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Original article

Efficacy of a simply resting box baited with crude fruit and leaf ethanol extracts of *Phytolaccadodecandra* (L' Herit) in capturing and killing of indoor mosquitoes (Diptera: Culicidae) at Korando, Western Kenya

Yugi Jared Owiti

School of Science and Technology, University of Kabianga, P. O. Box 20230-20300, Kericho, Kenya

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ABSTRACT

Effective capture and elimination of indoor resting mosquito population is important in the fight against mosquito borne diseases. This study aimed at evaluating the efficacy of a simply resting box baited with crude fruit and leaf ethanol extracts of *Phytolacca dodecandra* in attracting and killing indoor mosquitoes at Korando, Western Kenya. The study was conducted in three phases: pre-intervention, intervention and post intervention. Simple resting boxes made from galvanized wire frame measuring 30 cm × 30 cm × 30 cm, covered in blue and black tunic in and out and lined with carton boards were used. The boxes were baited with socks with strong human odour and 80 ml/100mls (e/w) solution of either crude ethanol fruit or leaf extracts of *P. dodecandra*, ethanol leaf extracts of *Azadiracta indica* or Deltamethrin. Deltamethrin and *Azadiracta indica* were used as positive and water as negative control. The treatments were applied at the intervention phase only. The boxes were left overnight in the houses and mosquitoes collected by 6.30 h. It was observed that more Culicines than Anopheline were captured irrespective of phase or treatment used. Mosquito densities reduced with phase of activity. *P. dodecandra* leaf extracts killed more mosquitoes than fruit or *A. indica* leaf extracts though the number were less than that of Deltamethrin or WHO threshold of >80% mortality. In conclusion, the simple resting boxes were effective in collecting and killing indoor mosquitoes though lethality did not matched the WHO threshold. With improved structural set up and use of pure extracts of *P. dodecandra*, the resting boxes can serve as effective tools for capture, elimination and management of mosquito borne diseases.

1. Introduction

Mosquitoes are important vectors of mosquito borne diseases (Tainchum et al., 2015; Wilke and Marrelli, 2015), that are of major public health importance in nearly all the tropical and subtropical countries (Becker et al., 2010; Braack et al., 2018). The need to develop new tools to manage the densities of these mosquitoes is important if the wars against diseases borne by mosquitoes are to be won. Currently the most popular method of mosquito control is the use of treated surfaces such as insecticide treated bed nets (ITNs), long lasting insecticide treated bed nets (LLITNSs) and indoor residual sprays (IRS). However, the synthetic chemicals used on these surfaces are harmful (Nkya et al., 2013) as they have been known to cause serious environmental pollution (water, air and soil), negatively impact human health, are non-target organisms (animals, plants, and fish) specific (Biswas et al., 2014) and less effective to target mosquitoes (Liu, 2015).

Many methods have been sought and tested in order to try and salvage the situation. One of such strategy is the use of mosquito traps for surveillance as well as management of mosquito population (WHO, 2018a,b). The traps come in different models (Pombi et al., 2014), layout (Okumu et al., 2010), baits (Kweka et al., 2013; Kweka et al., 2009) and are likely to be customized for particular habitats. The traps have been used to target indoor resting gravid females (Barrera et al., 2017; Johnson et al., 2017) and as an auto-dissemination devices for transiting females (Buckner et al., 2017). For the latter, its believed exposed mosquito either die immediately or if it exits (capture–release) (WHO, 2009), it auto-disseminate (amplify) the contaminants to other habitats it comes into contact with, exposing others that comes into contact

E-mail address: jowiti@kabianga.ac.ke

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with these surfaces. This in the end prevents other adults from emerging (Caputo et al., 2012). In either case, a proportion of the reproductive individuals are eliminated leading to reduced abundance (Sriwichai, et al., 2015) and by extension the number of infectious bites (Lorenzi et al., 2016).

The human bait trap (Human landing catch) is another method of sampling mosquitoes. It is accurate in measuring of man-vector contact (Service, 1993) as it involves real time collection of mosquitoes that attempt to feed (Jiang et al., 2007) and as such it is considered the gold standard for mosquito sampling. However, the method is hardly in use today due to ethical concerns (WHO, 2003) and available traps such as ovitraps (Johnson et al., 2017), surveillance traps (Mboera, 2006; Silver and Service, 2008; Brown et al., 2018; Degefa et al., 2019) and others (Rapley et al., 2009; Okumu et al., 2010; Gorsich et al., 2019) continue to significantly report different capture efficiencies (Kline, 2006). This is not only a challenge but a clarion call for urgent development and test of novel designs that could provide the needed consistency and accuracy in mosquito capture and elimination.

The current study was to develop a simple resting box and use the same to capture and kill indoor resting mosquitoes. This was to comply with the advocacy of the Global Technical Strategy for Malaria Elimination (GTS) (WHO, 2015) and Global Vector Control Response initiative (GVCR) (WHO, 2017) for integration of surveillance and elimination strategies against mosquitoes and mosquito borne diseases. However, the choice to use crude extracts of *Phytolacca dodecandra* (hereafter Endod), was to encourage use of botanicals which in addition to being highly efficient, are less likely to create dependence and resistance among disease vectors and above all are environmentally friendly (Pavela, 2014, 2016; Singh and Kaur, 2018; Saravi and Shokrzadeh, 2011). Endod plant extracts are rich in botanicals (Altemimi et al., 2017) and when closely monitored (Oladipupo et al., 2019) are likely to serve as safe and biodegradable bio-insecticides (Asadollahi et al., 2019) without running the risk of resistance. In addition, the study also intended to evaluate the efficacy of simply resting box baited with Endod plant part (fruit and leaf) crude ethanol extracts in capturing and killing indoor resting mosquitoes at Korando, Western Kenya.

2. Materials and methods

2.1. Study site

The current study was conducted at Korando "B" Village (Fig. 1). The geographical coordinates of the site is S 0°4′47.23716; E 34°40′38.3047. It is domiciled at Kisumu Central Location, Otonglo Division, Kisumu North District, Kisumu County, Western Kenya. It is 11.4 km away from Kisumu central business district (CBD) along Kisumu-Busia/Mumias Road. Most residents here are of Luo ethnic group engaged in a plethora of economic activities including agriculture and fishing to eke a living. The people live in variously designed house structures. The site was settled upon due to the fact that there is an intense mosquito activity that has often led to intense malaria transmission all year-round with peaks between March–June and October–November (Beier et al., 1994).

2.2. Sourcing for Deltamethrin (KOTab 1-2-3®) and plant materials acquisition and extraction

Deltamethrin (KOTab 1-2-3®) and fresh mature green fruits and leaves of Endod and leaves of *Azadirachta indica* (hereafter Neem) were sourced, identified and voucher number deposited as earlier described (Yugi et al., 2014). The parts were prepared and extracted following procedures used by Das et al. (2010) and Yugi et al. (2014, 2015) to obtain crude extracts in the form of a paste after freeze drying. The paste was then aliquoted into units of 80 mgs each, wrapped in aluminium foil and placed in zipped plastic bags (Each bag containing aliquots of a particular plant part). The bags were then put in airtight glass bottles to serve as stock quantity.

2.3. Selection of houses for experimentation

Houses for experimentation were sampled using a randomized complete block design. Four blocks of about 1 km^2 apart were identified and homesteads within numbered and selected randomly using lottery technique. Four houses per block were selected for inclusion and for each treatment (Fig. 2). The houses were included in the study based on; Type of material used to make the walls, the texture of the interior wall and whether the interior is painted or not. Also considered was the type of eve, whether open or closed, mosquito interventions method (IRS done or not) and presence or absence of mosquito nets and the type (whether ITNs or LLTNs). Houses with a history of use of an intervention were not used for experimentation.

2.4. Mosquito resting boxes

Simple resting boxes for the capture and exposure of mosquitoes were made using galvanized wire frame measuring 30 cm × 30 cm × 30 cm in a design similar to one described by Crans (1989). Bright blue and black pieces of cloth were sown to the outside and inside respectively and square pieces of cartons placed between the tunics on the faces of the cuboid for reinforcement (Fig. 3). The black tunic was used to attract mosquitoes (Bidlingmayer & Hem, 1980; Okumu et al., 2010; Lima et al., 2014) in addition to making the interior of box dark.

2.5. Experimental set up

The experiment was conducted during the dry seasons for a period of two months (60 days) in three phases; pre-intervention, intervention and post-intervention. For the pre-intervention, 24 resting boxes were placed individually in 24 houses. None of the boxes had treatment. For the Intervention, twelve boxes (12), four (4) of which was sprayed with a solution of a particular treatment (experimental) and twelve (12) other boxes sprayed with water only (control) were individually placed in the selected houses (Table 1). For the post-intervention, the activity was conducted as described for pre-intervention phase. All boxes were baited with socks with heavy human odour (the socks having been worn for four consecutive days by human volunteers). Each sock was pinned at the roof of each box and the box placed on the floor at a corner (Fig. 4) in a house with no preference as to where the opening faced (Mboera, 2005). The boxes were however, sited in areas with no or minimal interference if any for the duration of the study. The experiments were replicated four (4) times.

2.6. Mosquito bioassay

A four by four factorial experimental design was used in the bioassays (Kothari, 2004). Stocks extracts of [Endod (mature fruit and leaf) and Neem (leaf)] and Deltamethrin powder were mixed with distilled water at a ratio of 80 mg/100 ml (t/w) and the solution used to spray the interior of the resting boxes. Twelve (12) treated (4 with a particular treatment) and twelve (12) control boxes were used in the intervention phase. The experimental boxes were left to stay for a maximum period of two days in a house before being shuffled. Shuffling was done among houses with similar treatment.

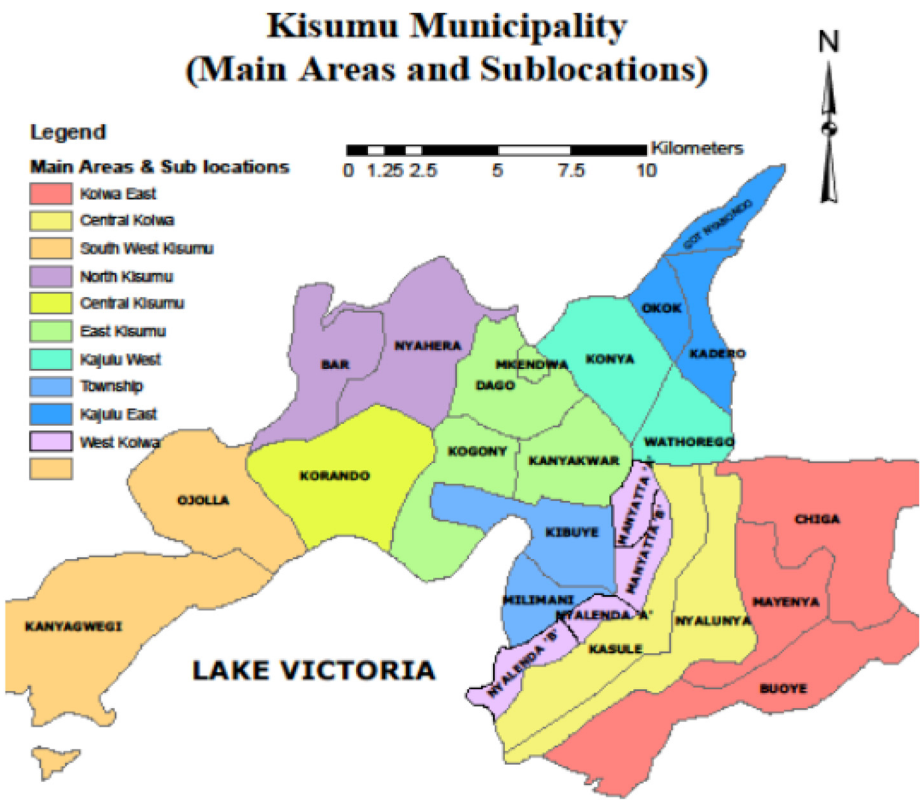

Fig. 1. Map of Kisumu County showing Korando "B" Village.

Fig. 2. Houses sampled for mosquito collection.

Fig. 3. Assembling components of simple mosquito resting boxes (modified after Harbisonet al., 2006) in readiness for surveillance.

The controls were however, undisturbed. After the boxes in each of the four experimental houses had been fully shuffled, the experimental boxes were exchanged with control boxes in such a way that experimental boxes were placed in houses that had control boxes and vice versa. The procedure was repeated until all the four treatments were used in each house. Priority however, was given to boxes sprayed with extracts of Endod and Neem. The shuffling and rotation was done to avoid positional and interpersonal bias.

For all the phases, resting boxes were set in place by 6.30 pm every evening and left to stay in the houses overnight. Trapped

Table 1
Placement schedule for Treated simple resting boxes within field selected houses.

S/N	Treatment used	No. of houses for each treatment
1	Endod fruit extracts	4
2	Neem leaf extracts	4
3	Deltamethrin	4
4	Control	12

mosquitoes were aspirated from the boxes twelve (12) hours later that is at 6.30 am (EAT) every morning. A torch was used to light the boxes and all mosquitoes therein collected using a battery powered (procopak) aspirator. The captured mosquitoes were put in plastic holding cups (Fig. 5) labeled with treatment used against and thereafter transported to the laboratory for processing.

At the laboratory, Gillies and Coetzee (1987) pictorial identification key containing line graphics of morphological characters in couplets was used to place the mosquitoes into species. Dead mosquitoes were counted and disposed of and those alive put in recovery paper cups appropriately labeled and provided with sugar solution for nutrition. These mosquitoes were monitored for mortality overnight. WHO threshold of >80% was used to pass a sentence of effectiveness for the extracts (WHO, 2006).

2.7. Data analysis

Data obtained from the bioassays was entered in excel sheets and the relationship between the effect of the trap and extracts on captured and killed mosquitoes determined using descriptive statistics. One way analysis of variance (ANOVA) and student T test were used to assess the level of significance of effect of the extracts on densities and mortality. Comparative attractiveness of the extracts to the mosquito species was determined using student T test. All statistical analysis was performed using Statistical Package for Social Scientists (SPSS) version 22.

3. Results

The current study was conducted for a period of two months (60 days) in three phases: pre, during and post intervention. Treatment was applied at the intervention phase only. It was observed that a high proportion of culicine were captured compared to anopheline mosquitoes. The densities of captured mosquitoes, reduced as the activities proceeded from pre to post intervention with densities significantly differing ($p < 0.05$) irrespective of the phase or species (Table 2).

A high density of culicine as opposed to anopheline mosquitoes preferred the resting boxes to the general interior of the houses though the densities were not significantly different ($p < 0.05$) irrespective of treatment or species (Table 3). The highest and lowest densities of the mosquitoes were recorded for boxes sprayed with Deltamenthrin and Endod plant part extracts respectively with the number of captured mosquitoes differing significantly irrespective of treatment ($p < 0.05$) except for Deltamethrin ($p > 0.05$) (Table 4).

The boxes caught on average 50% of the mosquitoes irrespective of species except for boxes treated with Neem leaf extracts (Fig. 6). The densities however, were significantly different irrespective of treatment (Table 5). Deltamethrin and Neem leaf extract treated boxes had the highest and lowest record of mortalities of exposed mosquitoes respectively. Fatalities however, differed significantly ($p < 0.05$) irrespective of treatment or control (Table 6).

4. Discussions

In the current study two species (Anopheline and Culicine) of mosquitoes important as malaria and other mosquito borne diseases in Western Kenya were captured (Karungu et al., 2019). Of these, culicines were the majority and also the most susceptible to the treatments used. Crude Endod leaf extracts were more lethal compared to Endod fruits or Neem leaf extracts but not Deltamethrin to the exposed mosquitoes.

Anophelines are a group of mosquitoes that belong to the family Culicidae, genus Anopheles and are known to be the most competent malaria vectors (Cohuet et al., 2009), transmitting one of the most virulent human malaria parasite, *Plasmodium falciparum* (Macharia et al., 2018). *Anopheles arabiensis* and *An. gambiae*, members of *Anopheles gambiae* complex are dominant in Kisumu and the neighbouring Siaya County (Okara et al., 2010). On the other hand the drab coloured, culicine mosquito species are generally found in all zoogeographical areas (Bhattacharya and Basu, 2016) around human settlements (Ciota and Kramer, 2013) and are implicated in the transmission of arboviruses (Weissenböck et al., 2010; Ochieng et al., 2013).

In the current study, more culicine than anopheline mosquitoes were captured. This observation was consistent with that of Pombi et al. (2014), who captured a higher density of culex mosquitoes in and out of the experimental houses using a Sticky Resting Box (SRB). Culex mosquito species are widespread (Bhattacharya and Basu, 2016) and coupled with their highly opportunistic feeding nature as well as their adaptation to live near human habitats (Maxwell et al., 1990), it is not surprising they were the majority in the traps.

Overraly, very low numbers of mosquitoes were retrieved from the boxes. Two explanations would service for this observation. First it is obvious most of the mosquitoes left, leaving those that had visited the boxes shortly before capture. Earlier, Matowo et al. (2013) had demonstrated that mosquitoes spend about 30 min or less in resting boxes and it was logical to catch only a few mosquitoes that had visited the boxes 30 min before collection. In the current study the boxes were set and left in the experimental house overnight and were only visited once in the morning almost 12 h later. Given that the mosquitoes visited the boxes in

Fig. 4. Simple mosquito resting boxes in experimental houses.

Bilge blower 4" inline model 240 Plastic holding cups

Fig. 5. Equipment for aspirating and holding mosquitoes from simple resting boxes.

Table 2
Mosquitoes densities per intervention phase [Densities presented as % mean plus standard error of mean (SEM)].

Activity type	Mosquito type		df	F	P
	Anopheline	Culicine			
Pre-intervention	41.80 ± 0.80^{a}	58.20 ± 0.80^{a}	2	208.274	0.000
Intervention	40.30 ± 1.03^{a}	59.29 ± 1.04^{a}	2	167.941	0.000
Post-intervention	39.27 ± 0.47^{a}	60.73 ± 0.47^{a}	2	1053.236	0.000

Notes: df = degree of freedom; F = the F statistical factor; *P* = probability for the level of significance. P was taken as significant at $p < 0.05$. Rows having mean percentage mortality superscripted with the same letter "**a**" indicate significance influence of experimental phases to the densities of captured mosquitoes

Table 3
Mosquitoes aspirated from the simple resting boxes (Numbers presented in terms of % mean ± standard error of mean (SEM).

Mosquito species	Mosquitoes in the resting boxes (Mean ± SEM)	df	t	P
Anopheline	40.25 ± 0.44^{a}	867	90.540	0.000
Culicine	59.47 ± 0.45^{a}	867	132.293	0.000

Notes: df = degree of freedom; t = the t statistical factor for student *t* test; *P* = probability for the level of significance. *P* was taken as significant at $p < 0.05$ for a two tailed test. Rows having mean percentage mortality superscripted with the same letter "**a**" indicate a significant influence of resting boxes on densities of trapped mosquito species.

Table 4
Mosquitoes densities per treatment used in the resting boxes [Densities represented as % means plus standard error of means (SEM)].

Treatment type	Mosquito type		df	F	P
	Anopheline	Culicine			
Endod fruits	37.95 ± 2.20^{a}	62.05 ± 2.20^{a}	4	60.131	0.000
Endod leaves	38.27 ± 1.76^{a}	62.02 ± 1.80^{a}	4	88.827	0.000
Neem	36.76 ± 2.63^{a}	60.27 ± 2.73^{a}	4	38.448	0.000
Deltamethrin	50.00 ± 1.66^{b}	50.00 ± 2.84^{b}	4	0.000	1.000
Control	43.39 ± 1.27^{a}	56.75 ± 1.27^{a}	4	55.313	0.000

Notes: df = degree of freedom; F = the F statistical factor; *P* = probability for the level of significance. P was taken as significant at $p < 0.05$. Rows having % mean numbers of mosquitoes superscripted with a different letter "**b**" are not significantly different.

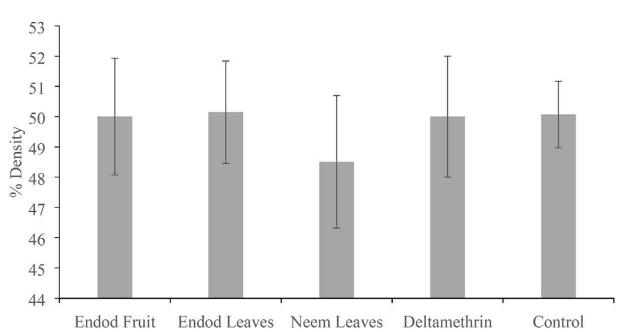

Fig. 6. Effect of treatment on mosquito densities [Densities are represented as % mean ± standard error of mean (SEM)].

Table 5
One sample *t* test representation on impact of treatment on mosquito densities.

Treatment used	df	t	p
Endod Fruit	111	25.980	0.000
Endod Leaves	111	29.737	0.000
Neem Leaves	111	22.129	0.000
Deltamethrin	111	24.995	0.000
Control	111	45.693	0.000

Notes: df = degree of freedom; t = the t statistical factor for student *t* test; *P* = probability for the level of significance. *P* was taken as significant at $p < 0.05$ for a two tailed test.

Table 6
Impact of lethal treatment on exposed mosquitoes [Densities represented as % means plus standard error of means (SEM)]

Treatment	Mortality (Mean ± SEM))	df	t	p
Endod fruits	53.98 ± 1.49[a]	55	36.275	0.000
Endod leaves	55.71 ± 1.00[a]	55	55.756	0.000
Neem	50.39 ± 1.01[a]	55	49.898	0.000
Deltamethrin	60.00 ± 1.27[a]	55	47.396	0.000
Control	16.38 ± 0.90[a]	55	18.186	0.000

Notes: df = degree of freedom; t = the t statistical factor for student *t* test; *P* = probability for the level of significance. *P* was taken as significant at $p < 0.05$ for a two tailed test.

anticipation for a blood meal (having been lured in by human foot oduor), it was natural for them to fly away shortly thereafter on realization that the box actually offered no such thing. Second, though human odour attractants was used, the fact that the sources were never replenished informs that the intensities and concentrations might have gone down leading to reduced attractiveness of the resting boxes. Indeed Chaiphongpachara et al. (2018) demonstrated using mush room attractants that the highest number of mosquitoes was attracted to a box with the highest dose of the lure or attractant, an observation similar to what was observed in the current study. However, in this study the odour lures were never refreshed or renewed and as would have been expected, after sometime the boxes would have yielded zero catches which was not the case. The boxes continually attracted mosquitoes to the last day, though the numbers were very low. This observation was consistent with Chaiphongpachara et al. (2018) and Cook et al. (2011) findings that observed that the attractant potential as lures was not a simple function of the amount used.

The above observations not withstanding and though it was impossible to ascertain the actual number of mosquitoes that visited the experimental boxes, one thing is for certain, all mosquitoes that landed and rested in the boxes picked an amount of the applied crude ethanol Endod plant part extracts (Matowo et al., 2013) and wherever they exited to, they died or contaminated surfaces or habitats they landed on. That is they biomagnified the effect of the toxicant. This however, remains just a speculation as it was not demonstrated. What is of certain is the fact that the simple resting boxes were attractive to the indoor resting mosquitoes due to probably three things; the colour of the interior (black), human oduor and the extracts.

Mosquitoes have been demonstrated to recognize colour by chemical receptor molecules in the eyes, and that colour is a significant determinant attraction (Lima et al., 2014). The black tunic must have aided in the attraction of the mosquitoes as had earlier been demonstrated by Bidlingmayer & Hem, (1980), Okumu et al. (2010) and Lima et al. (2014) who found that black was an effective attraction to mosquitoes.

That human have different attractive body oduor profiles (Verhulst et al., 2011) that make them attractive to mosquitoes (Takken and Knols, 1999; Olanga et al., 2010) is not in doubt (Rebollar-Téllez, 2005; Olanga et al., 2010) here. What is not certain is whether the plant extracts herein attracted the mosquitoes. Neem extracts have been demonstrated to repel as well as kill *An. gambiae* mosquitoes (Deletre et al., 2013) and from the observation made herein of boxes treated with Neem leaf extracts capturing less than 50% as well as killing slightly more than 50% of indoor mosquitoes, the findings herein is consistent with their repellant (Wannang et al., 2015; Macchioni et al., 2019) as well as toxic (Deletre et al., 2013) effects. Additionally, though, extracts of Endod had earlier proven toxic to laboratory cultured *An. gambiae* (Yugi et al., 2016) and *An. arabiensis* (Zeleke et al., 2017) mosquitoes, a feat that put Endod extracts in the same league as other plants with mosquito adulticidal potential (Mavundza et al., 2014; Ramkumar et al., 2016), the evidence herein of >50% capture of indoor mosquitoes, demonstrate repellency potential of Endod plant extracts towards mosquitoes.

The use of plant extracts for example straw mushroom, *Volvariella volvacea* (Chaiphongpachara et al., 2019) and Berk Mushroom, *Tremella fuciformis* (Chaiphongpachara et al., 2018) as alternative to animal-baited traps, homemade traps baited with heat, moisture and limburger cheese (Owino, 2011) would increase supervisory efficiency, reduce labour cost and risk of infection as is when passive seated human baits are used (Service, 1977). However, demonstrated efficacy of plant extracts as baits for mosquitoes are disappointingly few. A demonstration of extracts of Endod as having potential to attract mosquitoes as is inherent in this study is of double benefit. It is like having a double edged sword that cuts either way. In this study, the extracts not only augmented the capacity of the simple resting boxes to attract and capture but upgraded it to a killing machine for the exposed mosquitoes. Indeed the performance of the extracts exceeded that of other known box traps (Chaiphongpachara et al., 2018).

Phytochemical analysis has shown that terpenoids, phenolics (Matebie et al., 2019), saponins (Dorsaz and Hostettmann, 1986; Perret et al., 1999; Mmbando et al., 2010), alkaloids, proteins and amino acids, flavonoids, steroids, total phenols and tannins (Gani et al., 2016) are the chemical constituents inherent within Endod plant parts. In this study however, it is impossible to tell and therefore pinpoint with certainty the chemical constituent responsible for the observed lure of the mosquitoes into the resting boxes as none was experimental upon. This study submits therefore that the active Endod plant part chemical constituent that was responsible for lure of mosquitoes irrespective of species is unknown.

5. Conclusion

It is concluded that the simple resting boxes baited with crude ethanol extracts of Endod plant parts is indeed effective in capturing and killing indoor resting mosquitoes. It is further noted that with refinement of the trap, purification of extracts and possible use of a system that periodically replenishes both bait (human odour and Endod polyphenols), the simply resting box could be a dependable alternative tool for managing indoor resting mosquitoes and mosquito borne diseases.

Ethical clearance

Ethical clearance was sort and granted through a certificate Ref: No. GREC 092/38/2012 by the Great Lakes University of Kisumu (GLUK) Research Ethics Committee (GREC). Individual informed consent from head of homesteads was also obtained from all participants following verbal and written explanation of study aims and procedures in the participant's mother tongue (Dholuo). Individuals showed willingness to participate by either signing or thumb printing the consent document.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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