**Running Title:**

**CO-INTERCALATION OF INSECTICIDES IN BENTONITE**

# CO-INTERCALATION OF INSECTICIDES WITH HEXADECYLTRIMETHYLAMMONIUM CHLORIDE IN MOZAMBICAN BENTONITE

R.D. Merckel[1], W.W. Focke[1], M.M. Sibanda[1], P. Massinga Jr[2], and N.A.S. Crowther[3]

[1]Institute for Applied Materials, Department of Chemical Engineering, University of Pretoria, Pretoria, South Africa

[2]Faculty of Natural Sciences, Department of Chemistry, University Eduardo Mondlane, Maputo, Mozambique

[3]Department of Statistics, University of Pretoria, Pretoria, South Africa

**Abstract**

WHO-approved insecticides were co-intercalated with hexadecyl-trimethylammonium (HDTMA) chloride in bentonite in an attempt to improve their persistence. XRD and FT-IR confirmed successful co-intercalation of the insecticides. Bioassays revealed that the activity of co-intercalated insecticides deteriorated after just one week of accelerated aging at 40 °C and 90 % ℜH. However, when the pH of the bentonite slurry used for intercalation was reduced with acetic acid to pH = 4.5, the persistence life increased to 113 days. The performance ranking with respect to both knockdown and mortality was as follows: Co-intercalated α-cypermethrin > co-intercalated propoxur > co-intercalated DDT > DDT.

**KEYWORDS:** co-intercalated insecticides, organo-clays, insecticide hydrolysis

## Introduction

A World Malaria Report [1] states that half the world's population is at risk of contracting malaria, which has led to an estimated 863 000 deaths from the estimated 243 million malaria cases for 2008. Currently insecticides such as dichlorodiphenyl-trichloroethane (DDT) and its derivatives are used very effectively to control mosquitoes and thereby reduce the burden of malaria [2]. However, DDT is a persistent organic pollutant that causes continual ecological problems. Furthermore, exposure to DDT through residual spraying causes many serious health effects in humans [3].

Insecticides approved by the World Health Organization (WHO) offer safer alternatives than harmful insecticides such as DDT. However, due to volatilisation and weathering effects (such as biodegradation, photodecomposition and alkaline hydrolysis), larger dosages of these insecticides are often required. It has been shown that volatilisation and photodecomposition can be reduced by using montmorillonite-based clay (such as bentonite) containing appropriate organic cations. It is however possible that co-intercalated insecticides could be degraded if the clay contains locations on its structure that would cause alkaline hydrolysis [4].

The main objectives of this investigation were to:

- intercalate cationic surfactants into bentonite
- co-intercalate WHO approved insecticides into bentonite, and
- determine whether acidifying bentonite, using acetic acid, could help stabilize the co-intercalated insecticides

The montmorillonite clay used in the experiments was from the Boane region in Mozambique. Single chain cationic surfactants were used for intercalation of the clay. The insecticides were selected from those approved by WHO [5].

## Background

Indoor residual spraying (IRS) is one of the best methods to use against malaria and has received support by WHO [6]. Presently DDT is used for this method as it lasts longer than other insecticides currently approved by WHO [7]. DDT is however too stable and therefore is classed as a persistent organic pollutant that bio-accumulates in the ecosystem [8]. In order to eliminate the use of DDT in vector control, it is necessary to improve the stability of WHO approved insecticides. The co-intercalation of these insecticides into bentonite may improve their stability by protecting them from degradation caused by the environment until they are released from the clay [4].

It is known that bentonite containing montmorillonite is suited for intercalation via ion exchange of the cations present (mostly sodium and calcium) with organic cations [9]. Yaron-Marcovich, Nir & Chen [10] showed successful intercalation using organic alkylammonium cations. This caused the clay to become pillared [11] as illustrated in Figure 1. Such a structural change in the clay could be used to introduce a second neutral chemical species such as an insecticide. Clay which is intercalated with organic cations is known as an organo-clay.

Biological assays (or bioassays) are used to determine the effectiveness of insecticide formulations. The procedures for conducting bioassays, according to international standards, are documented by [6]. The same preparation and bioassay methods are used for the neat insecticides dosages and the co-intercalated samples. This involved spraying the actives onto a mud surface that is at least 5 mm thick a minimum diameter of 12 mm [6]. Plastic cones (open at both ends) are placed over these surfaces. Figure 2 shows the basic outline of the set up used for the bioassays.

The procedure according to [6] requires that 2 to 5 days old non-blood-fed female mosquitoes be used for bioassays. As shown in Figure 2, the mosquitoes are introduced into the cone and the entrance of the cone is plugged using cotton wool. To encourage the mosquitoes to rest on the surface of the substrate, the pans are placed at an angle of about 40 ° with reference to the ground. The mosquitoes are exposed to the prepared surface for 30 minutes [6] and are kept at a temperature of 27 °C ± 2 °C and a relative humidity of 80 % ± 10 % during the tests. After the 30 minute exposure time, the mosquitoes are collected and placed in 150 mL cups for 24 hours (being kept at the same conditions as mentioned above). After 1 hour of starting the tests the knockdown is recorded, and after 24 hours the mortality is recorded [6]. Bioassays are first used to determine the minimum concentration of substrate causing 100 % mortality. Once this is determined, the active is used at two to four times this concentration [6].

## Experimental

### *Procedure used for co-intercalation of insecticides*

HDTMA chloride was selected as the organic alkylammonium cationic surfactant. Sufficient dry surfactant was used so as to fully satisfy the cationic exchange capacity (CEC) of the bentonite, i.e. 70 mmol/100 g.

Environmental pH affects the stability of insecticides with the majority being most stable at pH ≈ 5. Therefore two sets of samples were prepared to compare the performance of the neat clay slurry with an acidified version. Three insecticides (DDT, propoxur and cyfluthrin) were dissolved into 5 g of acetone so that each insecticide would be present in the clay at 40 % (mol) with respect to the surfactant. See Table 1. Each insecticide solution was added to a solution of surfactant, containing 2.24 g of dry surfactant, and stirred vigorously in a test tube.

The insecticide solution was slowly dripped into 64 g ± 0.3 g of slurry (containing about 10 g ± 0.007 g bentonite) using a burette while the slurry was vigorously stirred with an overhead stirrer for approximately 12 minutes. For the second set of samples prepared, the same procedure was followed but with the slurry first being acidified with enough acetic acid to adjust the pH of the slurry to pH = 4.5. The amount of acetic acid was about 60 mL.

α-Cypermethrin was used instead of cyfluthrin in the second experiment, as it was later discovered through bioassays that α-cypermethrin was more effective than other pyrethroids [12]. It is also known that cyfluthrin and α-cypermethrin are analogous pyrethroids which have very similar physical and chemical properties so α-cypermethrin may be used instead [13]. After mixing, a part of the slurry was washed using 100 mL of distilled water by vigorously mixing the distilled water into the slurry for 1 minute. Each slurry sample was then centrifuged for 35 minutes using an industrial centrifuge (Model K, Damon/IEC Division).

For each sample, the liquid and solid layers formed during centrifugation were separated from each other and kept for further analysis. The liquid was tested for the presence of chloride ions using silver nitrate. The surfactant solution was also tested using silver nitrate. The solid layers (consisting of organo-clay) were dried on aluminium foil for 3 days in a fume hood, out of direct sunlight. The dried organo-clays were each powdered separately using a standard blade grinder. A fourth sample was prepared by grinding up plain bentonite in the same way.

The changes in the packing geometry of the organo-clays were measured using X-ray diffraction (XRD). To determine the presence of the insecticides in the organo-clays, Fourier-transform infrared spectroscopy (FT-IR) was used on the organo-clays.

*Preparing co-intercalated insecticides for bioassays*

To prepare samples for bioassays, mud blocks were prepared according to the relevant specifications [6]. For each co-intercalated insecticide, 3 mud blocks were formed. All the mud blocks were dried at ambient conditions for 3 days before treating them with organo-clays.

The required levels of insecticides used for IRS were determined based on the recommended amounts stated by [5] as summarized in Table 2. The amounts of insecticides actually used for the bioassays were 200 % of the recommended amounts suggested by [5]. The powdered co-intercalated insecticides were first dispersed into 10 mL of water before being sprayed.

*Conducting bioassays on co-intercalated insecticides*

Bioassays were conducted in a controlled environment, using accelerated aging conditions, at a temperature of 40 °C ± 2 °C and a relative humidity of 90 % ± 10 %. The treated mud blocks were covered with plastic cones according to the procedure recommended by [6]. Five day old female mosquitoes (anopheles arabiensis) were placed into groups of 25 mosquitoes each per sample tested. Each group of mosquitoes was exposed to an organo-clay for 30 minutes.

The exposed mosquitoes were placed into polystyrene cups after exposure and given food in the form of sugar water. After 1 hour from when the tests were started the number of knocked down mosquitoes were counted and recorded for each batch of mosquitoes. After 24 hours the total number of deaths in each batch was recorded. Two sets of bioassays were completed 1 week apart. In-between these tests, the samples were kept in a humidification cupboard at temperature of 40°C ± 2 °C and a relative humidity of 90 % ± 10 %. Further bioassays were conducted monthly until the insecticides lost their activity.

## Results and discussion

### *XRD and FT-IR analyses on organo-clays and bentonite samples*

Figures 3 to 5 show the diffractograms for the XRD analyses of untreated bentonite (denoted as "a") as well as the organo-clays prepared (denoted as "b"). The d-spacing for each organo-clay sample as well as for plain bentonite clay is summarized in Table 3. The d-spacing of the co-intercalated clays has increased from 1.58 nm to between 2.12 nm and 2.17 nm. This difference in d-spacing is attributed to successful intercalation of bentonite with HDTMA cations. This however may not confirm whether co-intercalation of any insecticide was successful.

### *Results for bioassays*

Results for bioassays for co-intercalated insecticides without acetic acid are summarized in Table 4. The results indicate that although the insecticides remain active after co-intercalating them into bentonite after 1 day, their activity declined rapidly after just 1 week. It is known that DDT has a long active life and easily lasts for 6 months [2], but since the bioassays did not reveal this (even taking into account that bioassays were completed under accelerated aging conditions), it is possible that the clay itself caused the degradation of the insecticides.

The bioassay results for the co-intercalated insecticides samples obtained from the acidified clay slurry are given in Table 5. These results showed an improvement in the performance of co-intercalated insecticides. From this, it is clear that it is necessary to adjustment the pH in order to obtain stable insecticides. It is further concluded that α-cypermethrin and propoxur could possibly outperform DDT using the techniques presented in this paper. This is indicated by the inactivity of DDT at month 4, while α-cypermethrin and propoxur still had some residual activity as shown in Table 5.

The data in Table 5 also reveal that activity decreased for a time and then increased again after some time. It has been suggested by Boul [14] that DDT may bind to clay whereby it loses its activity, although little is known about this principle.

*Statistical analysis of bioassays for acid-treated co-intercalated insecticides*

Statistical analysis was conducted to estimate the active life of the samples under the accelerated ageing conditions used here. This was taken as the time for the 1 h Knockdown or the 24 h Mortality to drop below 80 %. This information is plotted in Figure 6, where the first two bars are for the knockdown of mosquitoes, and the remaining bars are for the mortality of mosquitoes. Co-intercalated DDT failed to perform above the 80 % cutoff mark for knockdown activity. From Figure 6 it may be concluded that Mortality ranking was as follows:

Co-intercalated α-cypermethrin > co-intercalated propoxur > co-intercalated DDT > DDT

The error bars in Figure 6 indicate 95 % confidence intervals for Mortality and Knockdown as determined by logistics regression. Those for α-cypermethrin do not overlap with those of the other treatments. This indicates that the superior performance observed for this insecticide is statistically significant. Co-intercalated propoxur also performed better than co-intercalated DDT with regards to Knockdown since the 95 % confidence intervals do not overlap. However, it cannot be said with certainty whether co-intercalated propoxur performed better or worse than co-intercalated DDT when considering Mortality.

## Conclusions and recommendations

XRD and FT-IR results confirm successful co-intercalation of the insecticides. Bioassays following accelerated aging were used to quantify the performance of co-intercalated insecticides. Bioassays at first revealed that co-intercalated insecticides obtained without the addition of acetic acid remained active for a day after the organo-clays have been sprayed onto mud surfaces, but rapidly lost their effectiveness within a week. Reducing the pH of the clay to 4.5 with acetic acid increased the active life of co-intercalated insecticides. As a result of this, the performance of co-intercalated α-cypermethrin regarding both knockdown and mortality frequencies exceeded that of co-intercalated propoxur and co-intercalated DDT. Although co-intercalated propoxur performed better for knockdown that co-intercalated DDT the difference was not statistically significant. So it is not possible to say whether co-intercalated propoxur is better with regards to Mortality when compared to co-intercalated DDT.

## References

[1] World Health Organization (2008). World Malaria Report 2008, http://www.who.int/malaria/wmr2008/ [2010 January 12].

[2] Dunlap, T.R. (1981). DDT: Scientists, Citizens, and Public Policy, Princeton University Press: New Jersey, USA.

[3] Eskenazi, B., Chevrier, J., Rosas, L.G., Anderson, H.A., Bornman, M.S., Bouwman, H., Chen, A., Cohn, B.A., de Jager, C., Henshel, D.S., Leipzig, F., Leipzig, J.S., Lorenz, E.C., Snedeker, S.M. and Stapleton, D. (2009) Environ. Health Perspect., 177, 1359.

[4] El-Nahhal, Y., Nir, S., Marguilies, L. and Rubin, B. (1999). Appl. Clay Sci., 14, 105.

[5] World Health Organization (2009). WHO recommended insecticides for indoor residual spraying against malaria vectors, http://www.who.int/whopes/quality/en/ [2010 January 12].

[6] World Health Organization (2006). WHO gives indoor use of DDT a clean bill of health for controlling malaria, http://www.who.int [2010, January 12].

[7] Sadasivaiah, S., Tozan, Y. and Breman, J.G. (2007). Am. J. Trop. Med. Hyg., 77, 249.

[8] Bro-Rasmussen, F. (1996). Sci. Total Environ., 188, 45.

[9] Gereli, G., Seki, Y., Kuşoğlu, I.M. and Yurdakoc, K. (2006). J. Colloid Interface Sci., 299, 155.

[10] Yaron-Marcovich, D., Nir, S. and Chen, Y. (2003). Appl. Clay Sci., 24, 167.

[11] Park, J.K., Choy, Y.B., Oh, Kim, J.Y., Hwang, S.J., Choy, J.H. (2008). Int. J. Pharm. 359, 198.

[12] Hougard, J.M., Duchon, S., Darriet, F., Zaim, M., Rogier, C. and Guillet, P. (2003). Bull. World Health Organ. 81, 324.

[13] Williams, R.L., Bernard, C.E. and Krieger, R.I. (2003). J. Exposure Anal. Environ. Epidemiol., 2003, 112.

[14] Boul, H.L. (1995). N. Z. J. Agric. Res., 38, 257.

**List of Figures**

**List of Tables**

Table 1: Amounts of insecticides used for the co-intercalation experiments

| Insecticide Name | Amount of Insecticide (g) |
| --- | --- |
| DDT | 1.65 |
| Propoxur | 0.98 |
| Cyfluthrin | 2.03 |
| α-cypermethrin | 1.95 |

Table 2: WHO recommended dosages of insecticides for IRS

| Insecticide | Dosage ($g/m^2$) |
| --- | --- |
| Propoxur | 1.0 – 2.0 |
| DDT | 1.0 – 2.0 |
| α-cypermethrin | 0.02 – 0.03 |
| Cyfluthrin | 0.02 – 0.05 |

Table 3: XRD d-spacings for neat bentonite and co-intercalated clays

| Type of Clay | d-Spacing (nm) |
| --- | --- |
| Clay containing α-cypermethrin | 2.12 |
| Clay containing propoxur | 2.14 |
| Clay containing DDT | 2.17 |
| Bentonite | 1.58 |

Table 4: Anopheles arabiensis bioassay Mortality for co-intercalated insecticides prepared without acetic acid treatment

| Insecticide | Mortality, % | |
|---|---|---|
| | (1 day) | (7 days) |
| Cyfluthrin sample 1 | 73.91 | 0.00 |
| Cyfluthrin sample 2 | 94.74 | 0.00 |
| Cyfluthrin sample 3 | 100.00 | 3.70 |
| Propoxur sample 1 | 100.00 | 17.86 |
| Propoxur sample 2 | 100.00 | 0.00 |
| Propoxur sample 3 | 100.00 | 22.73 |
| DDT sample 1 | 90.48 | 16.00 |
| DDT sample 2 | 78.95 | 40.91 |
| DDT sample 3 | 29.41 | 0.00 |

Table 5: Anopheles arabiensis bioassay Mortality for co-intercalated insecticides prepared with acetic acid treatment

| Insecticide | Mortality, % | | | | |
|---|---|---|---|---|---|
| | 7 days | 28 days | 56 days | 91 days | 113 days |
| α-cypermethrin sample 1 | 100 | 92 | 89 | 65 | 4 |
| α-cypermethrin sample 2 | 100 | 100 | 92 | 54 | 8 |
| α-cypermethrin sample 3 | 100 | 100 | 100 | 67 | 12 |
| Propoxur sample 1 | 100 | 100 | 8 | 20 | 4 |
| Propoxur sample 2 | 100 | 100 | 10 | 36 | 8 |
| Propoxur sample 3 | 100 | 100 | 8 | 48 | 0 |
| DDT sample 1 | 100 | 96 | 100 | 88 | 0 |
| DDT sample 2 | 100 | 36 | 96 | 42 | 0 |
| DDT sample 3 | 97 | 88 | 96 | 43 | 0 |

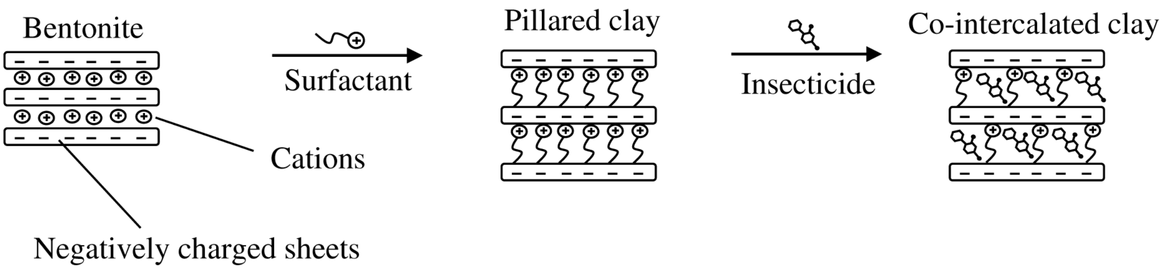


Figure 1: The concept of pillaring bentonite so as to introduce insecticides between the clay sheets through co-intercalation.

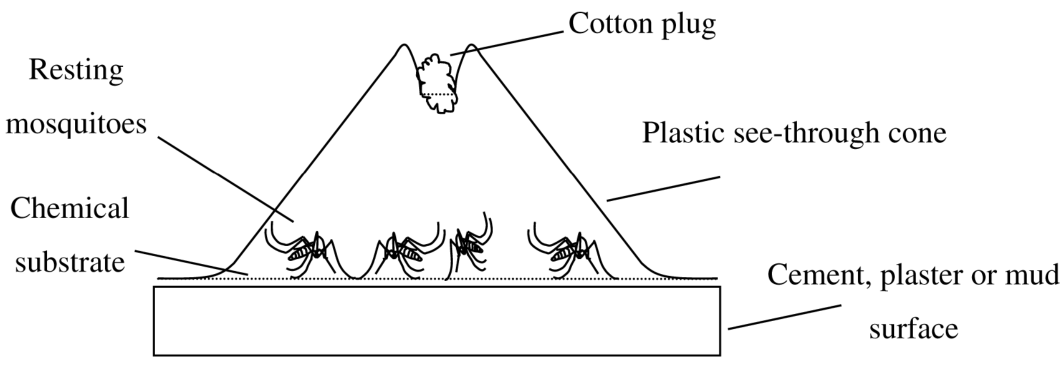


Figure 2: Basic set up of apparatus for conducting bioassays on mosquitoes.

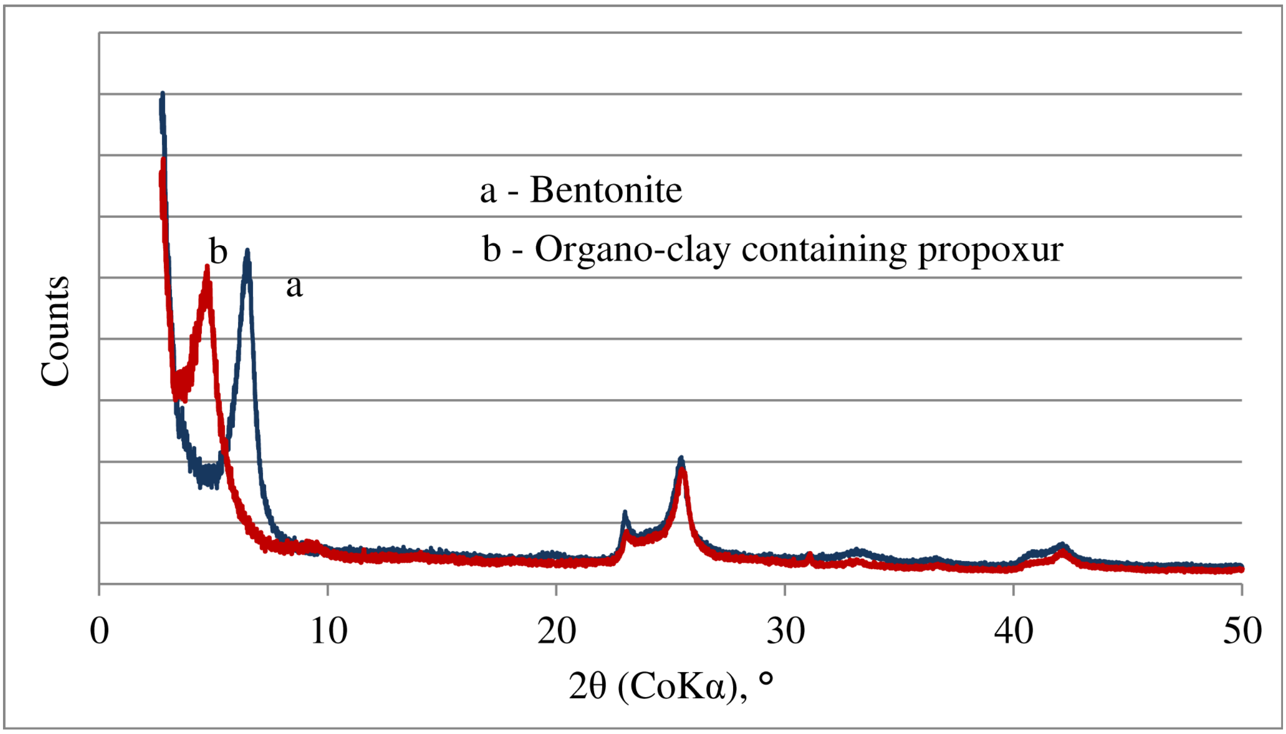


Figure 3: XRD diffractogram for clay prepared with HDTMA and propoxur compared to that of the neat clay.

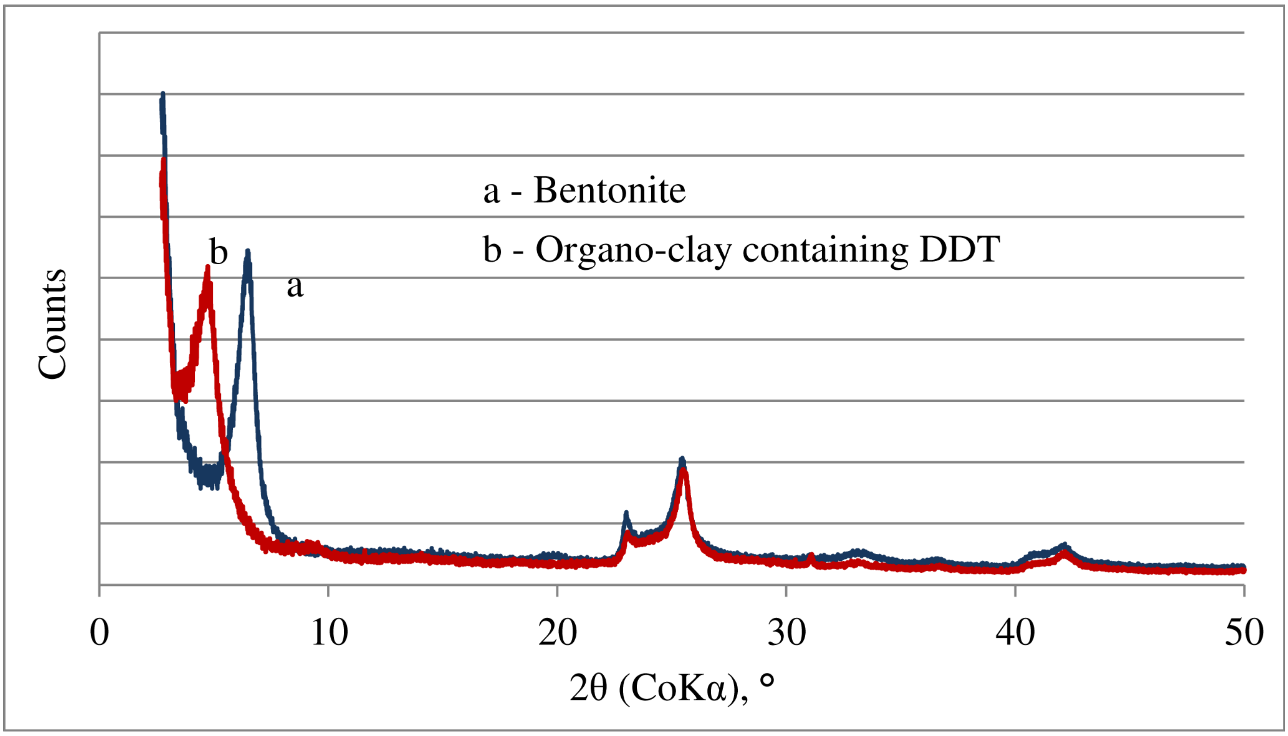


Figure 4: XRD diffractogram for clay prepared with HDTMA and DDT compared to that of the neat clay.

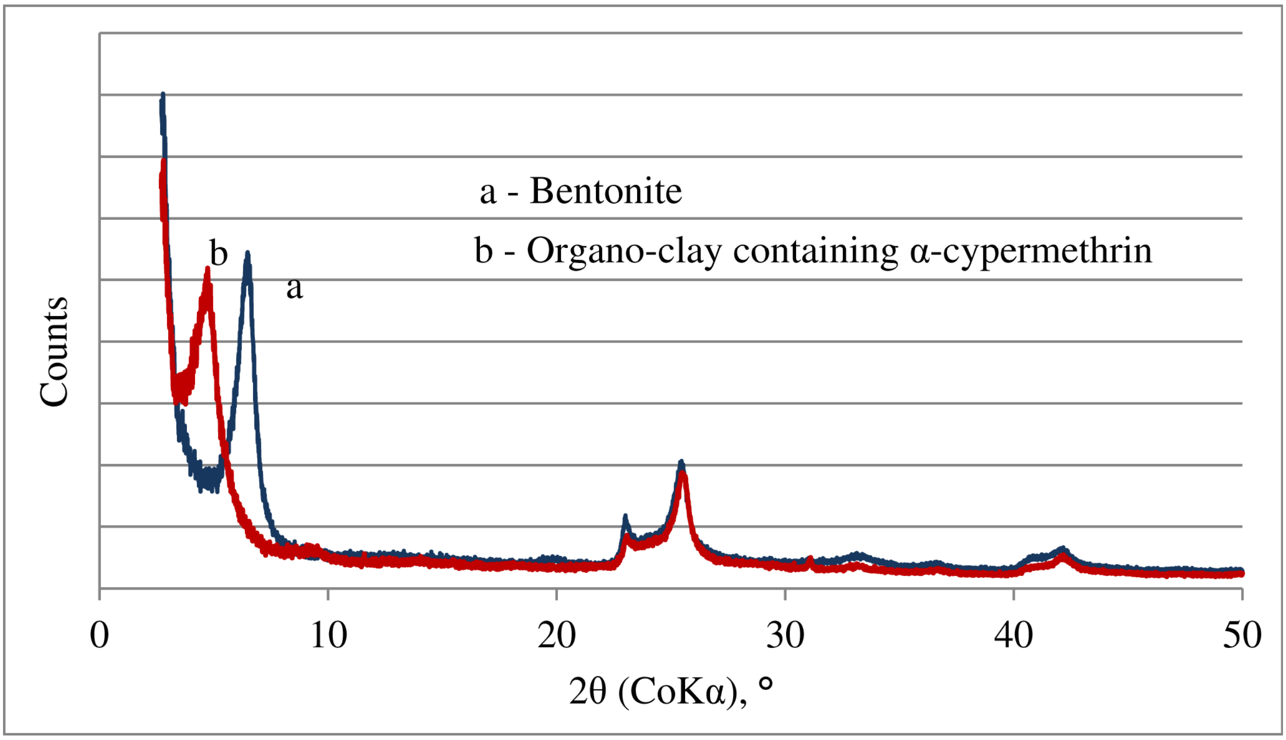


Figure 5: XRD diffractogram for clay prepared with HDTMA and α-cypermethrin compared to that of the neat clay.

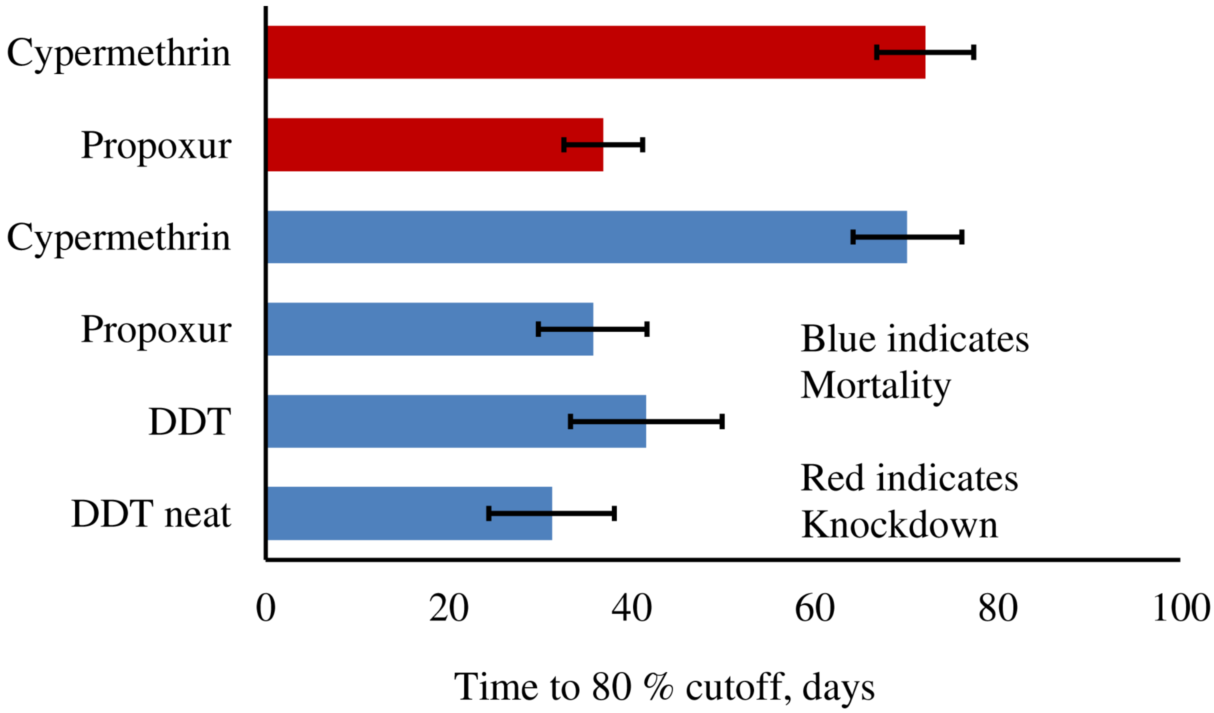


Figure 6: Time cut off for 80 % knockdown and 80 % mortality of mosquitoes, with error bars indicating the 95 % confidence interval.