Insecticide susceptibility tests conducted in Kamhororo, Masakadza and Chilonga villages in Zimbabwe during the 2011 malaria period

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Abstract

Insecticide susceptibility tests using World Health Organization papers treated with 4% dichloro-diphenyl-trichloro-ethane (DDT), 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 0.5% etofenprox, 0.15% cyfluthrin and 0.75% permethrin were conducted in Kamhororo, Masakadza and Chilonga villages, Zimbabwe. Three to 5-day old female *Anopheles gambiae sensu lato* adult mosquitoes were used.

Deltamethrin knocked down 100% of the mosquitoes from Kamhororo, Masakadza and Chilonga at 35 min exposure. DDT did not knock down 100% of the mosquitoes from Kamhororo and Masakadza but did so in Chilonga. One hundred percent knockdown was achieved for cyfluthrin when exposed to mosquitoes from Kamhororo (60 min), Masakadza (25 min) and Chilonga (25 min). Etofenprox knocked down 100% of the mosquitoes collected from Kamhororo (30 min), Masakadza (30 min) and Chilonga (55 min). Knockdown of mosquitoes due to deltamethrin, DDT, cyfluthrin, permethrin; lambda-cyhalothrin and etofenprox were different at different observation times. One hundred percent mortality due to deltamethrin, DDT, etofenprox, lambda-cyhalothrin and cyfluthrin was recorded for mosquitoes collected from all the 3 sites. One hundred percent mortality due to pemethrin was recorded for mosquitoes collected from Kamhororo and Chilonga but mortality was 98.5% for those collected from Masakadza. No knockdown or mortality occurred in the controls from each locality. The kd_{50} (knockdown of 50% of the mosquitoes) values were 24.4-73.7 min (DDT), 8-13 min (pemethrin), 9.4-16.3 min (cyfluthrin), 9.4-14.4 min (etofenprox), 8.7-13 min (lambda-cyhalothrin) and 12.1-15.9 min (deltamethrin). The kd_{90} (knockdown of 90% of the mosquitoes) values were 45.6-199.5 min (DDT), 14.7-26.5 min (pemethrin), 16.5-34.9 min (cyfluthrin), 21.8-24.4 min (etofenprox), 16.3-31.6 min (lambda-cyhalothrin) and 21-25.3 min (deltamethrin). No insecticide resistance was recorded from the 3 sites.

Introduction

Malaria control is largely based on the use of long-lasting insecticide-treated nets and indoor residual spraying, but the efficacy of these control methods is endangered by the appearance of insecticide resistance in vector mosquitoes. Malaria in Zimbabwe causes significant mortality and morbidity although control efforts aimed at the main vector, *Anopheles arabiensis*, are instituted annually (Midzi *et al.,* 2004, unpublished data). *Anopheles gambiae sensu stricto, Anopheles arabiensis,* and *Anopheles funestus sensu stricto* are the most important species for malaria transmission in Africa (Kawada *et al.,* 2011).

Insecticide resistance is a reduction in sensitivity of an insect population as reflected by repeated failure of an insecticide to achieve the expected level of control when used according to recommendations (WHO, 1998). Insecticide resistance is mediated by behavioral, metabolic or physiological factors that result from: reduction in insecticide penetration, an increased metabolism of insecticide by metabolic enzymes and/or modification of the insecticide target site (WHO, 1998). World Health Organization (WHO, 1998) standards state that a mortality of 98-100% indicates susceptibility (no resistance); 80-97%

suggests the possibility of resistance that needs to be confirmed and less than 80% indicates resistance. However, when more than 100 mosquitoes have been used per insecticide, less than 95% mortality strongly indicates resistance. However, no standards on knockdown times are specified to indicate resistance according to the WHO (1998). Pyrethroid insecticide resistance in *An. gambiae* is mainly associated with reduced target site sensitivity arising from a single point mutation in the sodium channel gene, often referred to as knock-down resistance (Awola *et al.*, 2007). The susceptibility status of *An. funestus* to insecticides remains largely unknown in most parts of Africa because of the difficulty in rearing field collected mosquitoes; but this is not the case with *An. gambiae* (Morgan *et al.*, 2010).

Although insecticides have been used for a very long time in Zimbabwe, there are very few instances when resistance has been recorded (Munhenga *et al.*, 2008). Three cases of insecticide resistance have been documented in Zimbabwe; one in Chiredzi involving benzene hexa-chloride (Green, 1982), one involving dichloro-diphenyl-trichloro-ethane (DDT) in Gokwe (Masendu, 2004; Masendu *et al.*, 2005, unpublished data) and one involving DDT and permethrin in Gokwe (Munhenga *et al.*, 2008). Munhenga *et al.* (2008) observed insecticide resistance to permethrin from *An. arabiensis* mosquitoes collected from Gwave, a locality 11 km from Kamhororo and 16 km from Masakadza. Munhenga *et al.*, (2008) also recorded DDT resistance in Gwave (68.4% in 2006) but this reversed in 2008 (96% mortality).

Insecticide susceptibility tests did not show any significant increase in the resistance status for either permethrin or DDT but an improvement in susceptibility over a 3-year period (Awola *et al.*, 2007). Chanda *et al.* (2011) detected insecticide resistance to DDT, deltamethrin, lambda-cyhalothrin and permethrin in both *An. gambiae ss* and *An. funestus ss* collected in Zambia. Abilio *et al.*, (2011) detected insecticide resistance to lambda-cyhalothrin, permethrin and bendiocarb in *An. funestus* collected in Mozambique. *An. funestus* mosquitoes were resistant to 0.75% permethrin and 0.05% deltamethrin (Morgan *et al.*, 2010). There was suspected resistance to 4% DDT but these mosquitoes were fully susceptible to bendiocarb, malathion and dieldrin (Morgan *et al.*, 2010).

Djogbenou *et al.* (2011) observed full susceptibility to chlorpyrifos-methyl and very few samples displayed resistance to carbosulfan. Yewhalaw *et al.* (2011) observed that *An. arabiensis* mosquitoes were resistant to DDT, permethrin, deltamethrin and malathion, but susceptible to propoxur. Djogbenou *et al.* (2011) noted that insecticide susceptibility differs with geographical variation and this must be taken into account in the vector control strategies. For this reason, we conducted insecticide susceptibility tests in 3 different locations in Zimbabwe.

Materials and methods

Study areas

Mosquito collection was performed in Midlands province, Gokwe South district, Kamhororo village (17°51'S, 28°38'E), Masakadza village (17°49'S, 28°36'E) and Masvingo province, Chiredzi district, Chilonga village (21°13'S, 31°39'E). The Kamhororo River runs through the village of the same name. It starts as an artesian well and flows for over 14 km. This is the major source of water for washing and domestic animals. There are no agricultural activities taking place along the river. However, cotton is grown extensively in the village and a lot of crop spraying takes place. Chances of pesticides getting into the river system are high when washing clothes and spraying equipment; washing facilities have been provided but the water flows back into the river.

Mosquito larval collection was performed from hoof prints (a large number of cows are present and they drink this water. Masakadza village, 5 km from Kamhororo, is also on the Kamhororo River, but mosquitoes were collected from a swamp that also started from an artesian

well and flows for 1 km (this does not flow into the Kamhororo River). Water is used for washing (there are no designated washing facilities) and watering animals. Cotton growing is also widespread in the village. No agricultural activities near the swamp are conducted. Both Kamhororo and Masakadza are in dry areas where rainy water is limited. Chilonga village is spanned by the expansive Runde River that flows for over 50 km. Kitchen gardening is the most common method of farming in the villages although the river passes through large sugar estates in the low veldt district of Chiredzi.

Mosquito collection

Mosquito larvae were collected from breeding sites using larval scoops and placed in white plastic dishes (Figure 1). The collected larvae were morphologically identified and separated for rearing; the Kamhororo field insectary was used for Kamhororo and Masakadza mosquitoes, the Chilonga field insectary was used for Chilonga mosquitoes. The identified *An. gambiae sl* mosquitoes were reared according to Awola *et al.* (2007) and the adults were provided with 10% sugar solution on cotton wool placed as a wick in a 50 mL glass bottle. Unfed 3-5 day old *An. gambiae sl* adults from the same study area were pooled together as this is the time/stage at which a sizable mosquito sample was obtained.

Susceptibility tests

WHO papers treated with 4% DDT, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 0.5% etofenprox, 0.15% cyfluthrin and 0.75% permethrin were used according to the WHO (1998). The WHO (1998) states that knock-down rates should be measured every 10 min up to 60 min, but we made observations every 5 min so that we could detect even small differences. The WHO (1998) also states that in the event that 80% knockdown is not achieved after 60 min, the samples should be held for a further 20 min. We did not do this because two-thirds of the study sites had 80% of the mosquitoes knocked down within 60 min. The WHO (1998) states that 20-25 mosquitoes should be placed in each exposure tube (125 mm in length and 44 mm in diameter) but we used 15-20 mosquitoes before recording mortality after 24 h. All adult mosquitoes were removed from exposure tubes, provided with sugar water and held for 24 h. A total of 360, 240 and 236 mosquitoes from Masakadza, Kamhororo and Chilonga were posed to treated papers, respectively. The controls consisted of 50 mosquitoes in each study site. All exposure tubes were held in the vertical position. The insecticide treated papers were used once.

Figure 1. Collection of mosquito larvae.

Determination of kd_{50} and kd_{90}

Kd_{50} (min required to 50% knockdown of the mosquitoes) and kd_{90} (min required to achieve 90% knockdown of the mosquitoes) were calculated using Probit Analysis. This uses the regression principle and correlates fixed time with knockdown response. In circumstances in which the data are not normally distributed or do not follow a regression pattern, extrapolation is made beyond the period of observation.

Data analysis

Data was analyzed using analysis of variance (ANOVA) at 95% confidence limit.

Results

There was no knockdown or mortality from the 150 control mosquitoes used in this study.

Effect of deltamethrin on knocking down mosquitoes

Deltamethrin knocked down 100% of the mosquitoes from Kamhororo, Masakadza and Chilonga after 35 min exposure to deltamethrin (Figure 2). One hundred percent mortality was recorded and no insecticide resistance was observed.

There was no significant difference in knockdown of mosquitoes from Chilonga/Masakadza (P=0.13) and Chilonga/Kamhororo (P=0.42) after 5 min exposure to deltamethrin but a significant difference was seen in comparison with those from Kamhororo/Masakadza (P=0.03) (Table 1). There was no significant difference in knockdown of mosquitoes from Chilonga /Masakadza (P=0.22) and Chilonga/Kamhororo (P=0.09) at the 10 min observation time-point but a significant difference was seen in comparison with those from Kamhororo/Masakadza (P=0.003). There was no significant difference in knockdown of mosquitoes from Kamhororo/Masakadza (P=0.64), Chilonga/Kamhororo (P=0.47) and Chilonga/Masakadza (P=0.33) at the 15 min observation time-point. There was no significant difference in knockdown of mosquitoes from Kamhororo/Masakadza (P=0.59), Chilonga/Kamhororo (P=0.33) and Chilonga/Masakadza (P=0.301) at the 20 min observation time-point.

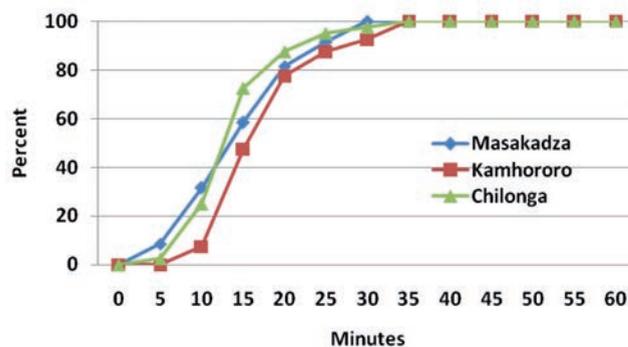There was no significant difference in knockdown of mosquitoes from Kamhororo/Masakadza (P=0.69), Chilonga/Kamhororo (P=0.49) and Chilonga/Masakadza (P=0.72) at the 25 min observation time-point. There was no significant difference in knockdown of mosquitoes from Kamhororo/Masakadza (P=0.27), Chilonga/Kamhororo (P=0.59) and Chilonga/Masakadza (P=0.27) at the 30 min observation time-point. There was no difference in knockdown rates from 35-60 min for mosquitoes collected from either of the study sites.

Effect of dichloro-diphenyl-trichloro-ethane on mosquito knockdown

One hundred percent knockdown was not achieved for mosquitoes collected from Kamhororo and Masakadza apart from those from Chilonga when exposed to DDT (Figure 3). One hundred percent mortality was recorded and no insecticide resistance was observed.

There was no significant difference in knockdown of mosquitoes from Chilonga/Masakadza (P=0.42), Chilonga/Kamhororo (P=0.42) and Masakadza/Kamhororo (P=0.27) after 5 min exposure to DDT (Table 2). A significant difference was found in knockdown of mosquitoes from Chilonga/Masakadza (P=0.012) and Kamhororo/ Masakadza (P=0.048) compared with those from Chilonga/Kamhororo (P=0.42) for which no significant difference was found at the 10 min observation time-point. There was no significant difference in knockdown of mosquitoes from Kamhororo/Masakadza (P=0.057), Chilonga/Kamhororo (P=0.52) and Chilonga/Masakadza (P=0.81) at the 15 min observation time-point. There was no significant difference in knockdown of mosquitoes from Kamhororo/Masakadza (P=0.057), Chilonga/Kamhororo (P=0.085) and Chilonga/Masakadza (P=0.078) at the 20 min observation time-point. Knockdown of mosquitoes from Kamhororo/Masakadza (P=0.06), Chilonga/Kamhororo (P=0.14) and Chilonga/Masakadza (P=0.32) were not significantly different at 25 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.16), Chilonga/Kamhororo (P=0.1) and Chilonga/Masakadza (P=0.11) were not significantly different at 30 min observation time.

Knock down of mosquitoes from Kamhororo/Masakadza (P=0.01), Chilonga/Kamhororo (P=0.02) and Chilonga/ Masakadza (P=0.03) were significantly different at 35 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.005), Chilonga/Kamhororo

Figure 2. Knockdown rate of mosquitoes due to exposure to deltamethrin.

Table 1. Knockdown of deltamethrin at each exposure time.

Knockdown (min)	Chilonga (%)	Kamhororo (%)	Masakadza (%)
0	Mean 0 Range 0	Mean 0 Range 0	Mean 0 Range (0)
5	Mean 2.5[ab] Range (0-5)	Mean 0[b] Range (0)	Mean 8.5[a] Range (5-10)
10	Mean 25%[cd] Range (20-30)	Mean 7.5[d] Range (5-10)	Mean 31.5[c] Range (30-35)
15	Mean 72.5[e] Range (65-80)	Mean 47.5[e] Range (20-75)	Mean 58.5[e] Range (50-65)
20	Mean 87.5[f] Range (85-90)	Mean 77.5[f] Range (70-85)	Mean 81.5[f] Range (75-85)
25	Mean 95[g] Range (90-100)	Mean 87.5[g] Range (80-95)	Mean 91.5[g] Range (80-100)
30	Mean 97.5[h] Range (95-100)	Mean 92.5[h] Range (85-100)	Mean 100[h] Range (100)
35	Mean 100 Range (100)	Mean 100 Range (100)	Mean 100 Range (100)

Same letter in the same row denotes no significant difference; different letter in the same row denotes significant difference.

(P=0.02) and Chilonga/Masakadza (P=0.045) were significantly different at 40 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.000) and Chilonga/Kamhororo (P=0.006) were significantly different at 45min observation time apart from Chilonga/Masakadza (P=0.07) that were not significantly different. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.001) and Chilonga/Kamhororo (P=0.01) were significantly different at 50min observation time apart from those from Chilonga/Masakadza (P=0.097) that were not significantly different. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.004), Chilonga/Masakadza (P=0.04) and Chilonga/Kamhororo (P=0.02) were significantly different at 55 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.000), Chilonga/Kamhororo (P=0.000) and Chilonga/Masakadza (P=0.03) were significantly different at 60 min observation time.

Effect of cyfluthrin in knocking down mosquitoes

Cyfluthrin knocked down 100% of the mosquitoes from Kamhororo (60 min), Masakadza (25 min) and Chilonga (25 min) (Figure 4). One hundred percent mortality was recorded and no insecticide resistance was observed.

Knock down of mosquitoes from Chilonga/Masakadza (P=0.77) and Masakadza/Kamhororo (P=0.31) were not significantly different at 5 min exposure to cyfluthrin apart from those from Chilonga/Kamhororo (P=0.037) that were significantly different (Table 3). Knock down of mosquitoes from Chilonga/Masakadza (P=0.8), Kamhororo/ Masakadza
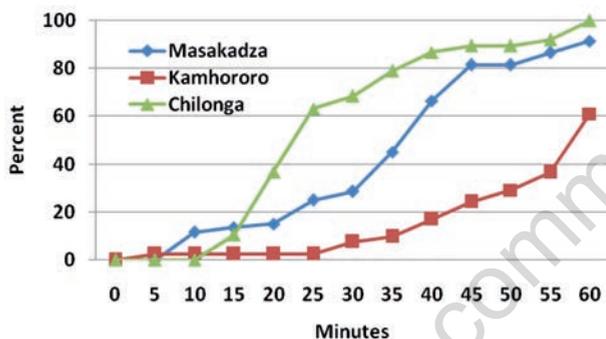

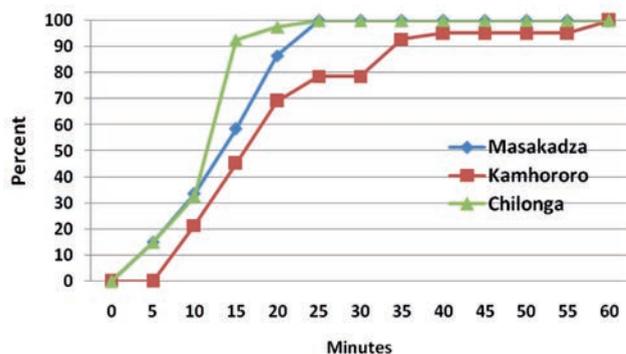**Figure 3. Knock-down rate of mosquitoes due to dichloro-diphenyl-trichloro-ethane.**

Figure 4. Knockdown of mosquitoes due to exposure to cyfluthrin.

Table 2. Knockdown of dichloro-diphenyl-trichloro-ethane at each exposure time.

Knockdown (min)	Chilonga (%)	Kamhororo (%)	Masakadza (%)
0	Mean 0 Range 0	Mean 0 Range 0	Mean 0 Range (0)
5	Mean 0[a] Range (0)	Mean 2.4[a] Range (0-5)	Mean 0[a] Range (0)
10	Mean 0[be] Range (0)	Mean 2.4[de] Range (0-5)	Mean 11.5[c] Range (10-15)
15	Mean 10.5[f] Range (0-22.2)	Mean 2.4[f] Range (0-5)	Mean 13.5[f] Range (10-15)
20	Mean 36.8[g] Range (27.8-50)	Mean 2.4[g] Range (0-5)	Mean 15[g] Range (10-20)
25	Mean 63.2[h] Range (38.9-94.4)	Mean 2.4[h] Range (0-5)	Mean 25[h] Range (15-35)
30	Mean 68.4[i] Range (50-94.4)	Mean 7.5[i] Range (4.8-10)	Mean 28.5[i] Range (15-45)
35	Mean 78.9[j] Range (72.2-94.4)	Mean 9.8[k] Range (9.5-10)	Mean 45[l] Range (35-50)
40	Mean 86.8[m] Range (83.3-100)	Mean 17[n] Range (10-23.8)	Mean 66.5[p] Range (60-70)
45	Mean 89.5[st] Range (88.9-100)	Mean 24.3[q] Range (23.8-25)	Mean 81.5[rt] Range (80-85)
50	Mean 89.5[wx] Range (88.9-100)	Mean 29.2[u] Range (23.8-35)	Mean 83.5[vx] Range (80-85)
55	Mean 92[a] Range (94.4-100)	Mean 36.6[b] Range (28.6-45)	Mean 86.5[c] Range (85-90)
60	Mean 100[d] Range (100)	Mean 61[e] Range (60-61.9)	Mean 91.5[f] Range (90-95)

Same letter in the same row denotes no significant difference; different letter in the same row denotes significant difference.

Table 3. Knockdown of cyfluthrin at each exposure time.

Knockdown (min)	Chilonga (%)	Kamhororo (%)	Masakadza (%)
0	Mean 0 Range 0	Mean 0 Range 0	Mean 0 Range (0)
5	Mean 15[a] Range (12-18)	Mean 0[a] Range (0)	Mean 15[a] Range (5-35)
10	Mean 32.5[b] Range (22.5-42.5)	Mean 21.4[b] Range (14.3-27.2)	Mean 33.5[b] Range (15-65)
15	Mean 92.5[d] Range (90-95)	Mean 45.2[c] Range (38.1-50)	Mean 58.5[c] Range (35-75)
20	Mean 97.5[f] Range (95-100)	Mean 69[e] Range (63.6-71.4)	Mean 86.5[ef] Range (70-100)
25	Mean 100[g] Range (100)	Mean 78.6[h] Range (72.7-81)	Mean 100[g] Range (100)
30	Mean 100[ij] Range (100)	Mean 78.6[j] Range (72.7-81)	Mean 100[i] Range (100)
35	Mean 100[km] Range (100)	Mean 92.8[k] Range (90.5-100)	Mean 100[lm] Range (100)
40	Mean 100[n] Range (100)	Mean 95.2[p] Range (90.9-95.3)	Mean 100[n] Range (100)
45	Mean 100[q] Range (100)	Mean 95.2[r] Range (95.3-95.5)	Mean 100[q] Range (100)
50	Mean 100[s] Range (100)	Mean 95.2[s] Range (95.3-100)	Mean 100[s] Range (100)
55	Mean 100[r] Range (100)	Mean 95.2[r] Range (95.3-100)	Mean 100[r] Range (100)
60	Mean 100[t] Range (100)	Mean 100[t] Range (100)	Mean 100[s] Range (100)

Same letter in the same row denotes no significant difference; different letter in the same row denotes significant difference.

(P=0.53) and Chilonga/Kamhororo (P=0.42) were not significantly different at 10 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.44 and Chilonga/Masakadza (P=0.11) were not significantly different at 15 min observation time apart from those from Chilonga/Kamhororo (P=0.017) that were significantly different. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.2) and Chilonga/Masakadza (P=0.41) were not significantly different at 20 min observation time apart from those from Chilonga/Kamhororo (P=0.02) that were significantly different. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.049) and Chilonga/Kamhororo (P=0.03) were significantly different apart from those from Chilonga/Masakadza at 25 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.049) and Chilonga/Kamhororo (P=0.03) were significantly different at 30min observation time apart from those from Chilonga/Masakadza.

Knock down of mosquitoes from Kamhororo/Masakadza (P=1.8×10^{-5}) and Chilonga/Kamhororo (P=0.000) were significantly different at 35 min observation time apart from those from Chilonga/Masakadza. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.02) were significantly different apart from those from Chilonga/Kamhororo (P=0.09) and Chilonga/Masakadza at 40 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=9.3×10^{-6}) and Chilonga/Kamhororo (P=0.000) were significantly different at 45 min observation time apart from those from Chilonga/Masakadza. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.27), Chilonga/Kamhororo (P=0.42) and
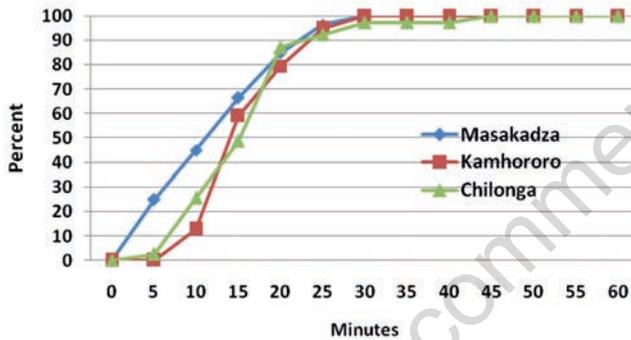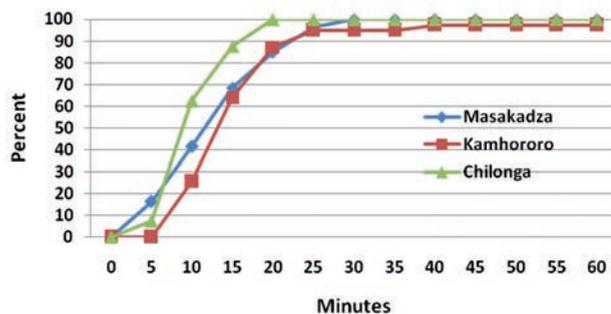

Figure 5. Knockdown of mosquitoes due to etofenprox.

Figure 6. Knockdown rate of mosquitoes due to permethrin.

Table 4. Knockdown of etofenprox at each exposure time.

Knockdown (min)	Chilonga (%)	Kamhororo (%)	Masakadza (%)
0	Mean 0 Range 0	Mean 0 Range 0	Mean 0 Range (0)
5	Mean 2.6[a] Range (0-48)	Mean 0[a] Range (0)	Mean 25[b] Range (20-30)
10	Mean 25.6[c] Range (23.8-27.8)	Mean 12.8[c] Range (0-26.3)	Mean 45[c] Range (35-55)
15	Mean 48.7[d] Range (38-61)	Mean 59[d] Range (40-78.9)	Mean 66.5[d] Range (50-80)
20	Mean 87.2[e] Range (83.3-90.5)	Mean 79.5[e] Range (65-94.7)	Mean 85[e] Range (75-90)
25	Mean 94.2[f] Range (88.9-95.2)	Mean 94.9[f] Range (95-100)	Mean 96.5[f] Range (95-100)
30	Mean 97.4[g] Range (94.4-100)	Mean 100[g] Range (100)	Mean 100[g] Range (100)
35	Mean 97.4[h] Range (94.4-100)	Mean 100[h] Range (100)	Mean 100[h] Range (100)
40	Mean 97.4[i] Range (94.4-100)	Mean 100[i] Range (100)	Mean 100[i] Range (100)
45	Mean 100[j] Range (100)	Mean 100[j] Range (100)	Mean 100[j] Range (100)

Same letter in the same row denotes no significant difference; different letter in the same row denotes significant difference.

Table 5. Knockdown of permethrin at each exposure time.

Knockdown (min)	Chilonga (%)	Kamhororo (%)	Masakadza (%)
0	Mean 0 Range 0	Mean 0 Range 0	Mean 0 Range (0)
5	Mean 7.5[a] Range (5-10)	Mean 0[a] Range (0)	Mean 16.5[b] Range (15-20)
10	Mean 62.5[c] Range (60-65)	Mean 25.6[c] Range (15.8-35)	Mean 41.5[c] Range (30-50)
15	Mean 87.5[d] Range (80-95)	Mean 64[d] Range (47.4-80)	Mean 68.5[d] Range (55-79)
20	Mean 100[e] Range (100)	Mean 87.2[e] Range (78.9-95)	Mean 85[e] Range (80-94.7)
25	Mean 100 [g] Range (100)	Mean 94.9[f] Range (94.7-95)	Mean 96.5[fg] Range (95-100)
30	Mean 100[h] Range (100)	Mean 94.9[i] Range (94.7-95)	Mean 100[h] Range (100)
35	Mean 100[h] Range (100)	Mean 97.4[i] Range (94.7-95)	Mean 100[h] Range (100)
40	Mean 100[j] Range (100)	Mean 97.5[j] Range (95-100)	Mean 100[j] Range (100)
45	Mean 100[j] Range (100)	Mean 97.5[j] Range (95-100)	Mean 100[j] Range (100)
50	Mean 100[j] Range (100)	Mean 97.5[j] Range (95-100)	Mean 100[j] Range (100)
55	Mean 100[j] Range (100)	Mean 97.5[j] Range (95-100)	Mean 100[j] Range (100)
60	Mean 100[j] Range (100)	Mean 97.5[j] Range (95-100)	Mean 100[j] Range (100)

Same letter in the same row denotes no significant difference; different letter in the same row denotes significant difference.

Chilonga/Masakadza were not significantly different at 50 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.27), Chilonga/Kamhororo (P=0.42) and Chilonga/Masakadza were not significantly different at 55 min observation time and this was the same at 60 min.

Effect of etofenprox in knocking down mosquitoes

One hundred percent knock down was achieved for mosquitoes collected from Kamhororo (30 min), Masakadza (30 min) and Chilonga (55 min) when exposed to etofenprox (Figure 5). One hundred percent mortality was recorded and no insecticide resistance was observed.

Knock down of mosquitoes from Chilonga/Masakadza (P=0.01) and Masakadza/Kamhororo (P=0.006) were significantly different at 5 min exposure to etofenprox apart from those from Chilonga/Kamhororo (P=0.4) that were not significantly different (Table 4). Knock down of mosquitoes from Chilonga/Masakadza (P=0.09), Kamhororo/Masakadza (P=0.08) and Chilonga/Kamhororo (P=0.44) were not significantly different at 10 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.72), Chilonga/Masakadza (P=0.31) and Chilonga/Kamhororo (P=0.7) were not significantly different at 15 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.79), Chilonga/Masakadza (P=0.61) and Chilonga/Kamhororo (P=0.69) were not significantly different at 20 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.72), Chilonga/Kamhororo (P=0.66) and Chilonga/Masakadza (P=0.24) were not significantly different at 25 min observation time.

Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Masakadza (P=0.27) and Chilonga/Kamhororo (P=0.42) were not significantly different at 30 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Kamhororo (P=0.42) and Chilonga/Masakadza (P=0.27) were not significantly different at 35 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Kamhororo (P=0.42) and Chilonga/Masakadza (P=0.27) were not significantly different at 40 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Kamhororo (P=0.42) and Chilonga/Masakadza (P=0.27) were not significantly different at 50 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Kamhororo (P=0.42) and Chilonga/Masakadza (P=0.27) were
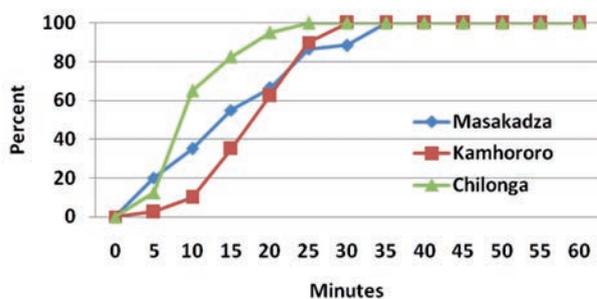
not significantly different at 55 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza; Chilonga/Kamhororo and Chilonga/Masakadza were not significantly different at 60 min observation time.

Effect of permethrin in knocking down mosquitoes

One hundred percent knock down was achieved for mosquitoes collected from Kamhororo (20 min), Masakadza (30 min) and Chilonga (20 min) when exposed to permethrin (Figure 6). One hundred percent mortality was recorded for mosquitoes collected from Kamhororo and Chilonga apart from those from Masakadza (98.5%). No insecticide resistance was recorded from the 3 sites.

Knock down of mosquitoes from Chilonga/Masakadza (P=0.04) and Masakadza/Kamhororo (P=0.003) were significantly different at 5 min exposure to permethrin apart from those from Chilonga/Kamhororo (P=0.09) that were not significantly different (Table 5). Knock down of mosquitoes from Chilonga/Masakadza (P=0.09), Kamhororo/Masakadza (P=0.21) and Chilonga/Kamhororo (P=0.06) were not significantly different at 10 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.08, Chilonga/Masakadza (P=0.16) and Chilonga/Kamhororo (P=0.31) were not significantly different at 15 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.96), Chilonga/ Masakadza (P=0.09) and Chilonga/Kamhororo (P=0.24) were not significantly different at 20 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.2) and Chilonga/Masakadza (P=0.49) were not significantly different at 25 min observation time apart from those from Chilonga/Kamhororo (P=0.000) that were significantly different.

Knock down of mosquitoes from Kamhororo/Masakadza (P=2.25×10^{-5}) and Chilonga/Kamhororo (P=0.000) were significantly different at 30 min observation time apart from Chilonga/Masakadza. Knock down of mosquitoes from Kamhororo/Masakadza (P=2.25×10^{-5}) and Chilonga/Kamhororo (P=0.000) were significantly different at 35 min observation time apart from those from Chilonga/Masakadza. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Kamhororo (P=0.42) and Chilonga/ Masakadza were not significantly different at 40min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/ Kamhororo (P=0.42) and Chilonga/Masakadza were not significantly dif-

Figure 7. Knockdown rate of mosquitoes due to exposure to lambda-cyhalothrin.

Table 6. Knockdown of lambda-cyhalothrin at each exposure time.

Knock down (min)	Chilonga (%)	Kamhororo (%)	Masakadza (%)
0	Mean 0 Range 0	Mean 0 Range 0	Mean 0 Range (0)
5	Mean 10[ab] Range (0-20)	Mean 2.5[b] Range (0-5)	Mean 20[a] Range (15-30)
10	Mean 65[c] Range (60-70)	Mean 10[d] Range (10)	Mean 35[cd] Range (15-60)
15	Mean 82.5[f] Range (80-85)	Mean 35[e] Range (25-45)	Mean 55[ef] Range (15-80)
20	Mean 95[g] Range (90-100)	Mean 62.5[g] Range (45-80)	Mean 66.7[g] Range (25-90)
25	Mean 100[h] Range (100)	Mean 90[h] Range (90)	Mean 86.7[h] Range (60-100)
30	Mean 100[i] Range (100)	Mean 100[i] Range (100)	Mean 88.3[i] Range (65-100)
35	Mean 100[j] Range (100)	Mean 100[j] Range (100)	Mean 100[j] Range (100)

Same letter in the same row denotes no significant difference; different letter in the same row denotes significant difference
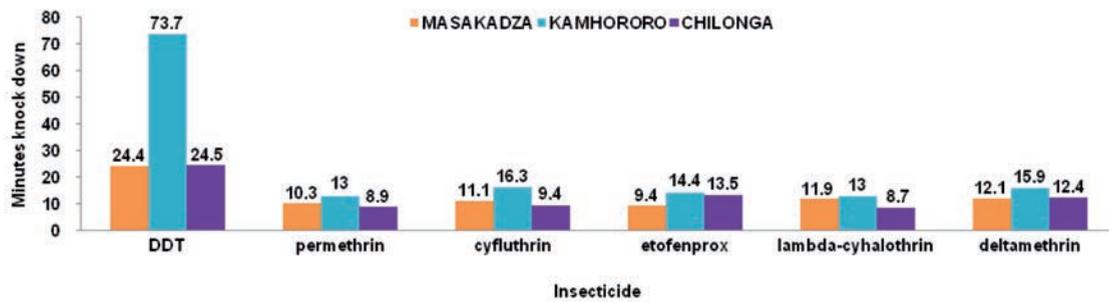
Figure 8. Kd_{50} values in minutes. Dichloro-diphenyl-trichloro-ethane (DDT) values were extrapolated by Probit analysis because 100% knockdown was not achieved.
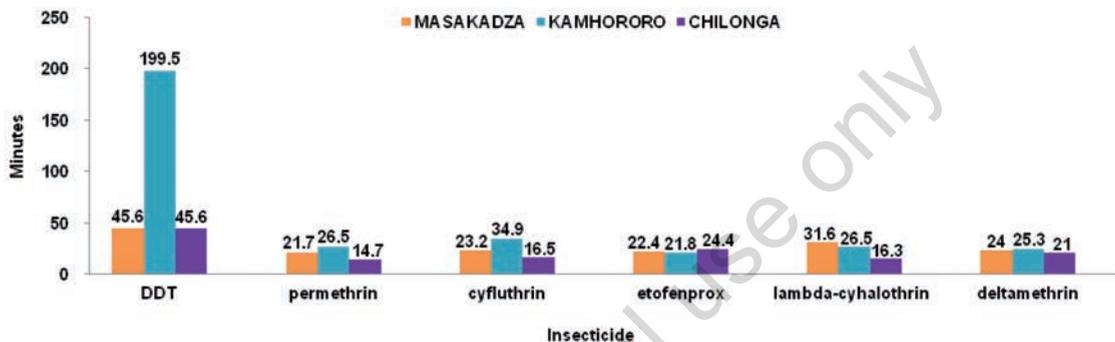

Figure 9. Kd_{90} values. Dichloro-diphenyl-trichloro-ethane (DDT) values were extrapolated by Probit analysis because 100% knockdown was not achieved.

ferent at 50 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/Kamhororo (P=0.42) and Chilonga/ Masakadza were not significantly different at 55 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza, Chilonga/ Kamhororo (P=0.42) and Chilonga/Masakadza were not significantly different at 60min observation time.

One hundred percent knock down was achieved for mosquitoes collected from Kamhororo (30 min), Masakadza (35 min) and Chilonga (25 min) when exposed to lambda-cyhalothrin (Figure 7). One hundred percent mortality was recorded for mosquitoes from all the study sites and no resistance was recorded.

Knock down of mosquitoes from Chilonga/Masakadza (P=0.67) and Chilonga/ Kamhororo (P=0.54) were significantly different at 5 min exposure to lambda-cyhalothrin apart from those from Masakadza/Kamhororo (P=0.038) that were significantly different (Table 6). Knock down of mosquitoes from Chilonga/Masakadza (P=0.18) and Kamhororo/Masakadza (P=0.23) were not significantly different at 10 min observation apart from those from Chilonga/Kamhororo (P=0.008) that were significantly different. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.51) and Chilonga/Masakadza (P=0.37) were not significantly different at 15 min observation time apart from those from Chilonga/Kamhororo (P=0.04) that were significantly different. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.89), Chilonga/Masakadza (P=0.37) and Chilonga/Kamhororo (P=0.22) were not significantly different at 20 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.85), Chilonga/Masakadza (P=0.49) and Chilonga/Kamhororo (P=0.9) were

not significantly different at 25 min observation time. Knock down of mosquitoes from Kamhororo/Masakadza (P=0.49), Chilonga/Kamhororo and Chilonga/Masakadza (P=0.49) were not significantly different at 30 min observation time. One hundred percent knockdown of mosquitoes was reported for mosquitoes collected from the 3 study areas.

Determination of kd_{50} and kd_{90}

The kd_{50} values were 24.4-73.7 min (DDT), 8-13 min (pemethrin), 9.4-16.3 min (cyfluthrin), 9.4-14.4 min (etofenprox), 8.7-13 min (lambda-cyhalothrin) and 12.1-15.9 min (deltamethrin) (Figure 8).

The kd_{90} values were 45.6-199.5 min (DDT), 14.7-26.5 min (pemethrin), 16.5-34.9 min (cyfluthrin), 21.8-24.4 min (etofenprox), 16.3-31.6 min (lambda-cyhalothrin) and 21-25.3 min (deltamethrin) (Figure 9).

Discussion

The time required to knock-down 100% of the mosquitoes was compared and the results indicated that there was no difference when mosquitoes were exposed to different sources of deltamethrin. DDT only managed to knock-down 100% of the mosquitoes collected from Chilonga and could not knock-down mosquites from either Kamhororo or Masakadza. This might be due to the great pressure being placed on the mosquitoes from Kamhororo and Masakadza through intensive application of pesticides for cotton growing while this is not the case

with Chilonga (approx. 600 km from these 2 sites). It is worth noting that Gwave (a village not very far away from Kamhororo and Masakadza where insecticide resistance has been detected) serves as a potential reservoir of DDT resistant mosquitoes, as observed by Masendu (2004), Masendu *et al*. (2005, unpublished data) and Munhenga *et al*. (2008). Problems with not achieving 100% knockdown when DDT was used might be an indication of knock-down resistance, as observed by Awola *et al*. (2007). However, Djiegbe *et al.* (2011) demonstrated that a high frequency of resistant genes does not necessarily translate into resistance in *An. gambiae sl* mosquitoes. It is important to study this mechanism in the follow-up studies.

There was no great difference between the times required to knock-down 100% of the mosquitoes due to lambda-cyhalothrin and permethrin from the 3 sites. This is interesting because Munhenga *et al*. (2008) recorded insecticide resistance from mosquitoes from Gwave but this has not been observed for mosquitoes from Kamhororo and Masakadza in terms of knock-down time. The times required for 100% knockdown of mosquitoes from Chilonga and Masakdza (for cyfluthrin) were very similar but were abnormally high for Kamhororo; the reasons for this are not known. Interestingly, the time required for 100% knockdown of mosquitoes from Chilonga (etofenprox) was higher than that of Kamhororo and Masakdza; the reasons for this are not known. It may be linked to pest control on sugar estates since there is no sugar cane cultivation in either Kamhororo or Maskadza.

In general, knock-down rates of mosquitoes appeared to be differ according to their sources and this was also time dependent. This trend was also observed for the different insecticides under study. This highlights the need to study all the insecticide classes in order to understand this trend as this may provide useful information on the possibility of insecticide resistance developing in some localities in Zimbabwe. It is also important to cover all geographical areas since this study was only carried out in dry areas where malaria is prevalent.

Mortality rates are encouraging from all the study sites when considering all the insecticides used in this study. No insecticide resistance was observed at the study sites. It is important to monitor trends in pemethrin response for mosquitoes from Masakdza (near Gwave where pemethrin resistance has been reported by Munhenga *et al*. 2008). Interestingly, this trend was not observed in Kamhororo that is nearer Gwave than Masakdaza. Thus, the absence of DDT and permethrin resistance agrees with observations of Dabire *et al.* (2008).

The kd_{50} and kd_{90} values obtained from all the study sites (for DDT) appeared to be within the same range but these were abnormally high for mosquitoes collected from Kamhororo. This may signal future problems with DDT use in Kamhororo, but according to Munhenga *et al*. (2008), reversal of resistance may occur. We are not sure whether this will happen for mosquitoes collected from Kamhororo. Otherwise, our results show that the tested insecticides have reasonable knock-down rates in the study areas.

Use of Probit Analysis showed the difference between knock-down rates. One major observation from this program is that it extrapollates results when 100% knock down is not achieved and at times, this goes beyond the study time.

These results agree with earlier observations that cases of insecticide resistance are very rare in Zimbabwe, in agreement with Munhenga *et al.* (2008), Green (1982), Masendu (2004) and Masendu *et al.* (2005, unpublished data). Unfortunately, Munhenga *et al.* (2008) did not detect any knock-down resistance or mutations from Gwave but no information is available for Kamhororo. Knock-down rates from our study agree with findings of Djogbenou *et al.* (2011) that insecticide

susceptibility differs according to geographical variations. More studies on this should be conducted across the country.

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