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Synergistic effects of lambdacyhalothrin incorporated into 1, 4-dichlorobenzene for the control of sand fly and mosquito vectors in Baringo and Kirinyaga Counties, Kenya

Kaburi Josyline * [1,2], Ngumbi Philip[1], Irungu Lucy[2], Ndegwa Paul[2], Ingonga Johnstone[1], Bernard Osero[1], Dickson Libendi[3], Anjili Christopher[1]

1 Centre for Biotechnology Research and Development, Kenya Medical Research Institute P.O Box 54840, 00200, Kenya Nairobi,
2 School of Biological Sciences, University of Nairobi, P.O Box 30197, 00100, Kenya Nairobi,
3 School of Pure and Applied Sciences, Kenyatta University, P.O Box 43844-00100, Nairobi

E-mail : jcirindi@kemri.org / jcirindi@gmail.com

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Abstract

Current efforts at mosquito control of malaria rely heavily on insecticide-treated bednets, indoor residual spraying with insecticide, and application of chemical larvicides. No new public health insecticides have been developed for mainstream vector control in disease endemic countries for 30 years. Marigat study site is an area that is endemic for both cutaneous and visceral leishmaniasis while Mwea is a malaria endemic area. Both sand fly and mosquito collections were carried out in the study households before and after interventions using the CDC light traps. For laboratory bioassays, mosquitoes and sandflies were exposed to a dosage of 100 mg/m2 and their repellency and mortality monitored and scored over a period of 48 hours. For the field studies, vector collections were carried out before and after interventions. They were then counted identified and recorded. Results revealed high mortality rates of vectors exposed to the combination of PCB and ICON as compared to individual treatment of the two. The sand fly and mosquito densities decreased significantly when exposed to PCB/ICON combination as opposed to ICON and PCB alone. The mean numbers of sand flies and mosquitoes collected from houses with PCB/ICON combination was less as compared to PCB and ICON independently. From the results, there was a synergistic effect observed when lambda-cyhalothrin (ICON®) and 1, 4-dichlorobenzene (PCB®) are used in combination as compared to their individual treatments. This indicates that combinations are more effective in controlling vectors of malaria and leishmaniases diseases.

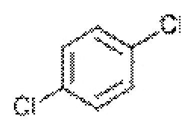
Key words : Lamdacyhalothrin, Malaria, Leishmaniasis, para-Dichlorobenzene

INTRODUCTION

In recent years the use of environment friendly and easy biodegradable insecticides has received much attention for control of medically important arthropods. Vector borne diseases such as malaria and leishmaniases still cause thousands of deaths and morbidity [1, 2]. Management of these disease vectors using most synthetic chemicals have failed because of insecticide resistance, vector resurgence and environmental pollution consequently, necessitating intensive efforts to find alternative methods of control [3].

Lambdacyhalothrin (ICON®) is a synthetic pyrethroid insecticide that has been shown to be effective in killing mosquitoes and phlebotomine sand flies when used in low doses [4, 5, 6]. It has also been used for the control of intra-domiciliary *Anopheles arabiensis* Patton in huts in South Africa [4]. It has no smell and has a long residual period of 6 months [4, 7] and unlike DDT it is not toxic to vertebrates [6, 5, 4, 8]. Unlike DDT no resistance has been reported for sand flies [9] and mosquitoes [8]. The observed efficacy of lambdacyhalothrin in protecting people from bites of sand flies by studies carried out in Venezuela [10], its ready acceptance by users as was shown in Brazil [11], and its cost effectiveness make it a more useful insecticide for the control of malaria and leishmaniasis.

1, 4-Dichlorobenzene (*para*-dichlorobenzene, *p*-DCB, PDB, 1, 4-dichlorobenzene) is an organic compoundwith the molecular formula $C_6H_4Cl_2$ and a structure shown below. This colourless solid has a strong sweet odour. It consists of two chlorine atoms substituted at opposing sites on a benzene ring. *p*-DCB is used as a pesticide and a deodorant, most famously in mothballs in which it is a replacement for the more traditional naphthalene ("National Pesticide Information Center - Mothballs Case Profile", 2009). *para* -Dichlorobenzene is also used as a precursor in the production of the polymer poly (p-phenylene sulfide) [12]



PCB is produced by chlorination of benzene using ferric chloride as a catalyst.

$$C_6H_6 + 2\,Cl_2 \rightarrow C_6H_4Cl_2 + 2\,\underline{HCl}$$

The compound is purified by fractional crystallisation, taking advantage of its relatively high boiling point of 174°C and melting point of 53.5 °C. 1, 4-dichlorobenzene is poorly soluble in water (10.5mg/100ml at 20°C) and is not easily broken down by soil organisms. Like many hydrocarbons, paracide® is lipophilic and would accumulate in the fatty tissues. There is no direct evidence of carcinogenicity according to environmental protection agent (EPA). 1, 4-dichlorobenzene is registered by USA-EPA for water use at a concentration of 75µg per litre and also as a pesticide. There is no report of insecticidal resistance against 1, 4-dichlorobenzene in sand flies and mosquitoes in Kenya.

1, 4-dichlorobenzene (Paracide®) when used as a deodorant and pesticide like lambdacyhalothrin lasts for six months. Incorporation of lambdacyhalothrin into 1, 4-dichlorobenzene under slow release basis will help not only to keep sand flies and mosquitoes away but also other household pests as well as freshening up the houses. The use of the lambdacyhalothrin fortified with 1, 4-dichlorobenzene, which are cheap will help repel the two vectors especially in the areas they are sympatric. This kind of control method was evaluated on vectordensities as well as feeding rates of the mosquitoes and sand flies both in the laboratory and field.

The study determined the feeding success of mosquitoes and sand flies and compared the efficacy of lambdacyhalothrin fortified with 1, 4-dichlorobenzene in the control of endophilicphlebotomine sand flies and *Anopheles* mosquitoes.

MATERIALS AND METHODS

Laboratory repellency and mortality of mosquitoes and sand flies

For the laboratory bioassay, laboratory bred sand flies and mosquitoes were exposed to ICON, PCB and their combination and the survival and mortality of adult vectors was determined under laboratory conditions.

A tunnel experiment was performed for the two vector species according to Kasili *et al*[13].

To perform the experiments, tunnels were constructed from plexi-glass cages with plaster of paris on their bases. Two such cages measuring 25 x 25 x 40 cm were attached to one another using a plexi-glass tube measuring 30 centimetres. One side of the cage was covered using a stocky net, which was also used to introduce either mosquitoes or sand flies. The second cage was used to introduce anesthetized hamster alone (control 1), Hamster plus lambdacyhalothrin/1, 4-dichlorobenzene block, Hamster plus lambdacyhalothrin block alone (control 2), Hamster plus paracide block alone (control for Number 2, and 3), lambdacyhalothrin block alone (control for Number 2), 1, 4-dichlorobenzene block alone (control for Number 2) and Hamster alone (control for1, 2, 3 and 4 because of the presence of CO_2).

Vector Repellency

All the mosquitoes and sand flies that traversed from the treated chamber were counted and recorded. The percentage repellency was calculated and compared with the ones collected from the untreated chambers.

Vector Mortality

Immediate mortality was determined by collecting and counting all mosquitoes and/or sand flies which died. The sand flies and mosquitoes which passed through the treated chamber was maintained for 48 hours and observed for possible delayed mortality. Time taken by the insects to die following exposure to lambdacyhalothrin positive sand flies and mosquitoes was recorded to determine the mortality rates.

Field studies

This study was carried out in two study sites, Marigat and Mwea. Baringo lies 250 km North West of Nairobi and covers an area of approximately 10,000 km^2 and is located north of the Equator in Kenya's Rift Valley Province. The area is semi-arid with subtropical climate. Rainy seasons are from March to September with peaks in May and August. Annual rainfall is below 300 mm while temperatures range from 17-42°C. Natural vegetation in Marigat is mainly composed of *Acacia* species either scattered or as forest in few cases, short bushes or patchy grassland. The ground is mostly bare soil or rocky with gullies in some areas. The Kenya government recently introduced *Prosofisjuliflora* to control desertification in the area which has changed the vegetation cover in the regions formerly devoid of vegetation. Rodent burrows are numerous in both vegetation covered and bare grounds. Termite mounts are common features in the area. There is a Perkerra irrigation scheme around Marigat that allows growing of various crops such as maize and vegetables. Animal husbandry is also practiced in the area whereby cattle, sheep and goats are kept.

Mwea a malaria endemic area that is not known to be endemic for leishmaniases was used to test the efficacy of the repellent on malaria vectors, particularly *An.gambie* and *An. funestus*. Mwea is found in Central Province, Kenya. Mwea Division is situated on the Eastern side of Mount Kenya at an altitude ranging between 1100 to 1350 metres above sea level. It is the centre of Mwea Rice Irrigation Scheme, a settlement scheme that produces 75% - 90% of the rice that is consumed in Kenya (NIB report). It covers an area of about 12,000 hectares. The division can be generally grouped into 2 distinct ecological settings, the low-lying irrigated rice paddies to the south and the elevated upland area to the north. Mwea Division has 3,270 families living in 60 villages [14].

In each of the study sites, 30 households separated by 100 metres from each other were selected. In the 30 experimental houses, treated disinfectant blocks were placed in all rooms where the inhabitants sleep and the kitchen was avoided because of smoke. Sand fly and mosquito collections were carried out in the study households before and after interventions. This was done using the CDC light traps Muirhead-Thomson [15].

Collections were carried out in houses for a minimum of 4 nights per month per area. CDC light traps were set in the houses at 1800 hours in the evening and collected before 0800 hours the following day during the known high transmission season of malaria and leishmaniasis [4]. The caught vectors were knocked down using ice. Collections were carried out for a period of 2 years in all the study sites. All the mosquitoes and sand flies collected were preserved separately in collection cups. They were counted and the numbers recorded down awaiting identification.

Data management

Data were entered in a computer using MS excel and thereafter imported into STATA 9.2, (STATACORP, TX USA) for analysis. Before the main analysis Fisher's exact tests were used to compare results for pre-interventions and post intervention. The average number of flies per trap (house) for each treatment was used as the units of analysis. Baseline data were compared with the intervention data using a paired *t* test. Data per treatment of sand flies and mosquitoes that fed/probed and on mortalities were compared by student *t* test.

RESULTS

Determination of mortality and survival rates of laboratory bred mosquitoes and sandflies exposed to lambdacyhalothrin incorporated into 1, 4-dichlorobenzene in the laboratory.

There was increase in mortality of mosquitoes from day one of treatment to 24 hours and steady increase in mortality to 48 hours for all treatments (Figure 1). A high number of mosquitoes exposed to ICON survived as compared to PCB but the mean difference was not significantly different (1.667±1.333, P=0.133). However there was high mortality rates observed in mosquitoes exposed to combination than PCB and ICON independently (2.667±1.706, P=0.0894; 4.333±2.789, P=0.0905)

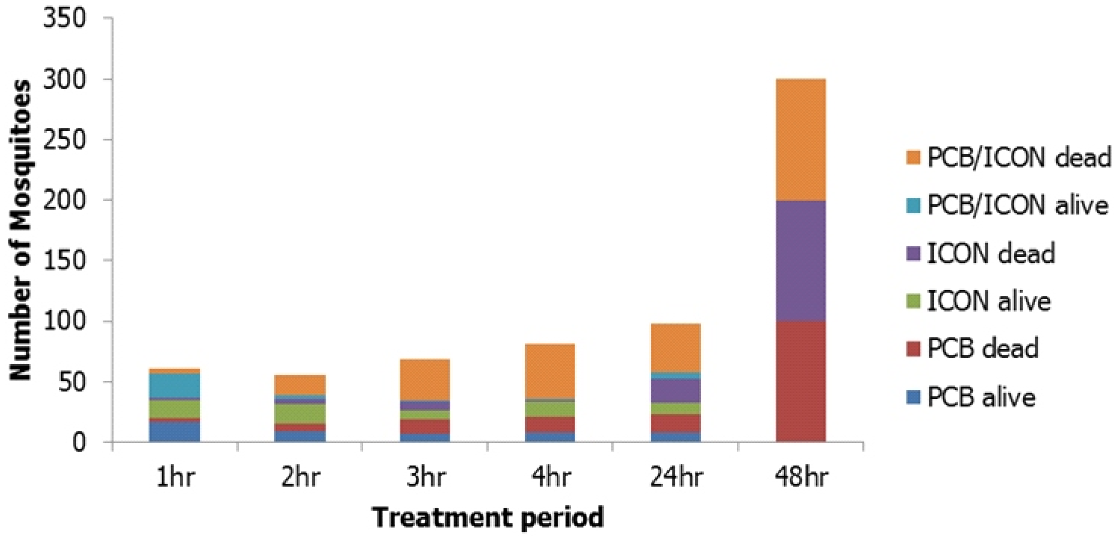


Fig 1: Trends of mosquito survival after treatment with PCB, ICON and PCB/ICON combination.

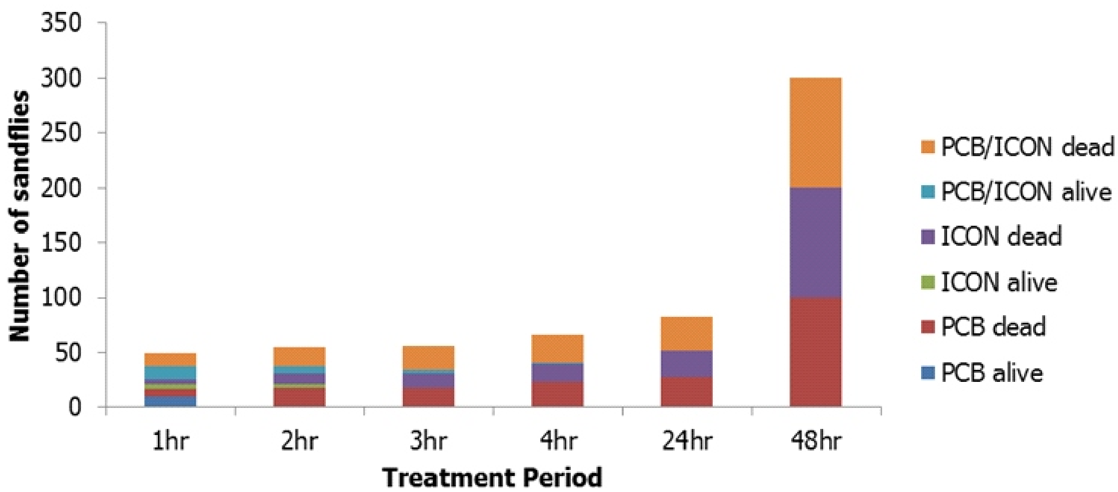


Fig 2: Mortality and survival rates of sand flies treated with ICON, PCB and their combination.

respectively.

Conversely there was a steady decrease in survival of mosquitoes from the first day of treatment to 48 hours for all treatments. There was observed high mortality of mosquitoes exposed to PCB as compared to ICON but the mean difference was not significant (2.5±2.32, P=0.1652). However there was high mortality of mosquitoes which were exposed to PCB/ICON combination as compared to PCB and ICON independently and their mean difference was significantly different (15.167±5.594, P=0.0211; 17.66±6.955, P=0.0259) respectively.

There was increase of sand fly density from day of treatment to 24 hours and steady increase in mortality to 48 hours for all treatments. Conversely there was a steady decrease in survival of sandflies from day of treatment to 48 hours for all treatments (Figure 2). The mean number of sandflies that survived was less in PCB/ICON combination as compared to PCB and ICON (2.1667 ±0.9098, P=0.0315 and 2.5 ±1.0567, P=0.0321 respectively.

The study showed high mortality of sandflies exposed to PCB as compared to ICON but their mean difference was significantly different (3.667±1.2824, P=0.0177). The mortality rates of sandflies exposed to PCB/ICON combination was high as compared to PCB (2.833±1.0462, P=0.0212) however vice versa when compared to ICON (6.5±1.36, P=0.0025).

Effects of PCB/ICON exposure on mosquitoes in Mwea study site.

There were a decreased number of mosquitoes after immediate exposure to ICON, PCB and PCB/ICON combination. The mean differences of mosquitoes trapped before and after immediate exposure to ICON, PCB and PCB/ICON combination were significantly different [(P=0.0117, 67.8±24.893, t=2.7237), (P=0.0266, 52.8±23.74, t=2.2235), (P=0.0138, 64.9±24.74, t=2.6229)] respectively.

Equally there was a significant reduction of mosquito densities after long term exposure (up to 6 months) to ICON

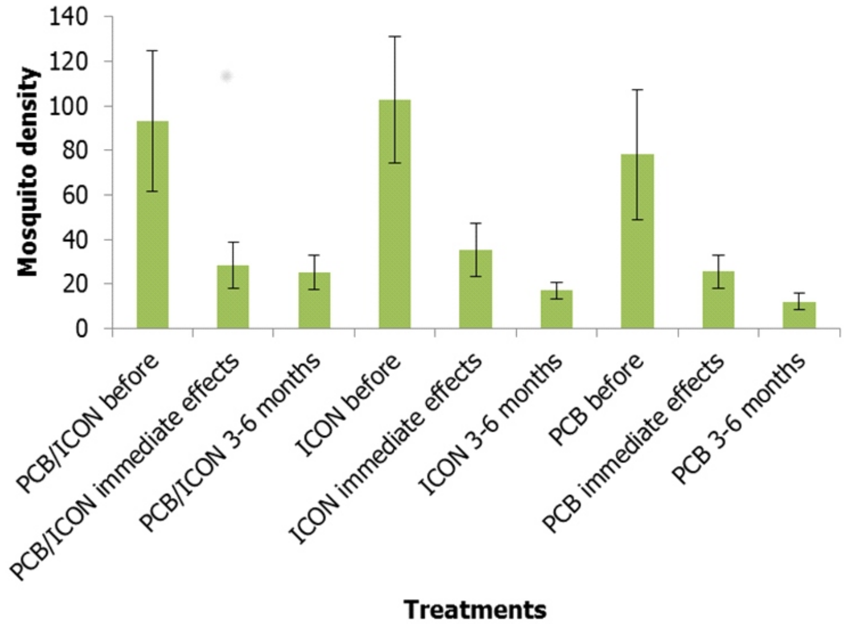


Fig 3: Mosquito densities in Mwea before and after intervention

(P=0.0232, 76.3±33.075, t=2.3069), PCB (P=0.0194, 66.2±27.4, t=2.4158) and PCB/ICON combination (P=0.0178, 68±27.5, t=2.4685) as shown in figure 3.

Effects of PCB/ICON exposure on sandflies in Mwea study site.

There was a notable significant difference in sand fly density after immediate exposure with ICON alone (P=0.0032, t=1.8125), but there was no significant difference in sand fly densities after immediate exposure with PCB and PCB/ICON combination (P>0.05) (Figure 4).

There was significant decrease in sand fly densities before and after six months of treatment of the household with ICON (P=0.0290, t=1.8125, SEM±25.9868), PCB (P=0.0248, t=1.8125, SEM±11.4632) but the densities of sand fly were not comparable before and after treatment with the combination of PCB and ICON (P=0.1131, t=1.8331, SEM±66.2999). The decrease of sand fly populations following exposure to ICON, PCB and ICON/PCB is a good indication that all the trial insecticides can be used in their control.

Effects of PCB/ICON combination exposure to sandflies in Baringo site

There was no significant decrease in sand fly densities before and after immediate exposure with ICON (67± 8.53, t= 1.3805, P=0.1004) and PCB (24.8±16.2, t= 1.5309, P=0.0801) as well as exposure after 6 months; ICON (67.4±48.0389, t= 1.4030

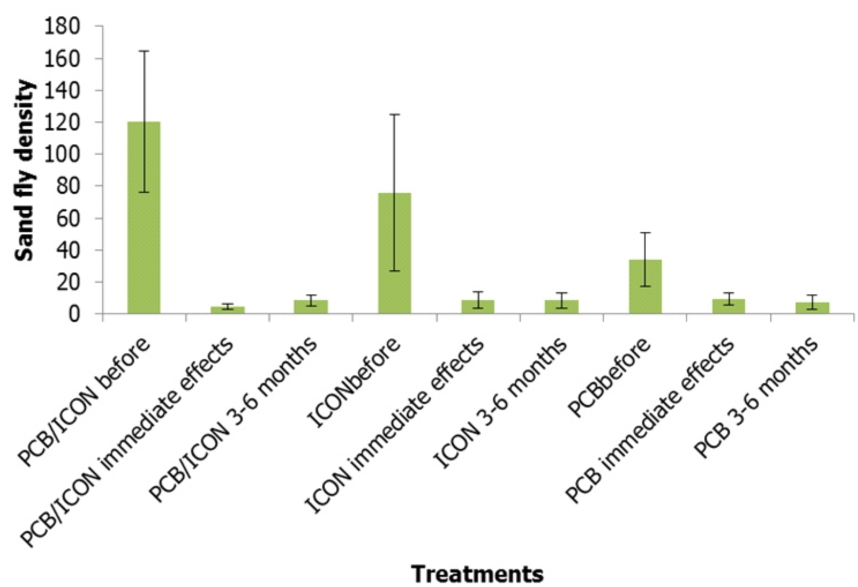


Fig 4: Sand fly densities in Mwea before and after interventions

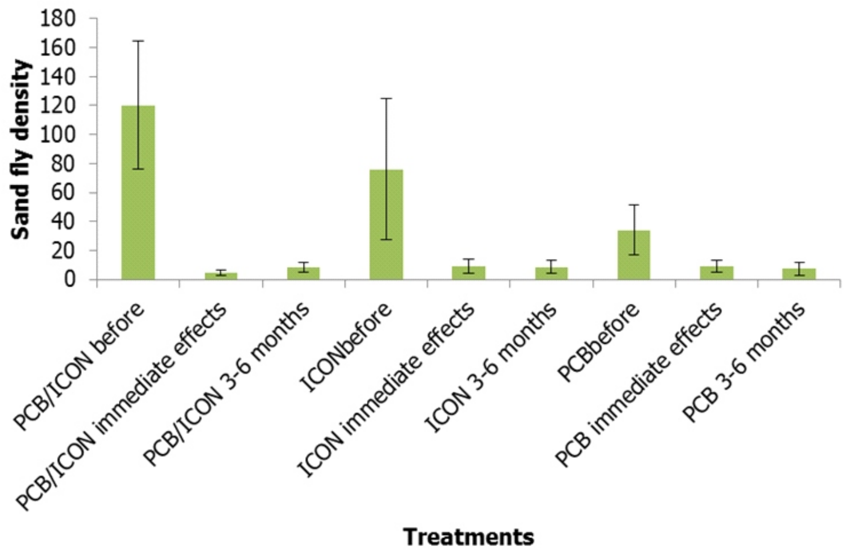


Fig 4: Sand fly densities in Baringo before and after interventions

P=0.0971) and PCB (26.8±16.13, t= 1.6614, P=0.0655). However there was a significant decrease in sand fly population after treatment with PCB/ICON combination (115.6±42.98, t= 2.6894, P=0.0124) as shown in figure 5.Conversely there was a significant decrease in sand fly densities before and after treatment with PCB/ICON combination after exposure for 6 months (111.6±41.434, t= 2.6934, P=0.0123).

Efficacy of insecticide treatments on mosquitoes over time in Baringo County

The mosquito densities decreased significantly when exposed to ICON and PCB/ICON combination as opposed to PCB alone for 3-6 months period (figure 6). The differences in mosquito densities seen in collection from the houses which had ICON and the combination of ICON/PCB recorded a reduction as opposed to PCB alone shows that PCB alone cannot be effective alone when used for more than 3 months. Overall significance in reduction of mosquito densities after treatment with ICON (45.4±18.043, P= 0.0165) and PCB/ICON (95.7±43.147, P= 0.0269) was observed, however there was no significant difference when mosquitoes were exposed to PCB (193.3±131.94, P= 0.0885)

DISCUSSION

Survival of arthropod vectors is one of the most important components of transmission of a vector-borne pathogen [16, 17, and 18]. Increased survival allows the vector to produce more offspring, to increase the chances of them becoming infected, to disperse over greater distances, to survive long enough to become infectious, and then to deliver more infective bites during the remainder of its lifetime.

PCB can be absorbed *via* ingestion, inhalation or dermal exposure. In mice, the oral route was found to be more rapid than inhalation in a study conducted with several human volunteers who were exposed to p-DCB through inhalation [19].

As a result, small changes in survival rate cause large changes in the rate of pathogen transmission [18, 20, 21, 22, 23].

In this study there was increase in mortality of mosquitoes from day one of treatment to 24 hours and steady increase in mortality to 48 hours for all treatments. A high number of mosquitoes exposed to ICON survived as compared to PCB but the mean difference was not significantly different (1.667±1.333, P=0.133). However there was high mortality rates observed in mosquitoes exposed to combination than PCB and ICON independently (2.667±1.706, P=0.0894; 4.333±2.789, P=0.0905) respectively.

Conversely there was a steady decrease in survival of mosquitoes from the first day of treatment to 48 hours for all treatments. There was observed high mortality of mosquitoes exposed to PCB as compared to ICON but the mean difference was not significant (2.5±2.32, P=0.1652). However there was high mortality of mosquitoes which were exposed to PCB/ICON combination as compared to PCB and ICON independently and their mean difference was significantly different (15.167±5.594, P=0.0211; 17.66±6.955, P=0.0259) respectively.

The high mortality of mosquitoes exposed to a combination of ICON and PCB at a dose of 40mg/m^2 and 60mg/m^2 respectively shows that this could be a better way of controlling *A. gambia* and probably other mosquito species. The increase of the mortality rates from 1st hour and full mortality rates in 24 hours shows that the slow release of the two insecticides facilitates the death of the insects. The mortality of sand flies exposed to ICON/PCB combination was lower than that of sand flies exposed to ICON and PCB singly, which could mean that mechanisms of insecticidal detoxification differs between sand flies and mosquitoes. The differences in reduction of mosquito densities seen in trap sites in the houses where ICON and ICON/PCB traps were placed showed that PCB alone was effective but not for more than 3 months. The decrease of sand fly populations following exposure to ICON, PCB and ICON/PCB is a good indication that all the trial insecticides can be used in their control.

In this study sandflies were exposed to ICON and PCB and their combination of the two for three months (immediate exposure) and compared to long time exposure (6 months). In Mwea exposure of sand flies to ICON significantly reduced their densities unlike exposure to PCB and ICON/PCB combination. This study therefore suggests that ICON was effective on sandflies in Mwea site after exposure for a short period. However the Mwea study gave contrary findings with that of Baringo site, where ICON/PCB combination significantly reduced the sand fly densities as compared to ICON and PCB alone. It has been shown that temperature affects the activity of lambda-cyhalothrin. The efficacy of ICON and PCB can be attributed to geographical location due to Mwea being a cooler region and Baringo being a much hotter region. This begs the need for further studies on how climatic conditions can affect the efficacy of the two insecticides. Furthermore exposure of sandflies to ICON and PCB for 6 months, showed a significant decrease in the densities. In comparison with the three months exposure this study suggests that efficacy of ICON and PCB is dependent on the longevity of time exposure. There was a significant decrease in mosquito densities after exposure to ICON and ICON/PCB combination for three months, however no significant decrease in their densities when exposed to PCB. This observation was also seen in their exposure for 6 months. It is therefore suggested that PCB alone cannot effectively kill mosquitoes but can synergize with ICON to be effective.

In Mwea there was a significant decrease in mosquito densities after exposure to ICON, PCB and their combination both immediate and long term exposures. This indicates that in Mwea site, PCB and ICON were able to kill mosquitoes individually the same way as when they were combined and this didn't need them to synergize each other to control the vectors. This is a peculiar finding that needs further investigation on geographical location, climatic differences and probably the genetic material of the mosquitoes in the two sites.

In the absence of pyrethroids, para-dichlorobenzene which is cheaper can be used to control mosquitoes and sandflies in malaria and leishmaniasis endemic regions in Kenya.

In Marigat the weather conditions were very harsh with very high temperatures in dry season and impassable roads in wet season. These were challenges associated with field related projects where researchers are forced to walk long distances to set traps and also collect them the following day. .

CONCLUSION

Lambdacyhalothrin and para-dichlorobenzene alone or in combination can be used safely for effective control of indoor-feeding sand fly and mosquito vectors of human disease.

In the absence of pyrethroidspara-dichlorobenzene which is cheaper, can be used to control mosquitoes and sandflies in malaria and leishmaniasis endemic regions in Kenya.

What is already known on this topic?

No new public health insecticides have been developed for mainstream vector control in disease endemic countries for 30 years.

Despite diligent application of available strategies, malaria still poses a global health burden, especially for those living in resource constrained countries in the developing world.

Resistance to insecticides is known to evolve rapidly and is already threatening the use of pyrethroids on bed-nets.

What this study adds to scientific knowledge

It is anticipated that the lambdacyhalothrin cubes developed for vector control which are cost effective, feasible, fragrant friendly and protective, will provide control of leishmaniasis in endemic areas. These nice smelling cubes will result in reducing vector-human contact or interrupting transmission of human leishmaniasis.

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COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

Josyline Kaburi, Benard Osero and Philip Ngumbi were involved in coordinating the study from proposal development, experimental design, data analysis and manuscript writing. Johnstone Ingonga and Dickson Libendiwere involved in experimental design. Lucy Irungu, Paul Ndegwa, and Christopher Anjili were involved in manuscript review.

All authors read and approved the final version of the manuscript.

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