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Characterisation of resistance mechanisms in the major malaria vector Anopheles arabiensis from southern Africa

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SUPPORTING THE NATIONAL MALARIA CONTROL PROGRAMME THROUGH BIOASSAYS AND INSECTICIDE RESISTANCE TESTING

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INTRODUCTION

The application of residual insecticides to surfaces has been the recommended measure against malaria vector mosquitoes for many years. On the other hand, insecticide treated mosquito nets, larvicides, mosquito coils, aerosols and mosquito repellents are being used as part of an integrated malaria control programme (WHO 1984). Mostly, the success of indoor house spraying depends on vector behaviour and the mosquitoes should rest mainly indoors and spend a considerable time on the insecticide sprayed surface. This ensures that the mosquitoes pick up an effective dose and eventually die. It is also important for the vector mosquitoes to be susceptible to the insecticides being used, thus, resistance should be ruled out. It is also desirable that the mosquitoes do not have a repellent effect.

The amount of insecticide remaining on treated mosquito nets is very important because it determines how fast mosquitoes die and how long this activity is sustained. We carried out bioassays on huts sprayed with DDT and mosquito nets treated with insecticide in both Gokwe North and South districts.

General objectives

To support the National Malaria Control Programme by performing bioassays and insecticide resistance tests in selected areas in Gokwe North and South districts.

Specific objectives

1. To perform insecticide resistance tests in Gokwe South district
2. To perform bioassays in DDT sprayed areas in Gokwe North and South
3. To perform bioassays on insecticide treated mosquito nets
4. To observe mosquito behaviour in DDT sprayed huts

MATERIALS AND METHODS

Study areas

Resistance tests were conducted from mosquitoes collected from Masakadza and Gwave areas.

Insecticide resistance tests (according to WHO 1998)

Resistance tests were performed by exposing 10 – 25 non-blood fed *Anopheles gambiae sensu lato* mosquitoes that were 2 days old. The exposure was done in WHO tubes for one a period of one hour and the knockdown rates were recorded at 5,10, 15, 20, 30, 50, 50 and 60 minutes. The mortality rates were scored after holding the mosquitoes for 24 hours. Any adults that are able to fly will be considered alive. A mortality rate of 98%-100% indicates that mosquitoes are susceptibility and therefore resistance is absent. Mortality rates of 80%–97% indicate that there is a possibility of resistance and confirmation is required. Any mortality less than 80% shows that resistance is present in the mosquito population. The papers treated with 4% DDT,

0.05% lambda-cyhalothrin, 0.05% deltamethrin, 1% permethrin, 0.75% permethrin and 0.1% bendiocarb were used.

Bioassays on sprayed structures

Mosquito bioassays were done on selected huts that have been sprayed with DDT in December 2006 (Gokwe South) and February 2007 (Gokwe North). Three WHO cones will be fastened on different positions on the wall and another 3 on the roof. Ten *An. gambiae s.l* mosquitoes were placed in each cone and knock down rate was monitored for 30 minutes before scoring mortality rates after 24 hours.

Bioassays on insecticide treated mosquito nets (according to WHO 2005)

A total of 5 noon blood fed (2–5-day old) *An. gambiae s.l* (mosquitoes were exposed to the net for 3 minutes using WHO cones. Three cones were used, one on top of the net, another at the middle of the net and the last one at the bottom of the net. Knock down rate was measured after 1 hour. Mortality rate was measured after 24 hours. Mosquito nets that have been washed 20 times are expected to cause greater than 80% mortality and/or greater than 95% knock down.

Mosquito behaviour in DDT sprayed huts

Mosquito behaviour studies were conducted by installing one Exit Window Trap (EWT) outside each selected hut (only window openings were used).

RESULTS

Midlands province: Gokwe North district

Table 1: The efficacy of DDT sprayed 10 months ago on *An. gambiae sl* mosquitoes at Kangazani village, Madzivazvido

Surface	**Knock down**	**Mortality**
Wall 1	1/10 (10%)	2/10 (20%)
Wall 2	1/10 (10%)	2/10 (20%)
Wall 3	3/10 (30%)	5/10 (50%)
Wall 4	0/9 (0%)	9/9 (100%)
Wall 5	0/10 (0%)	1/10 (10%)
Wall 6	0/7 (0%)	1/7 (14.3%)
Wall 7	2/8 (25%)	5/8 (62.5%)
Wall 8	1/9 (11.1%)	4/9 (44.4%)
Wall 9	0/9 (0%)	1/9 (11.1%)
Wall 10	2/8 (25%)	0/8 (0%)
Wall 11	0/10 (0%)	4/10 (40%)
Wall 12	1/10 (10%)	7/10 (70%)
Wall 13	1/9 (11.1%)	2/9 (22.2%)
Wall 14	2/9 (22.2%)	5/9 (55.6%)
Total	**14/128 (10.9%)**	**48/128 (37.5%)**

The knock down rates ranged from 0% to 30% with a mean of 10.9% (Table 1). Twenty – four hour mortality rates ranged from 0% to 100% with a mean of 37.5%. Mortality rates of *An. gambiae sl* mosquitoes exposed to DDT 10 months after spraying were very low.

Table 2: The efficacy of DDT sprayed 10 months ago on *An. gambiae sl* mosquitoes at Kangazani village, Madzivazvido

Surface	**Knock down**	**Mortality**
Roof 1	3/10 (30%)	5/10 (50%)
Roof 2	5/9 (55.6%)	8/9 (88.9%)
Roof 3	1/10 (10%)	9/10 (90%)
Roof 4	2/8 (25%)	7/8 (87.5%)
Roof 5	1/10 (10%)	5/10 (50%)
Roof 6	3/10 (30%)	4/10 (40%)
Roof 7	0/8 (0%)	4/8 (50%)
Roof 8	0/10 (0%)	3/10 (30%)
Roof 9	3/10 (30%)	6/10 (60%)
Roof 10	2/8 (25%)	7/8 (87.5%)
Roof 11	3/10 (30%)	3/10 (30%)
Total	**23/103 (22.3%)**	**61/103 (59.2%)**

The knock down rate of mosquitoes exposed to sprayed roofs ranged from 0% to 55.6% with a mean of 22.3% (Table 2). The mortality rates ranged from 30% to 90% with a mean of 59.2%. The mortality rates 10 months after DDT spraying were very low.

Midlands province: Gokwe South district

Table 3: The efficacy of DDT sprayed 12 months ago on *An. gambiae sl* mosquitoes at Mandava village, Svisvi

Surface	**Knock down**	**Mortality**
Wall 1	0/10 (0%)	7/10 (70%)
Wall 2	0/10 (0%)	6/10 (60%)
Wall 3	1/9 (11.1%)	8/9 (88.9%)
Wall 4	2/8 (25%)	5/8 (62.5%)
Wall 5	3/9 (33.3%)	9/9 (100%)
Wall 6	4/9 (44.4%)	6/9 (66.7%)
Wall 7	4/10 (40%)	7/10 (70%)
Wall 8	5/10 (50%)	4/10 (40%)
Wall 9	4/10 (40%)	5/10 (50%)
Wall 10	0/9 (0%)	6/9 (66.7%)
Wall 11	0/10 (0%)	0/10 (0%)
Wall 12	0/8 (0%)	2/8 (25%)
Total	**23/103 (22.3%)**	**48/103 (46.6%)**

Knock down rates ranged from 0% to 50% with a mean of 22.3% (Table 3). The mortality rates ranged from 0% to 100% with a mean of 46.6%. The mortality rates of *An. gambiae sl* mosquitoes exposed to DDT 12 months after spraying were very low.

Table 4: The efficacy of DDT sprayed 12 months ago on *An. gambiae sl* mosquitoes at Mandava village, Svisvi

Surface	**Knock down**	**Mortality**
Roof 1	8/8 (100%)	8/8 (100%)
Roof 2	11/11 (100%)	11/11 (100%)
Roof 3	10/10 (100%)	10/10 (100%)
Roof 4	8/10 (80%)	9/10 (90%)
Roof 5	9/10 (90%)	10/10 (100%)
Roof 6	5/7 (71.4%)	7/7 (100%)
Total	**32/37 (86.5%)**	**36/37 (97.3%)**

Knock down rate of mosquitoes exposed to sprayed roofs ranged from 71.4% to 100% with a mean of 86.5% (Table 4). The mortality rates ranged from 90% to 100% with a mean of 97.3%. Roof mortality rates 12 months after DDT spraying were very good.

Table 5: Efficacy of long lasting mosquito nets treated with deltamethrin (mostiquaire) and conventional nets treated with deltamethrin (K-O Tab) on *An. gambiae sl* mosquitoes at Mandava village, Svisvi

Treatment	**Period post treatment**	**Cone number**	**Knock down**	**Mortality**
K-O Tab	1 month	1	5/5 (100%)	5/5 (100%)
		2	5/5 (100%)	5/5 (100%)
		3	5/5 (100%)	5/5 (100%)
		Total	**15/15 (100%)**	**15/15 (100%)**
Long lasting nets (deltamethrin)	1 year	4	5/5 (100%)	5/5 (100%)
		5	5/5 (100%)	5/5 (100%)
		6	5/5 (100%)	5/5 (100%)
		7	4/4 (100%)	4/4 (100%)
		8	5/5 (100%)	5/5 (100%)
		9	4/4 (100%)	4/4 (100%)
		Total	**23/23 (100%)**	**23/23 (100%)**
	3 years 1 month	10	3/5 (60%)	5/5 (100%)
		11	2/5 (50%)	4/5 (80%)
		12	3/4 (75%)	3/4 (75%)
		Total	**8/14 (57.1%)**	**12/14 (85.7%)**
	3 years 2 months	13	1/5 (20%)	1/5 (20%)
		14	3/4 (75%)	3/4 (75%)
		15	3/4 (75%)	2/4 (50%)
		Total	**7/13 (53.8%)**	**6/13 (46.1%)**

Conventional mosquito nets treated with K-O Table caused 100% knock down and mortality rates of *An. gambiae sl* mosquitoes (Table 5). Long lasting mosquito nets treated one year ago caused 100% knock down and mortality rates. Long lasting mosquito nets treated 3 years and one month ago had knock down rates ranging from 50% to 60% with a mean of 57.1%, the mortality rates were 75% to 100% with a mean of 85.7%. The long lasting mosquito nets treated 3 years 2 months ago had knock down rates of 20% to 75% with a mean of 53.8%, mortalities were 20% to 75% with a mean of 46.1%.

Table 6: Insecticide resistance tests conducted with WHO papers treated with 0.75% permethrin on *An. gambiae sl* mosquitoes collected from Gwave gardens

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	4 (16%)	11 (44%)	23 (92%)	25 (100%)	25 (100%)	25 (100%)	20/25 (80%)
2	0 (0%)	0 (0%)	1 (4%)	4 (16%)	12 (48%)	22 (88%)	25 (100%)	25 (100%)	13/25 (72%)
3	0 (0%)	0 (0%)	6 (37.5%)	9 (60%)	15 (93.8%)	16 (100%)	16 (100%)	16 (100%)	12/16 (75%)
Total	**0 (0%)**	**0 (0%)**	**11 (16.7%)**	**24 (36.4%)**	**50 (75.8%)**	**63 (95.5%)**	**66 (100%)**	**66 (100%)**	**45/66 (68.2%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

The mean knock down rates were achieved after 40 minutes (Table 6). Bioassays conducted on papers treated with 0.75% permethrin showed that the Gwave *An. gambiae sl* mosquito population is not susceptible to this concentration, implying that resistance to this concentration exists.

Table 7: Insecticide resistance tests conducted with WHO papers treated with 1% permethrin on *An. gambiae sl* mosquitoes collected from Gwave gardens

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	6 (24%)	15 (60%)	19 (72%)	25 (100%)	25 (100%)	25 (100%)	25/25 (100%)
2	0 (0%)	0 (0%)	5 (22.7%)	9 (40.9%)	10 (45.5%)	19 (86.4%)	22 (100%)	22 (100%)	22/22 (100%)
Total	**0 (0%)**	**0 (0%)**	**11 (23.4%)**	**24 (51%)**	**29 (61.7%)**	**44 (93.6%)**	**47 (100%)**	**47 (100%)**	**47/47 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

Mean knock down rates were achieved after 40 minutes of mosquito exposure (Table 7). Bioassays conducted on papers treated with 1% permethrin showed that the Gwave *An. gambiae sl* mosquitoes are fully susceptible to this concentration and no insecticide resistance occurs at this concentration.

Table 8: Insecticide resistance tests conducted with WHO papers treated with 1% permethrin on *An. gambiae sl* mosquitoes collected from Masakadza

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	9 (39.1%)	16 (69.6%)	21 (91.3%)	23 (100%)	23 (100%)	23 (100%)	23/23 (100%)
2	0 (0%)	0 (0%)	7 (28%)	9 (45%)	14 (64%)	17 (68%)	25 (100%)	25 (100%)	25/25 (100%)
3	0 (0%)	0 (0%)	5 (20.8%)	10 (41.7%)	17 (70.8%)	21 (87.5%)	24 (100%)	24 (100%)	24/24 (100%)
Total	**0 (0%)**	**0 (0%)**	**21 (29.2%)**	**35 (48.6%)**	**52 (72.2%)**	**61 (84.7%)**	**72 (100%)**	**72 (100%)**	**72/72 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

Mean knock down rates were achieved after 40 minutes of mosquito exposure of mosquitoes from Masakadza (Table 8). Our results showed that the mosquitoes were fully susceptible to papers treated with 1% permethrin.

Table 9: Insecticide resistance tests conducted with WHO papers treated with 0.05% deltamethrin on *An. gambiae sl* mosquitoes collected from Gwave

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	1 (4%)	12 (48%)	18 (72%)	21 (91.3%)	24 (96%)	25 (100%)	25 (100%)	25/25 (100%)
2	0 (0%)	0 (0%)	9 (36%)	19 (76%)	22 (88%)	22 (88%)	25 (100%)	25 (100%)	25/25 (100%)
3	0 (0%)	0 (0%)	7 (28%)	16 (64%)	17 (68%)	21 (84%)	25 (100%)	25 (100%)	25/25 (100%)
Total	**0 (0%)**	**1 (1.3%)**	**28 (37.3%)**	**53 (70.7%)**	**60 (80%)**	**67 (89.3%)**	**75 (100%)**	**75 (100%)**	**75/75 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/24 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/49 (0%)**

One hundred percent knock down rate was recorded after 40 minutes when mosquitoes from Gwave were used on 0.05% deltamethrin (Table 9). One hundred percent mortality rate was recorded after 24 hours and the mosquitoes were fully susceptible to papers treated with 0.05% deltamethrin.

Table 10: Insecticide resistance tests conducted with WHO papers treated with 0.05% deltamethrin on *An. gambiae sl* mosquitoes collected from Masakadza

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	5 (22.7%)	15 (68.2%)	20 (90.9%)	21 (95.5%)	22 (100%)	22 (100%)	22/22 (100%)
2	0 (0%)	0 (0%)	6 (30%)	13 (72%)	23 (92%)	24 (96%)	25 (100%)	25 (100%)	25/25 (100%)
Total	**0 (0%)**	**0 (0%)**	**11 (23.4%)**	**28 (59.6%)**	**43 (91.5%)**	**45 (95.7%)**	**75 (100%)**	**75 (100%)**	**75/75 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

One hundred percent knock down was achieved after 40 minutes for mosquitoes from Masakadza (Table 10). One hundred percent mortality rate was recorded after 24 hours and the mosquitoes were fully susceptible to papers treated with 0.05% deltamethrin.

Table 11: Insecticide resistance tests conducted with WHO papers treated with 0.05% lambda-cyhalothrin on *An. gambiae sl* mosquitoes collected from Gwave

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	2 (4%)	9 (36%)	17 (68%)	23 (92%)	25 (100%)	25 (100%)	25 (100%)	25/25 (100%)
2	0 (0%)	5 (25%)	11(44%)	15 (60%)	21 (84%)	25 (100%)	25 (100%)	25 (100%)	25/25 (100%)
3	0 (0%)	3 (12%)	7 (28%)	12 (48%)	20 (80%)	25 (100%)	25 (100%)	25 (100%)	25/25 (100%)
4	0 (0%)	5 (25%)	6 (24%)	20 (80%)	21 (84%)	25 (100%)	25 (100%)	25 (100%)	25/25 (100%)
5	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	2 (13.4%)	8 (61.5%)	13 (100%)	13 (100%)	13/13 (100%)
6	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (23.1%)	7 (53.8%)	13 (100%)	13 (100%)	13/13 (100%)
Total	**0 (0%)**	**14 (11.1%)**	**33 (26.2%)**	**65 (59.5%)**	**90 (71.4%)**	**115 (91.3%)**	**126 (100%)**	**126 (100%)**	**126/126 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

All mosquitoes from Gwave were fully susceptible to 0.05% lambda-cyhalothrin and no resistance was detected (Table 11).

Table 12: Insecticide resistance tests conducted with WHO papers treated with 0.05% lambda-cyhalothrin on *An. gambiae sl* mosquitoes collected from Masakadza

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	7 (28%)	13 (52%)	19 (76%)	21 (84%)	25 (100%)	25 (100%)	25 (100%)	25/25 (100%)
Total	**0 (0%)**	**7 (28%)**	**13 (52%)**	**19 (76%)**	**21 (84%)**	**25 (100%)**	**25 (100%)**	**25 (100%)**	**25/25 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

All mosquitoes from Masakadza were fully susceptible to 0.05% lambda-cyhalothrin and no resistance was detected (Table 12).

Table 13: Insecticide resistance tests conducted with WHO papers treated with 0.1% bendiocarb on *An. gambiae sl* mosquitoes collected from Gwave

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	0 (0%)	1 (6.7%)	4 (26.7%)	9 (60%)	12 (80%)	15 (100%)	15/15 (100%)
2	0 (0%)	0 (0%)	0 (0%)	2 (10%)	6 (30%)	15 (75%)	17 (85%)	20 (100%)	20/20 (100%)
Total	**0 (0%)**	**0 (0%)**	**0 (0%)**	**3 (8.6%)**	**10 (28.6%)**	**24 (68.6%)**	**29 (82.9%)**	**35 (100%)**	**35/35 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/50 (0%)**

All mosquitoes from Gwave were fully susceptible to 0.1% bendiocarb and no resistance was detected (Table 13).

Table 14: Insecticide resistance tests conducted with WHO papers treated with 4% DDT on *An. gambiae sl* mosquitoes collected from Masakadza

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (16.7%)	10 (41.7%)	17 (70.8%)	24 (100%)	24/24 (100%)
2	0 (0%)	0 (0%)	0(0%)	0 (0%)	3 (15.8%)	12(63.2%)	14 (73.7%)	19 (100%)	19/19 (100%)
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (23.8%)	14 (66.7%)	18 (75%)	21 (100%)	21/21 (100%)
Total	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**12 (18.8%)**	**36 (56.3%)**	**49 (76.6%)**	**64 (100%)**	**64/64 (100%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/23 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/24 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/47 (0%)**

One hundred percent knock down and mortality rates were achieved when mosquitoes were exposed to papers treated with 4% DDT (Table 14).

Table 15: Insecticide resistance tests conducted with WHO papers treated with 4% DDT on *An. gambiae sl* mosquitoes collected from Gwave

	Knock down (minutes)								**Mortality**
Replicate	**0**	**5**	**10**	**15**	**20**	**30**	**40**	**60**	
1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (17.6%)	7 (41.2%)	11 (64.7%)	17 (100%)	16/17 (94.1%)
2	0 (0%)	0 (0%)	0(0%)	0 (0%)	6 (24%)	15(60%)	18 (72%)	25 (100%)	24/25 (96%)
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (20%)	9 (60%)	15 (100%)	15(100%)	14/15 (93.3%)
4	0 (0%)	0 (0%)	0 (0%)	2 (18.2%)	6 (54.5%)	10 (90.9%)	11 (100%)	11 (100%)	11/11 (100%)
Total	**0 (0%)**	**0 (0%)**	**0 (0%)**	**2 (2.9%)**	**18 (26.5%)**	**41 (60.3%)**	**55 (80.9%)**	**68 (100%)**	**65/68 (95.6%)**
Control 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/25 (0%)
Control 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0/24 (0%)
Total control	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/49 (0%)**

One hundred percent knock down rate was achieved after one-hour exposure to 4% DDT of mosquitoes collected from Gwave (Table 15). Resistant individuals exist in mosquitoes collected from Gwave and verification is required since 95.6% mortality was recorded.

Table 16: Mosquito behaviour in DDT sprayed huts: Gwafa village: Gwave area

		An. gambiae sl and condition			Total	Culex			Total
Trap	Occupants	Fully Fed	Half Gravid	Unfed		Fully Fed	Half Gravid	Unfed	
1	3	2	3	1	6	1	0	0	1
2	4	1	0	9	10	0	0	0	0
3	1	0	0	0	0	0	0	0	0
4	2	0	0	0	0	0	0	0	0
5	2	3	0	1	4	0	0	0	0
6	3	2	0	1	3	0	0	0	0
1	3	1	1	0	2	0	0	0	0
2	4	0	3	4	7	0	0	2	2
3	1	0	0	0	0	0	0	0	0
4	2	0	0	0	0	0	0	0	0
5	2	1	0	0	1	0	0	0	0
6	3	3	0	0	3	0	0	0	0
Total		**13**	**7**	**16**	**36**	**1**	**0**	**2**	**3**

Out of a total of 36 *An. gambiae sl* mosquitoes collected, 7 (19.4%) had rested in the huts 24 hours ago (Table 16). Thirteen mosquitoes (36.1%) entered the huts and managed to feed and 16 (44.4%) failed to take a visible blood meal. All occupants did not own mosquito nets. Two unfed Culex mosquitoes were collected from huts.

DISCUSSION

Bioassays conducted on walls sprayed with DDT 12 months ago in Gokwe South showed low mortality rates on the walls but very high mortality rates on the roofs. Walls are expected to have a low mortality rate than roofs 6 months after DDT application and the results are good. However, bioassays conducted on surfaces sprayed with DDT 10 months ago in Gokwe North showed very low mortality rates and this is attributed to poor spraying due to high turnover of Spray Operators.

Bioassays conducted on mosquito nets treated with K-O Tab showed that the insecticide is effective one month after application. Long lasting mosquito nets treated with deltamethrin one year ago were equally effective. However, results of long lasting mosquito nets treated 3 years 1 month and 3 years 2 months ago were not very good.

All insecticide resistance tests conducted on papers treated with 0.05% lambda-cyhalothrin showed a 100% susceptibility rate in mosquitoes collected from Gwave and Masakadza areas. Results on the exposure of mosquitoes collected from Gwave to 0.1% bendiocarb showed that there is no resistance. Tests conducted on mosquitoes collected from Gwave and Masakadza on papers treated with 0.05% deltamethrin showed full susceptibility. Bioassay results of mosquitoes collected from Gwave on papers treated with 0.75% permethrin showed resistance. However, when papers containing 1% permethrin were used, mosquitoes from Gwave and Masakadza were fully susceptible. Tests conducted using papers treated with 4% DDT on mosquitoes collected from Masakadza showed susceptibility but for those

mosquitoes collected from Gwave, the results indicated that resistant individuals still exist.

Mosquito behaviour is one of the aspects that have a direct link with performance of a programme. Indoor residual spraying assumes that the insecticide used has no repellent effect and kills mosquitoes on contact. Our results suggest that of all the mosquitoes that rested indoors, only 19.4% were found in exit window traps. It is also interesting to note that 36.1% of the mosquitoes that entered the huts managed to feed, indicating that endophagic behaviour is present and a combination of nets and IRS is meaningful. However, DDT prevented 44.4% of the mosquitoes from feeding.