Resistance to acaricides of *Boophilus* ticks from cattle in Ghana.

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Abstract

A survey focusing on the presence of resistance of *Boophilus* ticks against three different types of acaricides was carried out in Ghana. The three types of acaricides were Amitraz, Deltamethrin and Cypermethrin. The principles of the larval packet test, written by the FAO, and the method of the University of the Free State, South Africa, were used to measure the percentage mortality produced by the acaricides. Fifty five engorged female ticks were collected in the Volta Region. The ticks were kept at a temperature of 27º C and a relative humidity greater than 75% to oviposit. Approximately 13,320 seven-to-fourteen day-old larvae were tested by 198 filter papers. The filter papers were impregnated according to the FAO practical field manual with six different acaricide concentrations in olive oil and chloroform, and one control without acaricide. Duplicate filter paper packets were prepared for each test assay. After examination, a mean mortality of 100% was found for both cypermethrin and deltamethrin at the recommended doses, which was written on the packaging of the acaricides. A mean mortality of 80.8% was found for Amitraz at the recommended dose. At a concentration twice higher than the recommended dose, all the larvae died. In conclusion, the results indicated the presence of low levels of resistance against Amitraz of *Boophilus* ticks from the Volta Region of Ghana.

Chapter 1: Introduction

Background information

The country

Ghana is a coastal country situated in West Africa between 1º and 11º north of the equator and is bisected by the Greenwich meridian (Figure 1). The mean annual temperature varies between 22ºC and 33ºC with a seasonal variation. Most regions of Ghana have a tropical savanna climate, except a small area in the coastal west of tropical rain forest climate and a small area in the south east coastal plain of tropical steppe climate. Rainfall decreases from south to north [Walker and Koney, 1999].

Fig 1. Map of regions of Ghana

Agricultural structure

The cattle population has gradually increased over the years reaching about 1,248,861 animals in 1996, with an estimate for 2007 of 1,402,581 cattle. The highest density of cattle is found in the Upper East Region with 24 cattle per square kilometre, followed by the Greater Accra Region and Upper West Region with 21 and 15 cattle per square kilometre respectively. The other regions of Ghana have the following cattle densities: Eastern, Northern, Volta and Western Regions have three, six, five and less than one animal per square kilometre, while Ashanti, Brong Ahafo and Central Regions all have one animal per square kilometre [Koney, 2004].

Fig 2. Zebu bulls in Accra

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The West African Shorthorn (WAS) and its crosses are the dominant breed, making up about 90-96 percent of the total cattle population. The WAS are small, humpless animals and the adults weigh about 180-200 kg. Among the crosses the Sanga, a cross between WAS and Zebu, forms about 16 percent of the population. Pure Zebus and N'dama comprise about 2.6 and 2.1 percent respectively. The Sanga and Zebu (Figure 2) are mainly kept in the Volta and Greater Accra Regions [Koney, 2004].

Almost all cattle in the country are kept under a cow-calf operation system. The breeding herds are kept together with their calves until the calves have reached market weight or the breeding animals are removed. Breeding herds and their calves may be owned by several individuals who keep their animals together in the same kraal under the same management, or the animals are kept as family property and managed by the head of the family. In most of the areas, animals are kept in a kraal at night after grazing. These animals are kept under a traditional management system with a minimum of deworming and tick control. Animals graze improved or natural forage without supplementation in many cases and are exposed to the direct effect of rain and sun because their kraals are not roofed. Sometimes a few kraals have tree cover near the kraals which provide shade. Cattle faeces are not regularly removed from the kraal thus facilitating the breeding of flies. These flies contribute greatly to disease

conditions such as pink eye and eye worms. Wet and muddy conditions contribute to locomotive and intestinal problems. The animals are herded twice a day in the rainy season on communal unfenced land. In the dry season the animals are herded once a day and are also milked once a day [Koney, 2004].

Ticks in Ghana

In a field study carried out between 1994 and 1997 the distribution of ticks on livestock in Ghana was mapped. Tick species found on livestock were:

1 *Amblyomma variegatum*
2 *Boophilus decoloratus*
3 *Rhipicephalus senegalensis*
4 *Boophilus annulatus*
5 *Hyalomma marginatum rufipes*
6 *Boophilus geigyi*
7 *Hyalomma truncatum*
8 *Rhipicephalus evertsi evertsi*
9 *Rhipicephalus lunulatus*

Amblyomma variegatum is a 3-host tick and was found on every sample of the sheep, goats and cattle examined and was widely distributed in all vegetation zones. *A. variegatum* was the predominant tick species on cattle in all areas. *Boophilus* species (Figure 3) are 1-host ticks and were found mainly on cattle, with some on sheep, and were widely distributed by vegetation zone. *Hyalomma* species were found predominantly on cattle and widely distributed by vegetation zone. *Rhipicephalus e. evertsi* and *R. senegalensis* were found on sheep and cattle in all vegetation zones [Walker and Koney,1999].

Tick control

Ticks cause considerable losses to the livestock industry worldwide. The main reasons why ticks are controlled are disease transmission, tick paralysis or toxicosis and physical damage. The most common method of killing ticks is by the use of chemical acaricides. For the application of acaricides several methods exist: dusting, hand spraying, mechanical spray race, hand dressing, systemic acaricides (pour-on, injection, oral) and dipping [Koney, 2004]. Numerous tick-borne and tick-associated diseases have been reported to occur in cattle in Ghana [Bell-Sakyi et al., 2004]. Between May 1994 and December 1996 Giemsa-stained thin blood smears prepared monthly from ruminants were examined for presence of tick-borne haemoparasites in the Greater Accra Region of Ghana. Monthly and cumulative incidences in cattle were presented of:

1 Anaplasma sp.
2 Babesia bigemina
3 Ehrlichia ruminatium
4 Borrelia sp.
5 Eperythrozoon sp.

6 *Theileria mutans* and *Theileria velifera*

T. mutans was the most common parasite in cattle, with 100% incidence in calves by 10 months of age [Bell-Sakyi et al.,2004]. Dermatophilosis is also an important disease and is associated with *A. variegatum* infestation [Koney et al., 1996]. Diseases transmitted by *Boophilus* species are as follows:

1. *Boophilus annulatus*: *Babesia bigemina, Babesia bovis*
2. *Boophilus decoloratus*: *Babesia bigemina, Anaplasma marginale* and *Borrelia theileri*
3. *Boophilus geigyi*: the diseases transmitted by this tick are poorly known.

The prevalence of Anaplasma and Babesia found by Bell-Sakyi et al in cattle both in the longitudinal survey were cumulative incidences of 62% and 73% respectively and in animals throughout Ghana sampled on a single occasion were 12% and 6% respectively. The real prevalence is likely to be higher because the detection method used was not very sensitive [Bell-Sakyi et al., 2004].

Acaricide resistance in Ghana

In many parts of the world the development of acaricide resistant tick strains has with time rendered one chemical agent after another ineffective. To date, the only tick investigated for acaricide resistance in Ghana is the predominant species infesting cattle, *A. variegatum*. During a study between July 1992 and January 1994 in Ghana, the susceptibility of four acaricides was determined on 14 to 21 day old larvae of *A. variegatum* using the F.A.O. acaricide resistance kit. The four acaricides were Coumaphos, Diazinon, Dieldrin and Cypermethrin and the study was carried out in four cattle rearing areas in the Accra Plains of Ghana. *A. variegatum* resistance was detected to Diazinon and Dieldrin in all the four areas. *A. variegatum* showed some resistance to Cypermethrin at a concentration of 0.05% in three areas. All the larvae were susceptible to Coumaphos [Koney and Nipah, 2000].
Another study was carried out more recently on the susceptibility of *A. variegatum* ticks to acaricides in Ghana. The susceptibility of unfed and fed larvae, nymphs and adult females of *A. variegatum* ticks were tested using Shaw's filter paper dip method against four acaricides: chlorfenvinphos and dioxathion, chlorfenvinphos, gamma benzene hexachloride and amitraz at four different concentrations including the recommended dose rates. The concentration of the chemical that killed 50% of the test ticks in a given time (LC50) was measured. Gamma benzene hexachloride had a LC50 of 0.001629, while chlorfenvinphos and dioxathion combined and chlorfenvinphos alone had a LC50 of 0.001794 and 0.002258 respectively. Amitraz appeared to have a quick knock-down effect on larvae and nymphs but at the recommended dose rate, showed no mortality of the ticks at that stage. However, at a concentration of 0.040%, amitraz showed a 100% inhibition of oviposition and hatching of laid eggs. Gamma benzene hexachloride produced only 66% inhibition of oviposition while chlorfenvinphos and dioxathion combined and chlorfenvinphos alone produced 100% inhibition of oviposition at their recommended dose rates. Fed nymphs were more susceptible than the unfed nymphs [Natala et al., 2005].

Importance of study

The purpose of the present study was to determine the susceptibility of *Boophilus* ticks to acaricides. The susceptibility was measured with the larval packet test. *Boophilus* species were of particular interest, because all previous studies about susceptibility of ticks to acaricides in Ghana focussed on *Amblyomma* species. Recently the occurrence of *Boophilus microplus* has been reported in the neighbouring country Cote d'Ivoire [Madder et al, 2007]. If *Boophilus microplus* spreads to Ghana it will cause real problems for farmers, because this species is more aggressive than the local *Boophilus* spp. and is notorious for rapid development of acaricide resistance in Australia and Latin America [FAO, 1984]. The first report of *Boophilus microplus* resistance against amitraz was in Australia in 1980 and resistance is also reported in Mexico and South Africa [Jonsson and Hope, 2007]. Recent results of laboratory bioassays using modified larval packet tests (LPT) revealed up to 16.59-fold resistance to deltamethrin and up to 5.86-fold resistance to amitraz in New Caledonia [Barre et al, 2008]. Knowing about the existing resistance status of this genus in Ghana will help to plan a control strategy to deal with *B. microplus* when it arrives in the country. If any resistance is measured to one or more acaricides the farmers can be informed about it and they can use the acaricides to which the ticks are susceptible. Health damage due to ticks can be prevented through adequate tick control management and the use of effective acaricides.

Materials and methods

Collecting of ticks
The *Boophilus* ticks were collected at farms in the Volta region.
At each farm cattle were restrained (figure 3) and engorged female *Boophilus* ticks were collected with a maximum of five ticks from any one animal [Ducornez,2005].

Fig 3. Catching of cattle.

Incubation of the ticks
The engorged female ticks were held at a temperature of 27 ±1 °C and at a relative humidity greater than 75% for 2 weeks to allow them to oviposit (Figure 4). Six days after the start of oviposition the female ticks were removed from the eggs and the eggs were kept under the same conditions as the female ticks [da Silveira Novelino et al.,2007; LPT, 2008].

Fig. 4: Female *Boophilus* tick laying eggs.

Filter papers
Filter papers were used and prepared according to the practical field manual from the FAO. Pieces of Whatman 541 filter paper were used each of 5 cm x 10 cm. Two solvents were used, olive oil (sterilised by heating to 110ºC for 75 minutes) and chloroform [FAO, 1984]. The acaricides were bought in the local shops in Accra and were not 100% pure.

Details of the acaricides are as follow:

Commercial name: Amiraz 20
Name of manufacturer: Mobedco-Vet (Jordan)
Active ingredient: Amitraz in the form of emulsifiable concentrate
Batch number: 080302
Purity: 20% (w/v)
Manufacturing date: 3/2008
Packaging: 1 litre bottle

Commercial name: Pourothrin
Name of manufacturer: Hipra (Spain)
Active ingredient: Deltamethrin
Batch number: 060303
Purity: 7,5 gm/L
Manufacturing date: 3/2008
Packaging: 500 ml bottle

Commercial name: Hexiprametrin
Name of manufacturer: Mobedco-Vet (Jordan)
Active ingredient: Cypermethrin
Batch number: 3F4F-1
Purity: 50 mg/ml
Manufacturing date: 7/2006
Packaging: 100 ml

Details of chemicals used as solvents are as follow:

Commercial Name: Chloroform
Name of manufacturer: BDH (England)
Date opened: 18/04/1995
Packaging: 500 ml
Commercial Name: Olive oil
Name of manufacturer: Albert Heijn (Netherlands)
Batch Number: 164/22
Purity: pure olive-oil
Expiry Date: 12/2009
Packaging: 250 ml bottle

The olive oil and chloroform were mixed in the ratio of 1:2 by volume. Solutions of different concentrations of each acaricide were prepared by pipetting the appropriate volume of acaricide and adding it to the olive oil and chloroform to make the highest concentration. After that serial dilutions were made as detailed in Tables 3 – 7 (Figure 5).
The papers were impregnated by pipetting 0.67 ml of solution of the appropriate concentration on to the filter paper (Figure 6). The control packets (without acaricide) were prepared first, then from the lowest to the highest acaricide concentration. The papers were air-dried for 1 hour, by which time the chloroform had vaporised and left the chemical in the olive oil solution on the paper.

Fig. 5 Preparation of olive oil, chloroform and acaricide solution

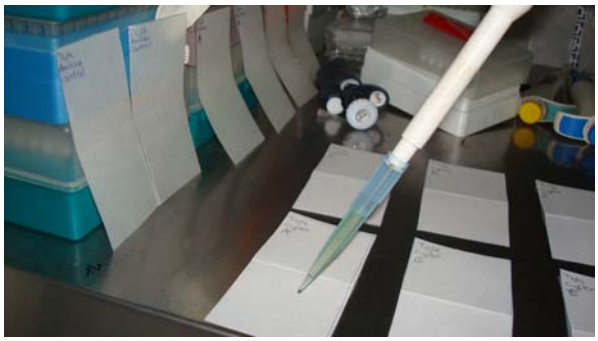
Fig. 6 Impregnation of filter papers

Details of acaricides used for preparation of larval packet test of University of Free State are as follow:

Commercial Name: Triatix 125
Active Ingredient: Amitraz
Batch Number: 707015
Purity: 12,5% w/v
Manufacturing date: 04/2006
Packaging: 1 litter bottle

Commercial Name: Curatik
Active Ingredient: Cypermethrin
Batch Number: 22117196EO
Purity: 15% w/v
Manufacturing date: 05/2006
Packaging: 500 ml bottle

Details on chemicals used as solvents are as follow:
Commercial Name: Chloroform
Batch Number: 1031999
Assay: min 99%
Date opened: 08/07/2008
Expiry Date: min 08/2009
Packaging: 1,5 liter bottle

Commercial Name: Olive Oil
Batch Number: 104772
Purity: BP Grade
Expiry Date: June 2009
Packaging: 200 ml bottle

Larval packet test
The method of the University of the Free State of South Africa was used. Seven to fourteen day-old larvae were used for testing. Duplicate filter paper packets (a and b) were prepared for each test and were placed onto a bamboo skewer to prevent them from falling over. The packets were sealed with three bulldog clips (Figure 7).
After drying for one hour, the top clip was removed from each packet and a cluster of approximately 100 larvae was picked up with a fine brush and eased into each packet with the aid of a glass rod, working from the lowest concentration up to the highest concentration to minimize contamination. After that the packets were resealed and stored at a temperature of 27ºC± 1 °C and a relative humidity greater than 75% for 24 hours. Mortality was determined by counting the dead and living larvae. Only the larvae capable of walking were considered alive. Number of ticks, alive, dead and total were recorded on the data capture forms provided [LPT,2008; FAO, 1984].

Fig 7 Filter paper packets

Data analysis

The mean percentage mortality was calculated for each acaricide at each concentration and shown in Figures 10 – 14 (appendix 2).

Two different larval packet tests were used, the first one was self-prepared with Ghanian acaricides and the second one was done with the packets from South Africa. Therefore data analysis is divided up into two experiments. During experiment 1 larvae from 50 ticks were tested in duplicate packets prepared using Ghanaian acaricides. Amitraz was used at concentrations of 0.00000018, 0.0000018, 0.000018, 0.00018, 0.00036 and 0.0018 ml/l. Deltamethrin was used at concentrations of 0.000000075, 0.00000075, 0.0000075, 0.000075, 0.00075 and 0.0075 ml/l. Cypermethrin was used at concentrations of 0.000005, 0.00005, 0.0005, 0.003, 0.010 and 0.05 ml/l. All the larvae used for this experiment were 7-13 days old at the day of testing. The tests were carried out during four days. At the first testing day the larvae were 7 days old, at the second testing day the larvae were 8 days, at the third testing day the larvae were 12 days old and the last testing day the larvae were 13 days old.

During experiment 2 larvae of 5 ticks were tested in duplicate packets provided by the University of the Free State and impregnated with Amitraz and Cypermethrin. Amitraz was used at concentrations of 0.0000025, 0.000025, 0.00025, 0.0025, 0.015, 0.025 and 0.25 ml/l and Cypermethrin was used at concentrations of 0.0000015, 0.000015, 0.00015, 0.0015, 0.015, 0.15 and 0.3 ml/l. All the larvae used for experiment 2 were 14 days old at the day of testing.

Chapter 3: Results

Fifty-five female ticks were collected, all laid eggs and all were used during the tests. It was not possible to indentify the ticks to species level. The results for each experiment and acaricide are presented in appendix 1 and the means presented as graph are given in appendix 2.

The mean percentages mortality of each acaricide at seven concentrations in each assay were calculated by counting all the living and dead larvae. Subsequently the mean percentages of each acaracide at the recommended doses were calculated and presented in Figure 8. A mean mortality of 80.8% forAmitraz at the recommended dose was found. At a concentration twice the recommended dose the mean mortality was 100%. The mean mortality for both Deltamethrin and Cypermethrin was 100% at the recommended doses. On the packaging of the Ghanaian Cypermethrin a recommended dose of 3-10 ml/l was written. Therefore doses of 3 and 10 ml/l were tested and at both doses all the larvae died, so the mean mortality was 100%. The last test was done using the test of South Africa which contains a different make of acaricide and different concentrations. Therefore the values of the different test are also calculated separately.
The results of the mean % mortality of each concentration of the three acaricides are shown in Figures 9 - 14.
Wednesday the 22nd of October, the first testing day, at the 0,0000018 concentration (concentration B) of Amitraz of test 1b four dead larvae were counted and no live ones. Because of the small number of larvae and the unexpected value it was unreliable and so this result was not included.
All the results of 28th of October of each acaricide were unexpected, because almost all the larvae were dead except the control larval packet test. Some contamination may have occurred during the impregnation. The control larval packet was impregnated at a different working table, an explanation why it gave these results.

Concentration (ml/l)	**Mean % mortality**
Amitraz: 0,00018	80,8
Deltamethrin: 0,0075	100
Cypermethrin low dose: 0,003	100
Cypermethrin high dose: 0,01	100

Table 1: mean % mortality at recommended doses

Concentration (ml/l)	**Mean % mortality**
Amitraz: 0,025	90,5
Cypermethrin: 0,015	99,1

Table 2: mean % mortality at recommended doses using packets from South Africa

Amitraz concentration (ml/l)	Mean % mortality
Control	50,0
0,00000018	73,3
0,0000018	64,66
0,000018	72,5
0,00018	80,8
0,00036	100
0,0018	100

Table 3: mean % mortality for Amitraz

Amitraz S-A concentration (ml/l)	Mean % mortality
Control	67,3
0,0000025	96,7
0,000025	99,1
0,00025	94,2
0,0025	94,5
0,015	69,3
0,025	90,5
0,25	97,7

Table 4: mean % mortality for Amitraz using packets from South Africa

Deltamethrin concentration (ml/l)	Mean % mortality
Control	61,1
0,000000075	55,9
0,00000075	60,4
0,0000075	68,9
0,000075	98,8
0,00075	100
0,0075	100

Table 5: mean % mortality for Deltamethrin

Cypermethrin concentration (ml/l)	Mean % mortality
Control	67,9
0,000005	63,0
0,00005	64,8
0,0005	95,8
0,003	100
0,010	100
0,05	100

Table 6: mean % mortality for Cypermethrin

Cypermethrin S-A concentration (ml/L)	Mean % mortality
Control	83,0
0,0000015	96,05
0,000015	59,5
0,00015	86,4
0,0015	52,5
0,015	99,1
0,15	100
0,3	100

Table 7: mean % mortality for Cypermethrin using packets from South Africa

Chapter 4: Discussion and conclusion

Roughly 13000 tick larvae were collected and examined for their susceptibility to acaricides with the larval packet test. Impregnation of the filter papers was done with locally purchased acaricides together with olive oil and chloroform. One assay was done with the larval packet test from South Africa to compare the results with the self prepared tests. After 24 hours incubation the dead and live tick larvae were counted and the percentage mortality was calculated.
Resistance to Amitraz was noticed in *Boophilus* ticks and this could be attributed to several factors including: prolonged application of Amitraz, poor management of acaricides in general, the use of weak acaricide concentrations or poor quality water used for diluting the acaricides [Koney and Nipah, 2000]. The finding of resistance to Amitraz in Ghana is in agreement with the observation made by Natala et al. (2004) who studied the effectiveness of Amitraz and other acaracides and concluded that the *Amblyomma* larvae did not show mortality at the recommended dose rate of Amitraz.
Research has showed that African cattle are generally highly resistant to ticks and other parasites and it is worthwhile to use selection programmes for tick resistance with breeds of cattle with naturally high resistance to ticks. Ideally ticks should be managed by a combination of techniques, supported where necessary by vaccination against tick borne diseases at a cost-effective level. [De Castro and Newson, 1993].
A recent review has concluded that commercial tick vaccines for cattle based on the *Boophilus microplus* Bm86 gut antigen are a feasible tick control method that gives an economically acceptable, environmentally friendly alternative to the use of acaricides. These tick vaccines result in reduced usage of acaricide and decreased tick infestations on cattle, as well as reduced transmission of tick-borne pathogens and increased productivity [de la Fuente et al, 2007].

A number of problems affecting the course of the study and the results were identified:

Number of ticks
During the period that the research was done (September and October) it was not possible to collect *Boophilus* ticks around Accra, therefore it was necessary to search for them elsewhere. Fifty five engorged female ticks were collected from cattle in the Volta Region. Although two more collections of ticks were planned, logistical problems with the control of the present study prevented this.

Impregnation of filter papers
During the preparation of the filter papers, the papers were impregnated with the different concentrations of acaricides. Every time the same concentrations were placed on the same working place and after every assay of one acaricide the working place was cleaned very well. Despite all this measures contamination could still be possible.

Cleaning of materials
As written in the protocol of the University of the Free State, the forceps and rods used to introduce the larvae into the packets were cleaned by dipping in an acetone solution. After dipping the instruments were dried by wiping with a tissue, but many larvae died directly after contacting the acetone cleaned materials. Without cleaning the chance of causing contamination would have been high.

Analyses
Some analyses were affected by low numbers of larvae and unexpected high mortality in some of the tests.

Conclusion
The percentage mortality values can be used to formulate a sensitive tick control strategy for farmers. Together with the data on *Amblyomma* tick resistance of other researchers (Koney and Nipah, 2000; Natala et al., 2005) and maybe results for other tick species and different types of acaricides in the future it will give a more complete view of sensitivity of ticks in Ghana to acaricides. It would be valuable to repeat the tests using a larger number of larvae and filter papers and reevaluate the results of this study.
The establishment of enzootic stability is created through the equilibrium between cattle, ticks and tick-borne diseases. Farmers should be encouraged to use selection programmes for tick resistance, by culling their most heavily-infested animals. Such selection programmes would minimize the use of acaricides and thus minimize the development of tick resistance to acaricides. Local breeds of cattle are more adapted and resistant to ticks and tick-borne diseases, thus farmers should be encouraged to use these breeds.
Farmers in Ghana mostly use acaricides of one type for a long time. Amitraz and Lindane are popular acaricides, because of low costs and easy available. Most of the farmers use the hand dressing application method, often by adding an unknown amount of acaricide and water. Withdrawal periods are not observed and farmers do not wear gloves during the preparation and application of the acaricides. Milking of cows and application of acaricides is often done at the same time and create a serious human health risk [Awumbila, 1996].
The use of acaricide dosage rates lower than the recommended doses will result in lower mortality rates, increased survival of nymphs and hatching of eggs and has serious implications in the field. This behaviour of farmers using sub-normal doses may lead to the rapid development of acaricide resistance. Therefore farmers should be educated to upgrade their knowledge of the proper use and handling of acaricides and they should be made aware of the development of resistance to acaricides by ticks and the serious consequences for the livestock farming industry.

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References

Awumbila B. (1996) Acaricides in tick control in ghana and methods of application. Tropical Animal. Health Production 28: 50S-52S

Barré N., Li A.Y., Miller R.J., Gaïa H., Delathière J.M., Davey R.B., George J.E. (2008). In vitro and in vivo evaluation of deltamethrin and amitraz mixtures for the control of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in New Caledonia. Veterinary Parasitology 155:110-119.

Bell-Sakyi L., Koney E.B.M., Dogbey O. and Walker A.R. (2004). Incidence and prevalence of tick-borne haemoparasites in domestic ruminants in Ghana. Veterinary Parasitology 124: 25-42.

de Castro J.J., Newson R.M. (1993). Host resistance in cattle tick control. Parasitology Today 9:13-7.

Ducornez S., Barré N., Miller R.J., de Garine-Wichatitsky M. (2005). Diagnosis of amitraz resistance in *Boophilus microplus* in New Caledonia with the modified Larval Packet Test. Veterinary Parasitology 130: 285-292.

FAO (1984). Ticks and tick-borne disease control: a practical field manual. Vol. 1: Chapter 5: Acaricide resistance: 264-297. FAO, Rome.

de la Fuente J., Almazán C., Canales M., Pérez de la Lastra J.M., Kocan K.M., Willadsen P. (2007). A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Animal Health Research Reviews. 8:23-28.

Harris E.G. (1977) Report to FAO on the Assessment of an Acaricide resistance Test Method and the Assembly and Distribution of Prototype Test Kits. Centre for Overseas Pest Research, London

Jonsson N.N., Hope M., (2007). Progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick *Boophilus microplus*. Veterinary Parasitology 146:193-198.

Koney, E.B.M., Morrow A.N., Heron I.D. (1996) Tropical Animal Health Production 28: 18S-25S

Koney, E.B.M and Nipah, G. (2000). *Amblyomma variegatum* resistance to acaricides in cattle in the Accra Plains of Ghana. Ghana Journal of Science 40:35-45.

Koney, E.B.M.(2004). Livestock production and health in Ghana. Second edition. Advent Press, Accra, pp 4-21.

LPT, (2008) Larval Packet Test kit protocol. University of the Free State, department of Zoology & Entomology, Faculty of natural & Agricultural Sciences, Bloemfontein South Africa.

Madder M., Thys E., Geysen D., Baudoux C., Horak I. (2007). *Boophilus microplus* ticks found in West Africa. Experimental and Applied Acarology 43: 233-234.

Natala, A.J., Agyei, A.D. and Awumbila, B. (2005). Susceptibility of *Amblyomma variegatum* ticks to acaricides in Ghana. Experimental and Applied Acarology 35: 259-268

da Silveira Novelino A.M., Daemon, E., and Soares, G.L. (2007). Evaluation of the acaricide effect of thymol, menthol, salicylic acid, and methyl salicylate on *Boophilus microplus* (Canestrini 1887) (Acari: Ixodidae) larvae. Parasitology Research 101:809-811

Walker, A.R. and Koney, E.B.M. (1999). Distribution of ticks (Acari: Ixodida) infesting domestic ruminants in Ghana. Bulletin of Entomological Research 89:473-479

Appendix 1

Concentrations (ml/l)

Amitraz	**Deltamethrin**	**Cypermethrin**
A= 0,00000018	A= 0,000000075	A= 0,000005
B= 0,0000018	B= 0,00000075	B= 0,00005
C= 0,000018	C= 0,0000075	C= 0,0005
D= 0,00018	D= 0,000075	D= 0,003
E= 0,00036	E= 0,00075	E= 0,010
F= 0,0018	F= 0,0075	F= 0,05

Wednesday 22nd of October.

Amitraz test 1/a	**Live**	**Dead**	**% dead/total**
Control	3	0	0
A	10	40	80
B	38	10	20,8
C	60	0	0
D*	8	15	65,2
E	0	113	100
F	0	100	100
Amitraz test 1/b	**Live**	**Dead**	**% dead/total**
Control	16	0	0
A	3	2	40
B	0	4	100
C	4	40	90,9
D*	8	15	65,2
E	0	83	100
F	0	89	100

Deltamethrin test 1/a	**Live**	**Dead**	**% dead/total**
Control	70	0	0
A	50	0	0
B	70	0	0
C	100	10	9,1
D	0	103	100
E	0	110	100
F*	0	180	100
Deltamethrin test 1/b	**Live**	**Dead**	**% dead/total**
Control	104	0	0
A	100	0	0
B	80	3	3,6
C	100	13	13
D	4	50	92,6
E	0	60	100

F*	0	70	100

Cypermethrin test 1/a	**Live**	**Dead**	**%dead/total**
Control	70	0	0
A	70	0	0
B	40	1	2,4
C	6	60	90,9
D*	0	190	100
E*	0	70	100
F	0	60	100
Cypermethrin test 1/b	**Live**	**Dead**	**%dead/total**
Control	60	5	7,6
A	100	0	0
B	60	1	1,6
C	9	52	85,2
D*	0	100	100
E*	0	100	100
F	0	80	100

Thursday 23rd of October

Amitraz test 2/a	**Live**	**Dead**	**%dead/total**
Control	4	62	93,9 Aceton
A	9	54	85,7
B	35	20	36,4
C	30	30	50
D*	8	70	89,7
E	0	109	100
F	0	40	100
Amitraz test 2/b	**Live**	**Dead**	**%dead/total**
Control	2	72	97,2 Aceton
A	4	58	93,5
B	6	67	91,8
C	1	100	99,0
D*	0	80	100
E	0	59	100
F	0	83	100

Deltamethrin 2/a	**Live**	**Dead**	**% dead/total**
Control	0	62	100 Aceton
A	2	72	97,3
B	3	62	95,4
C	9	106	92,2
D	0	88	100
E	0	74	100
F*	0	123	100

Deltamethrin test 2/b	**Live**	**Dead**	**%dead/total**
Control	0	54	100 Aceton
A	5	57	91,9
B	11	112	91,1
C	1	103	99,0
D	0	112	100
E	0	63	100
F*	0	71	100

Cypermethrin 2/a	**Live**	**Dead**	**%dead/total**
Control	0	54	Aceton100
A	10	61	85,9
B	5	72	93,5
C	0, movements	106	100
D*	0	107	100
E*	0	60	100
F	0	99	100
Cypermethrin test 2/b	**Live**	**Dead**	**%dead/total**
Control	0	75	Aceton
A	2	46	95,8
B	5	51	91,1
C	1	82	98,8
D*	0	66	100
E*	0	102	100
F	0	46	100

Monday 27th of October

Amitraz 3/a	**Live**	**Dead**	**% dead/total**
Control	11	10	47,6
A	12	47	79,7
B	12	54	81,8
C	0	71	100
D*	9	29	76,3
E	0	40	100
F	0	63	100
Amitraz 3/b	Live	Dead	**% dead/total**
Control	12	19	61,3
A	21	33	61,1
B	2	25	92,5
C	2	40	95,2
D*	3	23	88,5
E	0	46	100
F	0	37	100

Deltametrhin 3/a	**Live**	**Dead**	**% dead/total**
Control	0	48	100
A	32	42	56,8
B	11	43	79,6
C	0	186	100
D	0	98	100
E	0	134	100
F*	0	100	100
Deltamethrin 3/b	**Live**	**Dead**	**% dead/total**
Control	5	10	66,7
A	14	116	89,2
B	6	76	92,7
C	0	82	100
D	0	98	100
E	0	79	100
F*	0	70	100

Cypermethrin 3/a	**Live**	**Dead**	**% dead/total**
Control	0	100	100
A	2	70	97,2
B	0	121	100
C	0	70	100
D*	0	40	100
E*	0	30	100
F	0	30	100
Cypermethrin 3/b	**Live**	**Dead**	**% dead/total**
Control	0	60	100
A	1	114	99,1
B	0	70	100
C	0	70	100
D*	0	100	100
E*	0	30	100
F	0	30	100

Tuesday 28th October

Amitraz T4/a	**Live**	**Dead**	**% dead/total**
Control	18	10	35,7
A	0	33	100
B	1	11	91,6
C	0	56	100
D*	0	40	100
E	0	42	100
F	0	104	100
Amitraz T4/b	**Live**	**Dead**	**%dead/total**
Control	29	5	14,7
A	6	90	93,8
B	5	20	80
C	4	52	92,9

D*	0	30	100
E	0	48	100
F	0	10	100

Delta T4/a	**Live**	**Dead**	**% dead/total**
Control	15	15	50,0
A	1	52	98,1
B	0	119	100
C	0	90	100
D	0	44	100
E	0	43	100
F*	0	55	100
Delta T4/b			
Control	10	30	75,0
A	3	40	93,0
B	0	86	100
C	0	54	100
D	0	43	100
E	0	35	100
F*	0	20	100

Cyper T4/a	**Live**	**Dead**	**% dead/total**
Control	18	9	33,3
A	0	29	100
B	0	77	100
C	0	49	100
D*	0	60	100
E*	0	81	100
F	0	33	100
Cyper T4/b			
Control	19	28	59,6
A	0	32	100
B	0	60	100
C	0	46	100
D*	0	53	100
E*	0	51	100
F	0	105	100

Wednesday 29th october, larval packet test of University of the Free State

Zuid-Afrika Amitraz test/a	**Live**	**Dead**	**%**
Control	12	15	55,6
0,0000025	2	39	95,1
0,000025	0	34	100
0,00025	1	33	97,1
0,0025	0	13	100
0,015	36	27	42,9
0,025*	4	34	89,5
0,25	1	30	96,8
Zuid-Afrika Amitraz test/b			
Control	8	30	78,9
0,0000025	1	58	98,3
0,000025	1	51	98,1
0,00025	6	63	91,3
0,0025	4	32	88,9
0,015	3	65	95,6
0,025*	6	65	91,5
0,25	1 slow	70	98,6

Cypermetrhin T/a	**Live**	**Dead**	**%**
Control	9	47	83,9
0,0000015	1	31	96,9
0,000015	21	52	71,2
0,00015	7	89	92,7
0,0015	27	53	66,3
0,015*	0	136	100
0,15	0	80	100
0,3	0	54	100
Cypermethrin T/b			
Control	12	55	82,1
0,0000015	2	40	95,2
0,000015	24	22	47,8
0,00015	8	32	80,0
0,0015	73	46	38,6
0,015*	1	55	98,2
0,15	0	65	100
0,3	0	66	100

Appendix 2

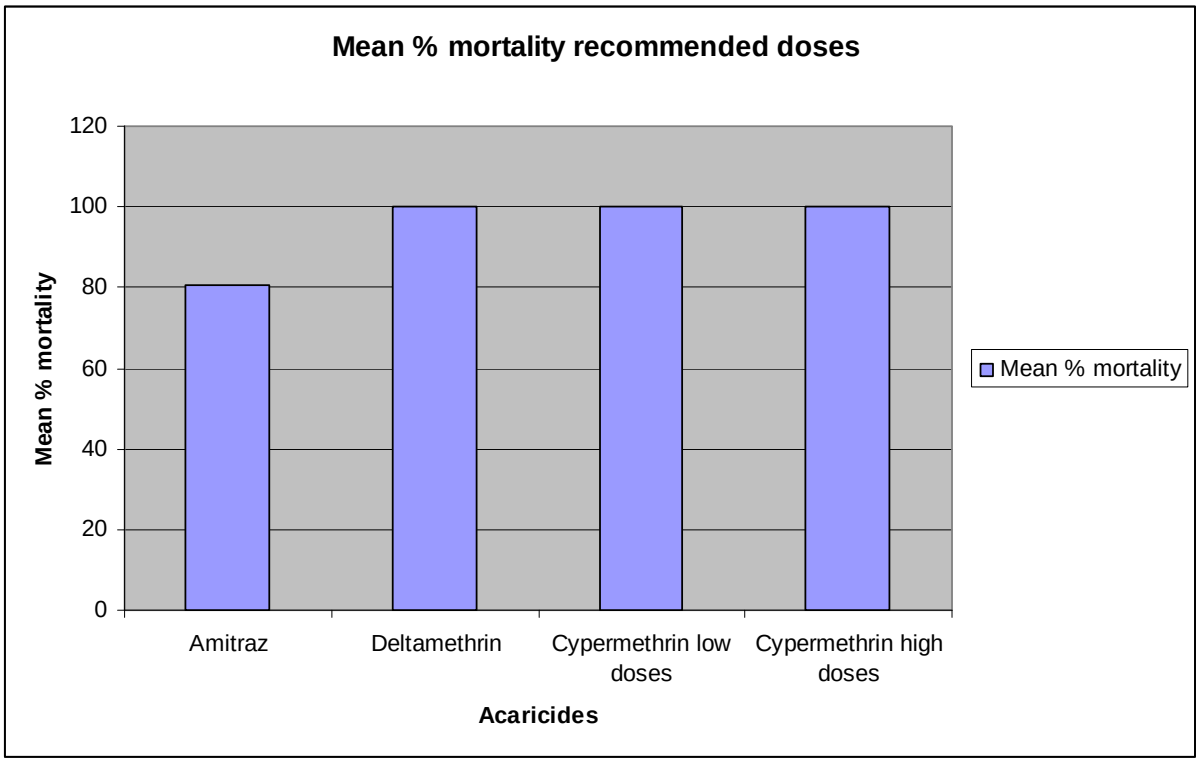

Fig. 8: mean % mortality at recommended doses

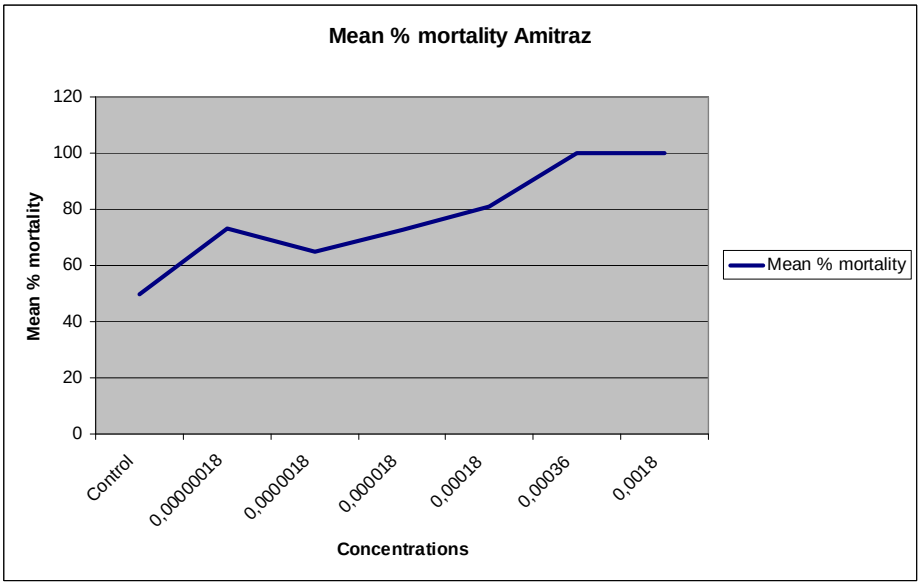

Fig. 10: mean % mortality for Amitraz

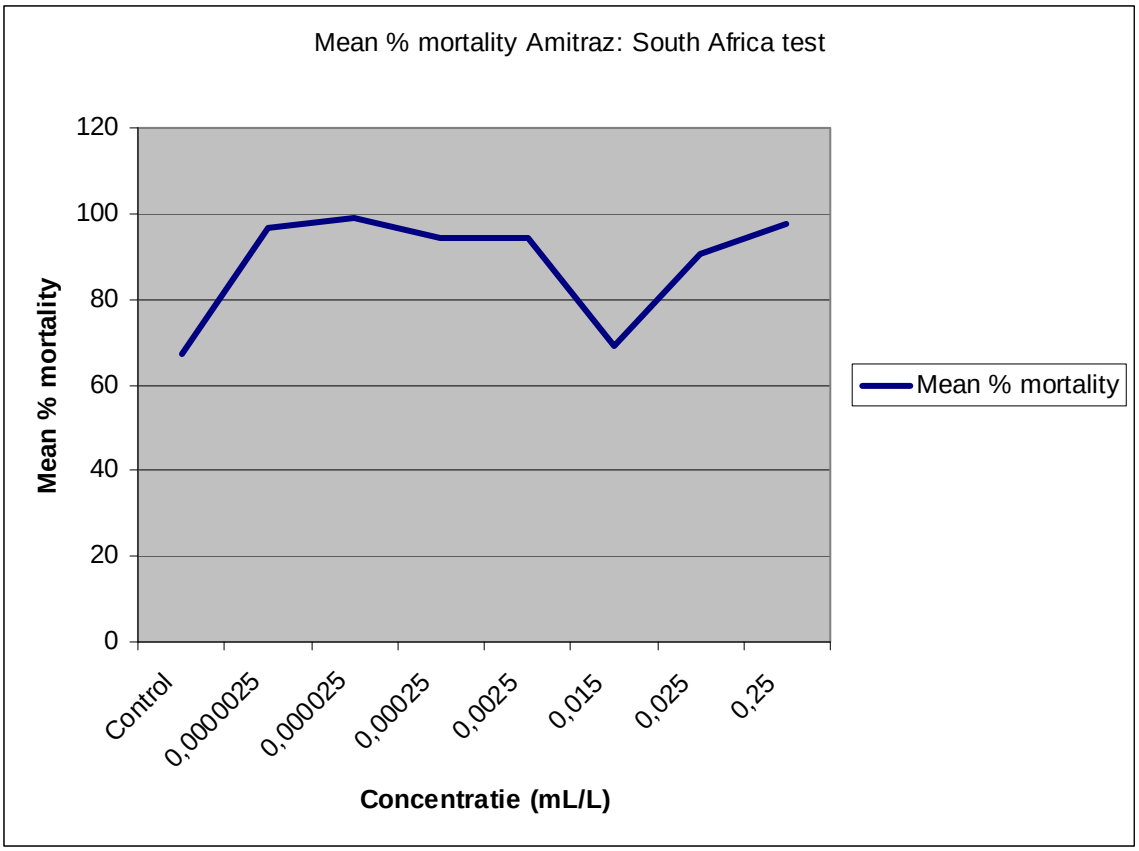

Fig. 11: mean % mortality Amitraz South Africa test

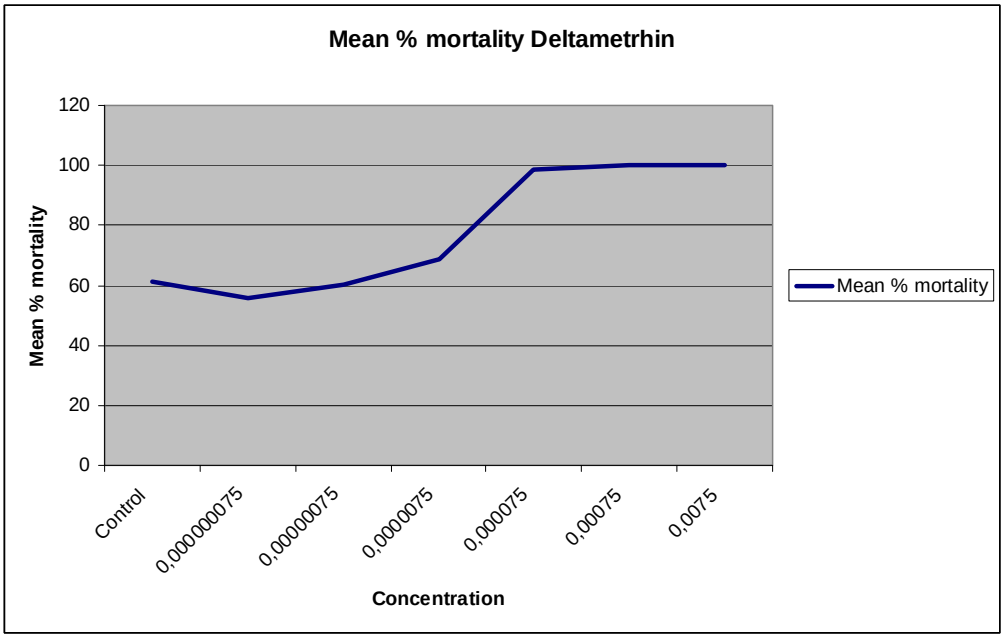

Fig. 12: mean % mortality Deltamethrin

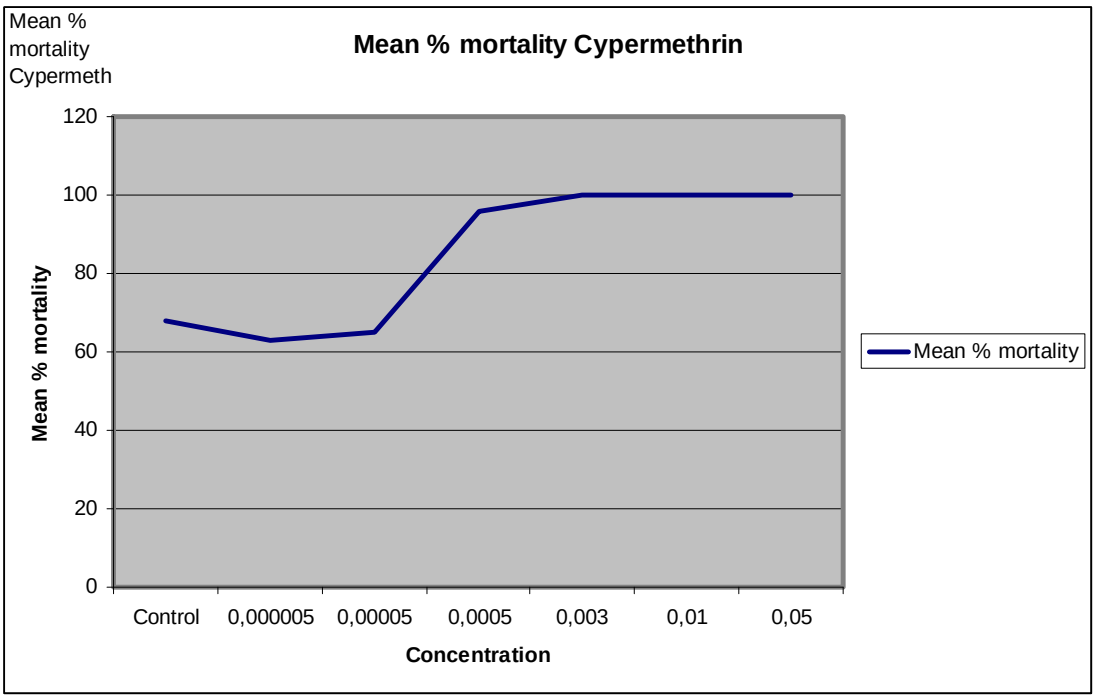

Fig. 13: mean % mortality Cypermethrin

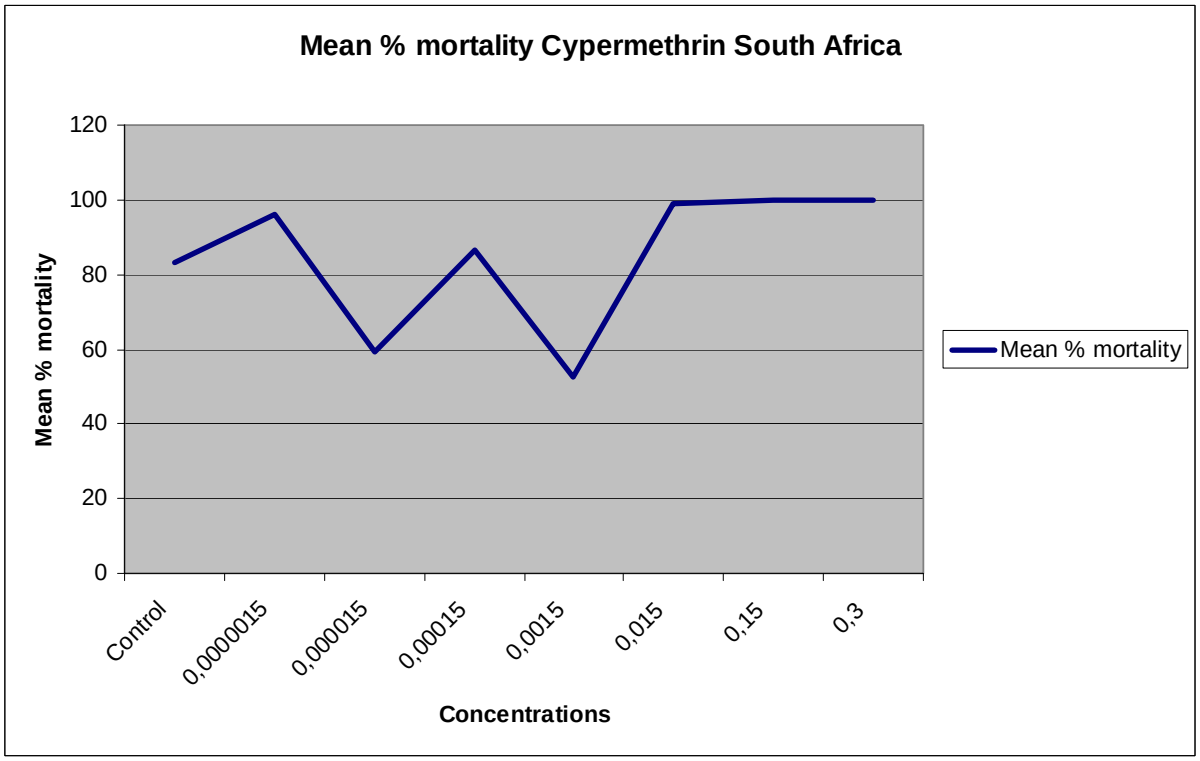

Fig. 14: mean % mortality Cypermethrin South Africa test