Vector-Borne Diseases & Treatment

Chapter 3

Susceptibility status of *Ornithodoros moubata* to different classes of insecticides (Acaricides) in six regions of mainland Tanzania

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Abstract

Understanding the insecticides susceptibility status of the commonly used insecticides for control of disease vectors is of paramount importance in setting up control agenda. In this chapter, the biological efficacy of commonly used insecticides (Acaricides) was evaluated under laboratory conditions against field collected populations of soft ticks (*Ornithdoros moubata*) from six regions of mainland Tanzania. The aim of this study was to assess the susceptibility status of *O. moubata* and ensure safe use of these pesticides for effective control. Six regions (namely Iringa, Morogoro, Arusha, Manyara, Shinyanga and Dodoma) with high infestations of O. moubata were selected. Within each region collections were carried out in two districts which are highly infested with *O. moubata*. Ticks collected from the community houses were transported to laboratory for rearing and insecticides susceptibility bioassays. Effectiveness of these insecticides were determined by exposing batches

of unfed 1st instar nymphs of *O. moubata* in five replicates on filter papers impregnated with serial dilutions of technical grade insecticides. The susceptibility status of eight field isolates of *O. moubata* was determined. The 24 and 48 hours mortality was higher and the insecticides were found to be effective.

The use of insecticides in control of *O. moubata* in Tanzania is seemed to have tolerance variability among *O. moubata* tested. More studies on genetic information on resistance to these insecticides should be established for each region for effective control planning for *O. moubata*.

Keywords: Tanzania; susceptibility; soft ticks; resistance; *O.moubata*

1. Background

Soft ticks of the genus *Ornithodoros* (also known as tampan ticks) belong to family Argasidae [1]. *Ornithodoros moubata,* the commonest soft tick is widely distributed in Tanzania [2-5]. These ticks inhabit mainly in human traditional huts with wall and floors with cracks, sands under trees where animals and humans often seek shelter during the day, they occur mainly in semi arid and arid areas [3]. They hide in cracks, crevices of walls and in floors with loose soils during the day and emerge at night for feeding on man or animal host present [1, 6]. They feed fast in presence of host and can survive for long duration of at least five or more years without food [1,7,8]. In Tanzania, *Ornithodoros moubata* as been found to be the vector for *Borrelia duttoni*, it has been reported that infestation rate of *Ornithodoros spp* by *Borrelia duttoni* to be more than 60% in Dodoma region where epidemics of tick-borne relapsing fever has been reported [9]. *O. moubata* are of medical importance as they cause nuisance and transmit tick borne relapsing fever (TBRF) caused by the spirochete *Borrelia duttoni*. Many cases have been reported from pregnant women (7.5% routinely attending Maternal and child health (MCH) centers in Dodoma) [4] and children, 75% and 55% in Dodoma and Mwanza regions respectively [10]. Spirochetes have been found in blood during febrile periods [11]. Transmission of TBRF by *O. moubata* is transovarial or transstadial [1,6]. Transstadial transmission occurs when immature and adult *O. moubata* suck blood from an infected host [12]. According to Service, houses infested with *Ornithodoros* species showed a remarkable density reduction when treated with insecticides (acaricides) sprays or dusts such as5% DDT, 3% Malathion, 5% carbaryl (Sevin), 0.5% Naled (Dibron), 0.5% Diazinon or 1% Propoxur [1,6].

Recently, newer acaricides with low human toxicity have demonstrated to be effective against *Ornithodoros* species both under laboratory and field conditions [13,14]. Vasil'eva and others reported that, *O. pappilipes* demonstrated reduced susceptibility with 40% mortality to Dieldrin, Malathion, Propoxur and Bendiocarb; while in DDT susceptibility was reduced to 60% mortality, and in deltamethrin mortality was observed to be 100%, completely susceptible to deltamethrin [14]. The field trial carried out in Magugu ward with Sevin powder (Carbaryl),

doom powder (Permethrin) and Doom spray (Permethrin) in houses infested with *O. moubata* in Matufa village found out that, the Permethrin formulation provided 100% mortality of the soft ticks within two weeks while Carbaryl provided 100% mortality of the ticks after three weeks [15]. Lambdacyhalothrin (Icon), used for indoor residual spray, permethrin and deltamethrin used on treated bed nets for the control of mosquitoes have been reported to reduce TBRF cases in Dodoma region in Tanzania [13].

In Tanzania, a surveys were carried out by Tropical Pesticides Research Institute (TPRI) between September 2003 and February 2004 in Dodoma, Shinyanga, Morogoro, Iringa, Arusha and Manyara regions revealed that, the communities use arbitrarily acaricides/insecticides meant for controlling agricultural, household pests and ticks of veterinary importance [16,17]. Moreover, the use of these chemicals was not based on susceptibility tests to ascertain their effectiveness. Such misuse of pesticides might have caused tolerance of Insecticides used in public health in *O. moubata* and the resistance may therefore interfere with the results of control measures in future. Also the current wide coverage and use of long lasting insecticides treated nets [18,19] have been the source of insecticides resistance to other ectoparasites observed in Tanzania mostly bed bugs [20-23]. The aim of this current chapter is therefore to report the efficacy of the commonly used insecticides (Acaricides) in controlling soft ticks *O.moubata* sampled from six regions of the mainland Tanzania.

2. Methods

2.1. Study area description

The regions involved in this study were Iringa, Morogoro, Arusha, Manyara, Dodoma, and Shinyanga. All these regions had reported relapsing fever cases before. In all infested region, a infested district was selected. In each district one ward was selected based on number of relapsing fever reported (i.e. the one with highest number of cases was prefereed) and within a ward two to three villages infested with *O. moubata* were surveyed. During the survey in each village, 20 houses were randomly selected for the assessment and collection of *O. moubata* as this is the maximum number of houses which could be managed. Samples of ticks collected from such houses provided representative samples as it was assumed that communities within the same village have similar practices and civilization. Tick surveys and collections were carried out between 2003 and 2004 while rearing of tick colonies and susceptibility tests were carried out from 2005 to 2008.

2.2. Tick collections

The regions with high infestations were selected based in the information obtained from ministry of health. In each region districts were selected and subsequently villages. From the selected houses in each village, loose soils were collected using a hand shovel from the sitting

room, bedside, kitchen (around fire place), and chicken roost areas at night. These sites were selected because people and chicken spend most of their time around while in the house, thus are preferred by ticks for easier blood meal access. The soils were taken outdoors and sieved to check for ticks as they tend to move away when exposed to light. Ticks were picked using forceps and kept in specimen tubes. Ticks were identified using morphological identification keys and subsequently reared while fed with rabbits and chicken for susceptibility tests [24]. Ticks collected from the field were divided into two batches. One batch was taken to Sokoine University of Agriculture (SUA) for parasitological work of *B. duttonii* in ticks and the other batch was reserved for rearing at Tropical Pesticides Research Institute (TPRI) for insecticides susceptibility tests.

2.3. Establishment of *O. moubata* tick colonies

The ticks (at various developmental stages) collected from the different areas in the field from six regions of Tanzania (Iringa, Morogoro, Dodoma, Arusha, Manyara, Shinyanga and Dodoma) (**Table 2**) were fed on rabbits (Chinchilla X New Zealand White breeds) or local chickens respectively. Ticks were the maintained under laboratory conditions at temperature between $28+^{0}1C$ and at a relative humidity above 85% at TPRI so as to obtain adequate amounts of larvae for susceptibility tests.

2.4. Evaluated chemical products

Technical grade of pesticide classes used were organophosphates, organochlorides, synthetic pyrethroids and carbamates (**Table 1**). The pesticides chosen for the tests are either widely used for control of other vectors or are having the promise to control the tick. Serial dilutions of technical grade pesticides (**Table 1**), were prepared using olive oil and trichloroethylene (Trilene) at 1:2 ratio as recommended by FAO (Stone et al., 1962). Trilene with olive oil alone was used to impregnate untreated control filter papers. Test papers were impregnated in the complete range of concentrations and were left to dry at room temperature (in a ventilated room) for 1 hour before exposing the ticks (Stone et al., 1962).

2.5. Susceptibility tests

First instar nymphs [10-14] day old were used for the tests. Batches of [10] nymphs of *O. moubata* per concentration were exposed to filter papers impregnated with insecticides (Anon 1971). Before and after treatment ticks were held at temperature between $28+^{0}1C$ and at a relative humidity above 85%. Mortalities at each concentration were determined at 24 hours and 48 hours after exposure. In discriminating the resistant population of ticks form different regions, we adopted the cutoff point method developed by World Health Organization [25]. The population found to have mortality below 90% was considered resistant after 48 hours of monitoring. In data analysis regression probit analysis was not d due to small sample of ticks

collected from houses in some areas.

2.6. Ethical clearance

Ethical and scientific approval to conduct the research was obtained from Tropical Pesticides Research Institute research ethics committee, Arusha, Tanzania. Informed consent was sought orally from the village communities, before collecting *O. moubata* in houses.

3. Results

3.1. Tick collections used to establish colonies of *O. moubata* for susceptibility tests

Ticks that were collected from Usolanga, Utosi, Hoza, Njoroki, Laghangareri and Nyabubinza villages were too few to continue the colonies, while adult ticks from Nyabubinza were fed but did not lay eggs thus their colony could not be established. Ticks collected from Endagichan could not stabilize under laboratory conditions. The number of ticks in highly infested areas were sufficiently available for use in the insecticides susceptibility test between March –June 2006 and March 2008 (**Table 2**). Ticks from less infested areas continued to be reared to increase their number to facilitate trials. The data shows that infestation of *O. moubata* in some places (Usolanga and Balang'dalalu) was as high as 80- 85% of houses (Table 2). Out of the total (3983) collected *O. moubata* collected 16% was used to raise the colonies for susceptibility tests at TPRI while the remaining ticks were used for parasitological work at Sokoine University of Agriculture. There were no ticks collected from Morogoro district.

3.1. Establishment of *O. moubata* tick colonies

Our laboratory observations shows that, it takes mean days of 18 for O. moubata eggs to develop into1st nymph instar; 10.5 days from engorged 1st nymph instar to 2nd nymph instar; 13 days from engorged 2nd nymph instars to 3rd nymph instars; 13 days from engorged 3rd nymph instars to 4th nymph instars; 13 days from 4th engorged nymph instar to 5th nymph instar; 12 days from 5th engorged nymph instar to adults and 19 days for engorged adults to lay eggs. In summary, our observations show that it takes about three months for *O. moubata* to complete one life cycle; as it took us a year to raise three generations from the few field collected ticks samples and some of them did not stabilize under laboratory conditions.

3.2. Susceptibility of *O. moubata* to commonly used insecticides

The mean mortality of *O. moubata* in a population in 24hrs and 48hrs after exposure obtained for each series of concentrations tested for each insecticide (in dosemortality) were subjected to analysis of variance one way as shown in Figures 1 to 9. In all regions higher mortalities were observed to all tested insecticides was observed. Mortalities due to Alphacypermethrin exposure for all regions was below cut off point of 90%. The mortality variation be-

tween 24 hours and 48 hours was statistically significant for each region (P<0.001). There was no statistical difference in 24hrs observation among regions (df =5, F=2, P=0.089), the same was for 48 hrs of monitoring post exposure (df=5, F=1.8, P=0.132) (**Figure 1**). Mortalities induced by exposure in Cyhalothrin were found to be statistically significant for comparison of 24 hours and 48 hours of monitoring in each region (P<0.001). There was no significant difference in mortalities among regions ticks in both 24hrs (df = 5, F=0.136, P=0.901) and 48hrs (df=5, F=1.953, P=0.101) among regions (**Figure 2**). Mortality in all regions for exposure in cyhalothrin was below 90%. In each region, the comparison of mortalities in 24 and 48 hours post exposure was significant different with more mortalities encountered in 48 hours (P<0.001) except for Manyara region (P = 0.211). The deltamethrin induced mortalities comparison in ticks among regions was statistically different in 24hrs (df = 5, F=5.4, P = 0.003) but was not statistically different 48 hrs post exposure (df=5, F=2.6, P=0.064) (*Figure 3*). Only in Manyara region mortality was above 90% in 48 hrs of monitoring. For permethrin, statistical difference were observed in each regions for mortalities in 24 and 48 hours post exposure (P<0.001). The mortalities induced by permethrin among the regions compared in 24hrs was statistically different (df=5, F=2.8, P=0.031), similar trend was observed in 48 hrs (df=5, F=6.7, P<0.001), only Manyara hours region had mortality above 90% after 48 hours while there rest were below 90% (**Figure 4**). In each region, the mortality induced by dieldrin in 24 and 48 hours was statistically different (P<0.001). There was significant responses in mortalities among the regions in 24 hrs post exposure (df=5, F=5.1, P=0.002) and similar trend in 48hrs (df=5, F=6.0, P=0.001), only Iringa and Shinyanga regions had mortalities above 90% (**Figure 5**). In each region mortality in 24 hrs and 48 hours post DDT exposure was low on no mortality at all (**Figure 6**). Mortalities varies significantly within each region (P<0.001). DDT induced low significant mortalities among regions in both 48hrs (df=5, F=5.7, P=0.001) and 72 hrs (df=5, F=7.8, P<0.001) post exposure but was not significant at 24hrs (df=5, F=2.1, P=0.097), and there was no mortality observed in Dodoma region in both 24 and 48 hrs (Figure 6). Mortalities induced by Fenithion in each region were statistically different between 24 and 48 hours of monitoring **(Figure 7**). The induced mortalities among the regions were no different statistically in 24 hrs (df=5, F=1.9, P=0.114) either in 48 hrs (df=5, F=2.43, P=0.058). Mortalities exceeded 90% only in 48 hours of monitoring in Shinyanga region (**Figure 7**). In each region, the mortality differences between 24 and 48 hrs was statistically different (P=0.01) induced by Malathion **(Figure 8**). Mortalities due to Malathion exposure among regions was not statistically different in 24 hrs (df=5, F=1.52, P=0.207) but significantly different in 48 hrs post exposure (df=5, F=3.3, P=0.006). Only Iringa region had high mortality exceeding cut off point of 90% after 48 hours (**Figure 8**). In each region the mortality different between 24hrs and 48 hrs were statistically different (P=0.001), except in Morogoro and Dodoma which were not different (P=0.209)(**Figure 9**). Low mortalities induced by Carbaryl were not statistically different among regions in 24 hrs (df=5, F=0.32, P=0.901) and also in 48hrs post exposure (df=5, F=1.95, P=0.101) (**Figure 9**).

4. Discussion

Standard test papers recommended by FAO for the assessment of ticks susceptibility was used to assess the susceptibility of *O. moubata*, the agent of tick-borne relapsing fever to a number of pesticides commonly used to control other arthropods of public health and veterinary importance. The results of most synthetic pyrethroids, organochlorines, organophosphates and carbamate insecticides used in these trials had reduced mortality efficacy in controlling *O. moubata* in the six regions, however their reduced efficacy varied from one insecticide to another in these regions which indicating different degrees of efficiency for controlling the vector in future. Similar efficacy of tested products have been observed in bedbugs tested from Manyara region [20]. The low mortality of *O. moubata* to pyrethroids (permethrin and deltamethrin) has been observed in Manyara region, this study observed high mortalities to due to low coverage of insecticides treated nets in this region during the study period, while in areas with high coverage of ITNs bed bugs and mosquitoes which have been exposed to pyrethroids for long time had low mortalities observed [21,22,26-28]. Mortalities due to fenithion and dieldrin was very high in Shinyanga region in all tested ticks tested while in Iringa region mortalities above cut of point was observed in the dieldrin and malathion 48 hours post exposure. Other insecticides observed mortalities below cut off points (i.e. below 90%). This mortalities variation observed in Iringa and Shinyanga regions might have been attributed with low usage or not used at all of these insecticides. Similar scenario of having insecticides induced mortality variations between regions has been observed in other arthropods of medical importance for similar classes of insecticides [26,29,30]. For those insecticides which have shown low mortality effect in controlling *O. moubata*, the use of similar insecticides or insecticides with similar active ingredients in public health or agriculture should be surveyed and changed or rotated for resistance management [31]. Similar scenarios of resistance to insecticides which have been used for the control of other pests of public health or agriculture have been found to be the source of pests resistance to insecticides [21-23,28,32,33]. The use of different strategies to delay or avoid insecticides resistance such as combination of insecticides, rotation of insecticide and mosaic strategy have been observed to combat insecticides resistance problem in mosquitoes and might work for other arthropods such as *O. moubata* [31]. The mixtures of acaricides/insecticide with different modes of action are suggested for use against vectors is one of the strategies could be used for delaying emergence of resistance [31,34]. Alternating the use of different classes of insecticides in community for control purposes might reduce tolerance of insecticides if well used and managed [31,34].

Laboratory trials have shown a low acaricides/insecticidal activity for most synthetic pyrethroids for *O. moubata* which has been shown with other insects such as mosquitoes [19, 35], tsetse flies [36], bedbugs [20,36] and cattle ticks [36]. Therefore application of synthetic pyrethroid insecticides on bed nets (ITNs) or long lasting Insecticides nets (LLINs) against

mosquitoes can play a significant role in controlling *O. moubata* by inducing reduced mortality due to frequent exposure to insecticides [13,21,22,37] and if not well managed can lead to insecticides resistance as observed in Tanga and coastal regions for bed bugs [21,22].

Disease Vector in human dwellings and his domestic animal as well as other insect pests are of great economic importance due to losses incurred such as death and for been sick which reduces production. The use of chemical has been one of the major method in controlling disease pests. In controlling *O. moubata*, treatments should be considered as a supplement to basic hygiene and house improvements will continue to be demployed in the near future as the principal measure for obtaining rapid and maximum control of a vector pest [38]. In delaying the emergence of resistance in soft ticks population, the use of insecticides with different mode of action in singly or in combination can be beneficial in resistance management. Cooperthion which has a mixture of acaricide and insecticide with different modes of actions for use against ticks and tsetse flies is one of the strategies of delaying emergence of resistance which have been working well in the field [39].

In public health departments, government should prepare community training manual, a package of training on house improvements which will be considered as the part of major disease vector control strategy. House types (i.e., the physical structure together with the materials and construction style that make up a human habitation) have been linked with the health negative outcomes [40-43]. The improvement and modernization of human houses construction have been found to be a major barrier in vector borne diseases, the transformation of houses from traditional houses (made of mud and thatches which are easily having cracks and dusty floor) to modern houses (houses built with blocks and concrete floor which is easy to clean) [40,41,44-47].

5. Conclusion

The findings of insecticides susceptibility status in these six regions survey have indicated that, there is reduces mortality for the insecticides classes tested against *O. moubata* in all regions. Further studies have to assess the possible ways to reinforce the management of *O. moubata* infestation and insecticide resistance. Although the findings of these studies show that, *O. moubata* can be controlled by several insecticides from Organochlorides, Organophosphates, Carbamates and synthetic pyrethroid groups; there was variation of insecticides classes used in mortality for different regions hence more efforts have to be included in house style improvement by public health officers.

6. Acknowledgement

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7.Tables

Table 1: Insecticides used for susceptibility tests of *O. moubata*

Pesticides Class	Chemical name	Purity of Technical material (a.i)
Organochlorine (OC)	DDT	10%
	Dieldrin	5%
Organophosphate (OP)	Fenthion	5%
	Malathion	10%
Carbamate (C)	Carbaryl	7.5%
Synthetic pyrethroid (SP)	Alphacypermethrin	95.7%
	Deltamethrin,	99%
	Permethrin	99%
	Lambdacyhalothrin	97%

Table 2: Collections of *O. moubata* from houses from different Regions of Tanzania for establishing colonies under laboratory conditions for susceptibility tests

Region	District	Ward	Village	No. of houses Sampled	No of houses Infested (%)	Total No. *O. moubata* collected	Total No. of ticks from the original samples allocated to TPRI
Iringa	Iringa Rural	Iddodi	Usolanga	20	16 (80%)	179	4
			Makadupa	20	9 (45%)	72	3
			Idodi	20	17 (85%)	**382**	7
	Mufindi	Sadani	Utosi	20	9 (45%)	122	5
			Igomaa	20	15 (75%)	**215**	7
Morogoro	Morogoro	Morogoro	Bonye	20	0	0	0
			Dakawa	20	0	0	0
	Mvomero	Kibati	Hoza	20	5 (25%)	55	3
			Pandambili	20	8 (40%)	314	13
Manyara	Hanang	Balang'dalalu	**Balang'dalalu**	40	27 (67.5%)	695	86
	Mbulu	Mbulu	Endagichan	20	14 (70%)	181	18
			Masieda	20	12 (60%)	440	16
Arusha	Karatu	Maleckchand	**Maleckchand**	20	5 (25%)	92	20
			Laghangareri	20	4 (20%)	72	5
	Monduli	Ketumbeine	Njoronyoki	13*	6 (46%)	330	36
			Namanga	2**	0	0	0
			Engarenaibor	1***	0	0	0
Shinyanga	Bukombe	Uyovu	**Nampalahala**	20	8 (40%)	**534**	186
			Kaniha	20	1 (5%)	**24**	9
	Maswa	Malampaka	**Gulunghwashi**	20	8 (40%)	**233**	59

			Nyabubinza	20	1 (5%)	43	19
Dodoma	Dodoma		**Makangwa**	-	Not done	**-**	100
			Mzula	-	Not done	-	20
Total				**396**		**3983**	**616**

* represents – sampled houses were 13 (coverage 65%) instead of 20 (100%) because of bad terrain which made accessibility to households difficult) and rough roads which made the survey team to spend the limited time in traveling

** and *** represents – few houses sampled due either resistance from the communities leadership, or absence of the ticks as per discussions between the team and the village leadership.

8. Figures

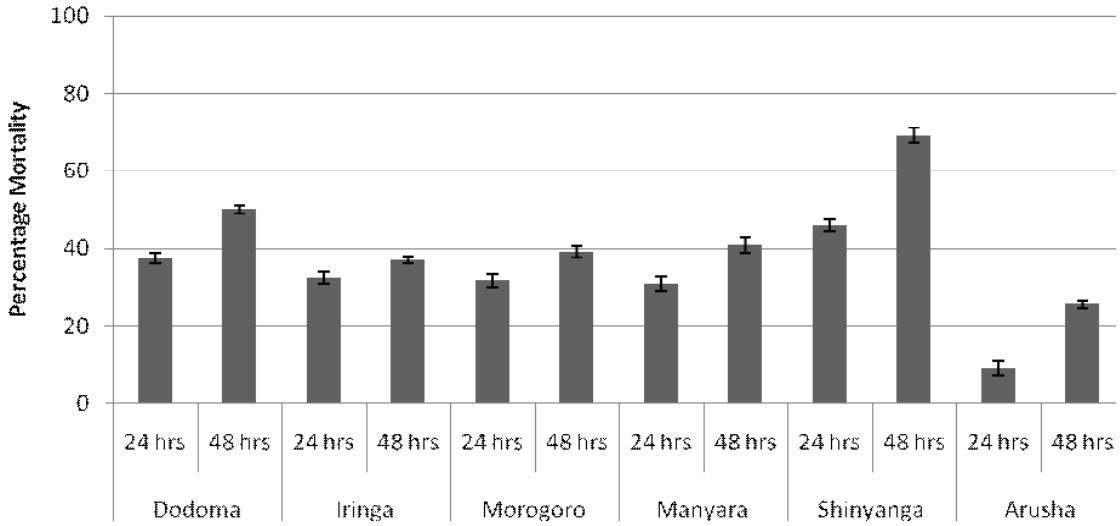

Figure 1: *O. moubata* mortality induced by Alphacypermethrin in 24 and 48 hours poste exposure

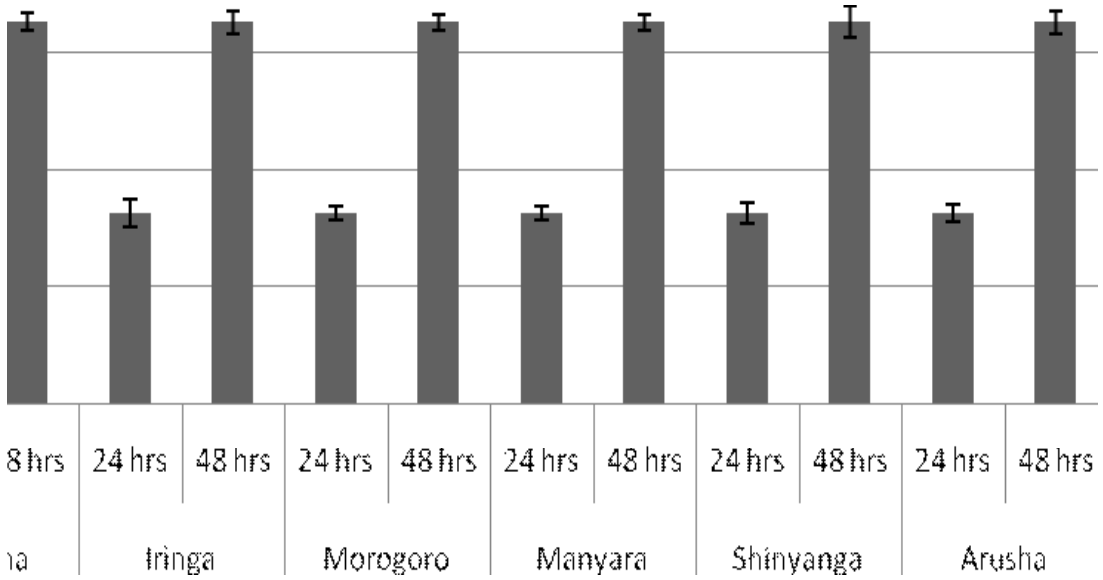

Figure 2: *O. moubata* mortality induced by Cyalothrin in 24 and 48 hours post exposure

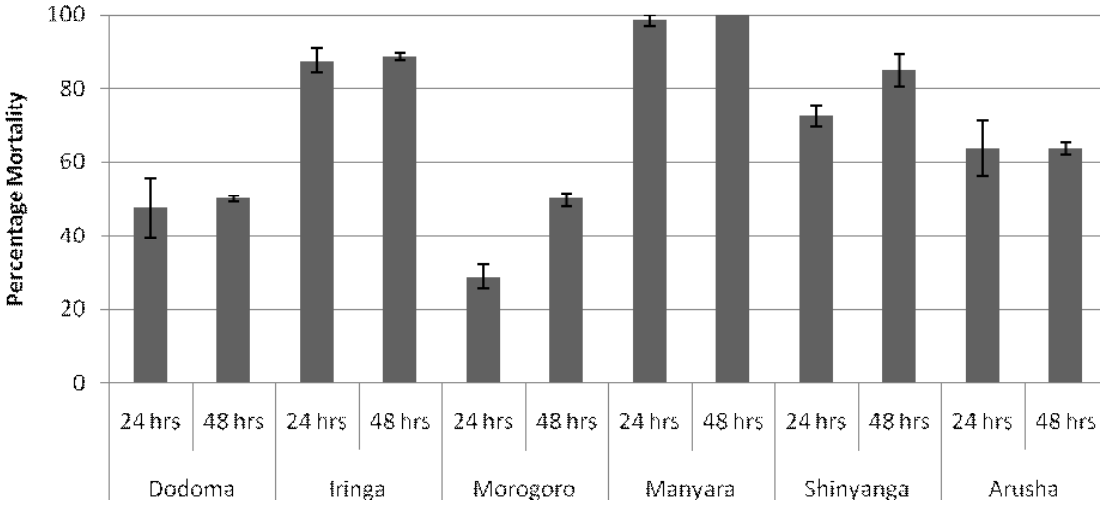

Figure 3: *O. moubata* mortality induced by Deltamethrin in 24 and 48 hours post exposure

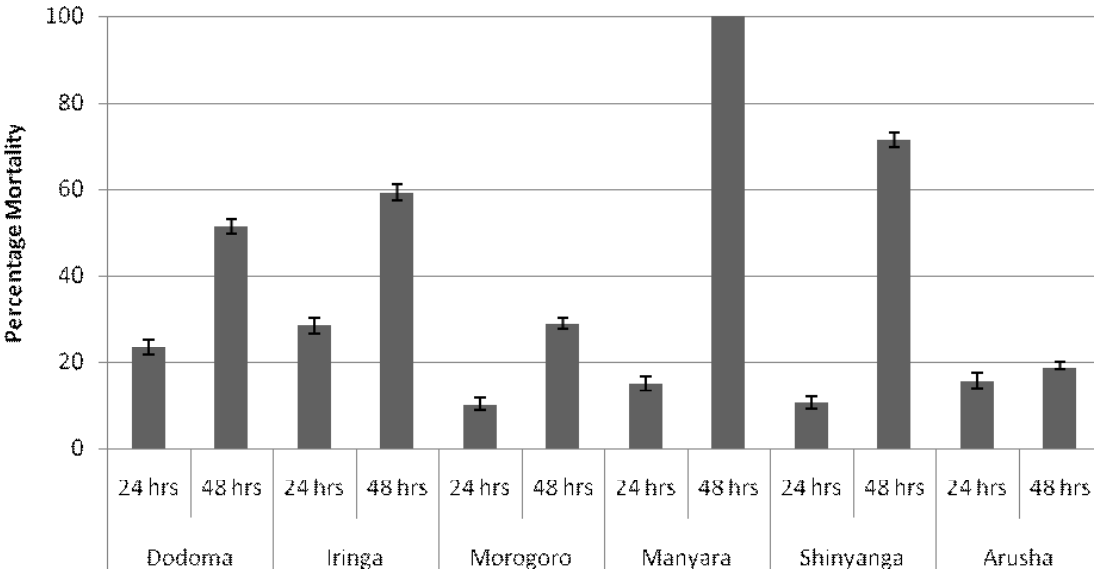

Figure 4: *O. moubata* mortality induced by Permethrin in 24 and 48 hours post exposure

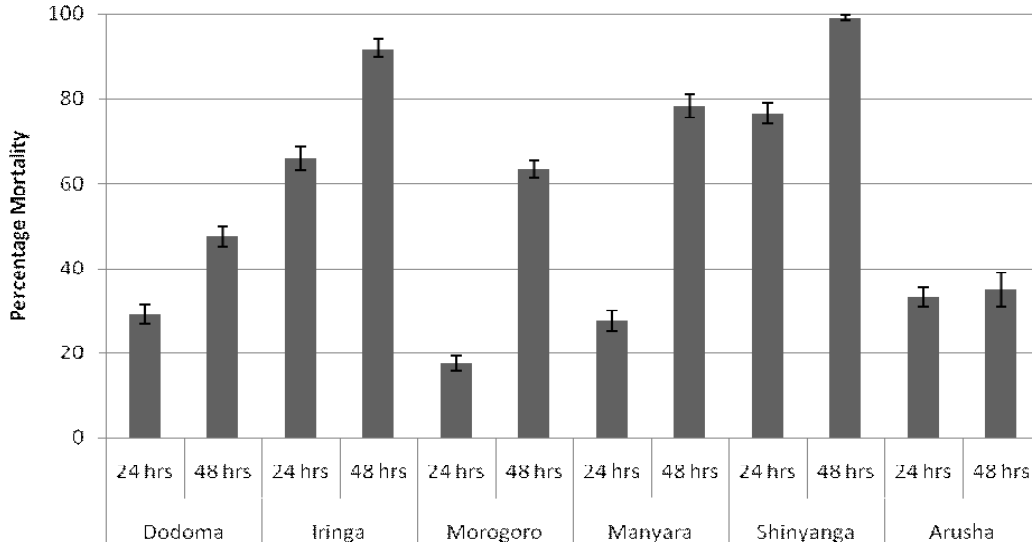

Figure 5: *O. moubata* mortality induced by Dieldrin in 24 and 48 hours post exposure

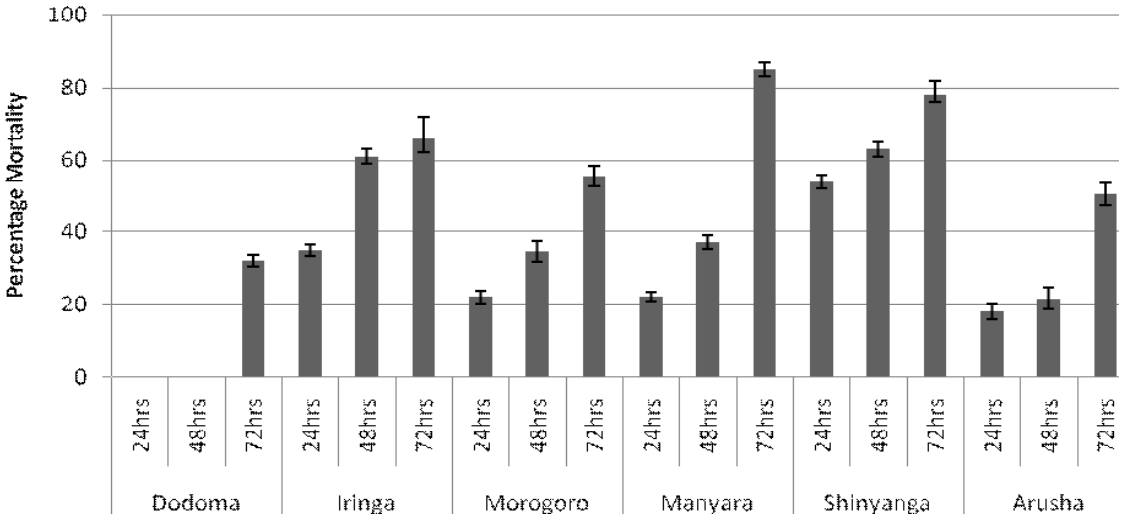

Figure 6: *O. moubata* mortality induced by DDT in 24 and 48 hours post exposure

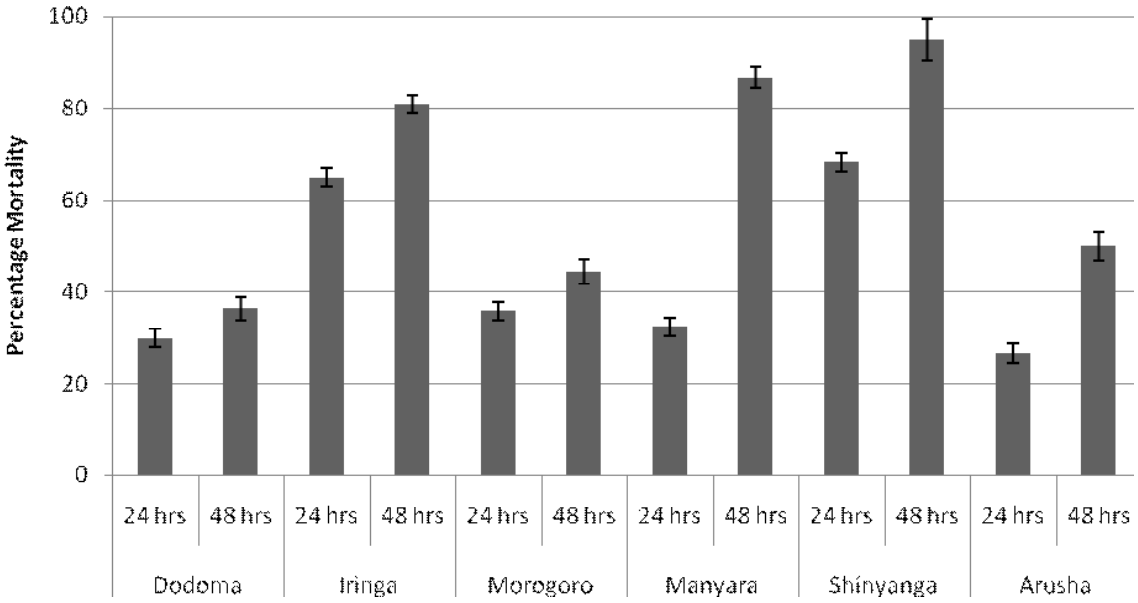

Figure 7: *O. moubata* mortality induced by Fenthion in 24 and 48 hours post exposure

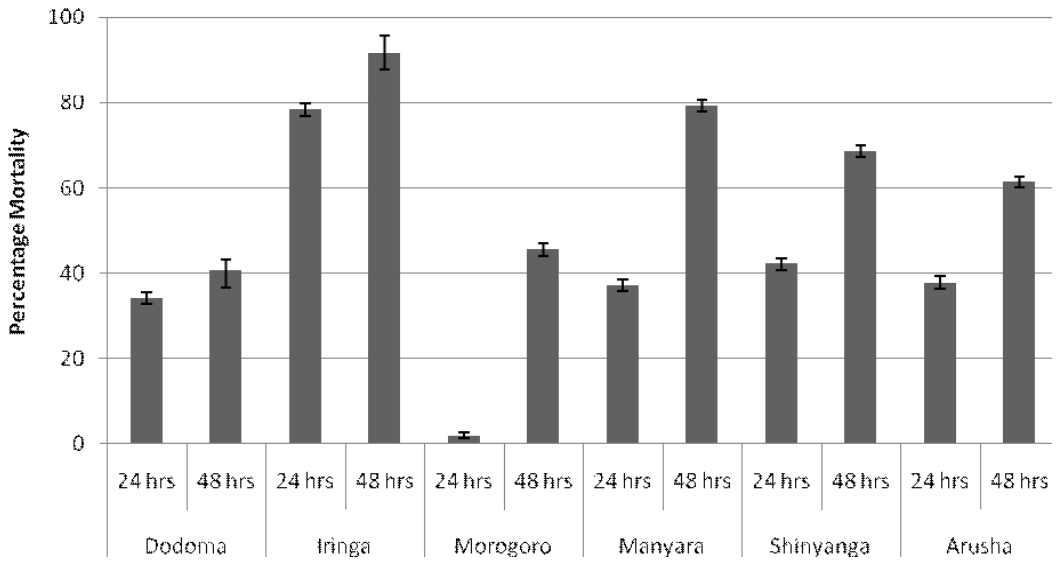

Figure 8: *O. moubata* mortality induced by Malathion in 24 and 48 hours post exposure

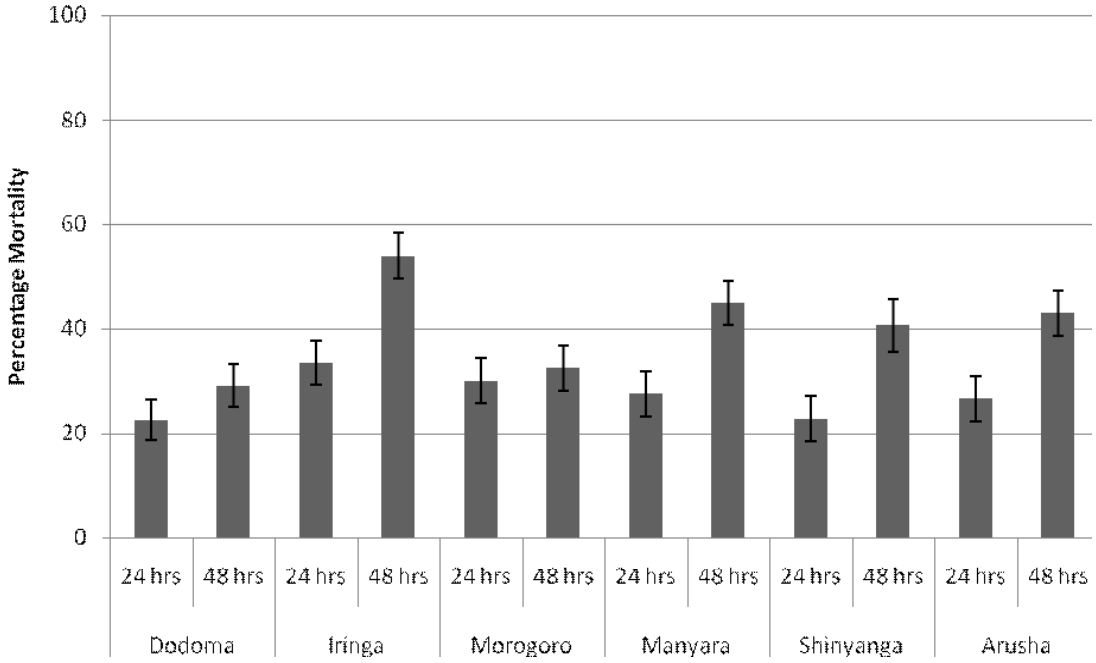

Figure 9: *O. moubata* mortality induced by Carbaryl in 24 and 48 hours post exposure

for maintenance of tick colonies and experimental animals while Mrs Katolina Ruhara is for typing some of the manuscript.

9. References

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