lab bred *Anopheles gambiae s.s.* was most sensitive to spinosad than *A. aegypti* which disagrees with the work of Onyido *et al* (2011) who reported that spinosad treatment was significantly more effective at controlling *Aedes spp* than the *Anopheles spp*. The toxicity of an insecticide increases as the particle size decreases and this is likely responsible for the higher speed of action and toxicity of spinosad. Spinosad was able to gain entry into the larvae and cause insecticidal action faster than Temephos. The attendant disadvantages with dust formulations such as inhalation hazards and problem of drift makes it undesirable for field. Hence it is suggested that the formulation should be improved upon to eliminate these disadvantages.

The higher susceptibility of the lab bred larvae of the test organisms agrees with the residual efficacy trials in Lagos State, Nigeria by Amajoh *et al* (2009) using Skeeter ABATE (5% Temephos). In their research, Temephos achieved complete control of culicine and anopheline larvae after 24 hours. It was observed that the laboratory-bred larvae were more susceptible than those obtained from the wild. This may be because lab - bred have not been exposed to different classes of insecticides; hence they would not have developed some form of resistance. It could also be because the lab bred larvae are not exposed to other environment factors.

Aedes aegypti larvae was found to be more susceptible to the test chemicals (Temephos) which is in agreement with the work of Jirakanjanakit *et al* (2005) who observed full susceptibility of *Aedes aegypti* larvae to Temephos in Cameroun. This result may be due to the recent introduction of Temephos in Africa countries for larviciding so the problem of resistance is yet to arise. The different morphological distortions observed may be important leads to understanding the mode of action of the chemicals.

This study has been able to establish the fact that spinosad as a larviciding tool will be effective in Lagos State. Spinosad has a number of advantages over Temephos. Besides its higher insecticidal action against mosquito larvae, it is biodegradable with no significant effect on non-target population. According to Wallace and Hypes (1981), Temephos treatment resulted in 20 -30 reduction in non-target aquatic invertebrate populations with Baetidae and chironomidae severely affected. There was no significant increase in fish fry mortality after exposure to 12ppm spinosad and it can be used in the control of fish

ectoparasites in aquaculture, either in the water or in fish feed, underlining their low toxicity to fish (Dick *et al.*, 2008).

One control strategy is larviciding which is the killing of immature mosquitoes by applying agents, collectively called larvicides, to control mosquito larvae and/or pupae. Most mosquito species spend much of their life cycle in the larval stage where they are highly susceptible to both predation and control efforts. They often are concentrated within defined water boundaries and immobile with little ability to disperse while the adult mosquitoes on the other hand, fly in search of blood meals, mates and water sources for egg laying which makes most of them inaccessible and widely distributed. Effective larviciding can thus reduce the number of adult mosquitoes available to infest humans through their bites.

Environmental management which ensures that the drains are cleared and permit free flow of water will help limit the use of larvicides to a few well-funded programs. Secondly, before larviciding can be carried out surveillance need to be carried out to determine the mosquito larval density and decide whether larviciding should be carried out or not. The technicalities and principles involved in surveillance show that larviciding is not a personal protective measure to control mosquitoes. Hence this work also recommends that Lagos State should be divided into Mosquito Control Districts where every member of the district will contribute their quota towards a successful larviciding programme funded by the government. The use of spinosad as a larvicide along with IRS and/or LLIN will result in a significant reduction in mosquito population and ultimately malaria morbidity.

RECOMMENDATIONS

Temephos granules and a suspension concentrate formulation of spinosad were both highly effective larvicides when used both singly and jointly against *Aedes aegypti* and *Anopheles gambiae*. Spinosad and Temephos should therefore be used singly rather than jointly. These compounds merit detailed evaluation for inclusion in integrated control programs targeted at *Aedes aegypti* and *Anopheles gambiae* in regions where they represent important vectors of human diseases.

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