Journal of Mosquito Research 2020, Vol.10, No.3, 1-10
http://emtoscipublisher.com/index.php/jmr

Research Article Open Access

Complementary Effects of Crude Ethanol *Phytolacca dodecandra* (L' Herit) Extracts with House Characteristics on Densities of Indoor Resting Mosquitoes in Western Kenya

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Journal of Mosquito Research, 2020, Vol.10, No.3 doi: 10.5376/jmr.2020.10.0003
Received: 21 Aug., 2020
Accepted: 30 Oct., 2020
Published: 20 Nov., 2020

Preferred citation for this article:
Yugi J.O., 2020, Complementary effects of crude ethanol *Phytolacca dodecandra* (L' Herit) extracts with house characteristics on densities of indoor resting mosquitoes in Western Kenya, Journal of Mosquito Research, 10(3): 1-10 (doi:10.5376/jmr.2020.10.0003)

Abstract Intensive use of insecticide treated nets, indoor residual sprays and timely treatment of malaria patients saw reduced malaria parasitaemia from 11% to 8% by 2015. However, the sorry housing characteristics in a number of homesteads in Kenya, threaten this achievement. This study meant to evaluate the complementary effect of crude ethanol *Phytolacca dodecandra* (Endod) extracts with housing characteristics on density of indoor resting mosquitoes. A completely random block design was used to sample experimental houses and a four by seven factorial design used to sample indoor resting mosquitoes using resting boxes treated with Endod extracts. House characteristics and Endod treatments were taken as independent, mosquitoes as dependent variable and unsprayed (plain) resting boxes as control. Of recovered mosquitoes, Culicines (4.41±0.03) were more abundant than Anophelines (2.65±0.04). High [(3.05 ± 0.14), anopheline (5.03±0.35), culicine] and least [(2.83±0.14), anopheline (3.48±0.21), culicine] mosquitoes were found in houses without and with highest number of nets respectively. The numbers however, differed significantly [(df=1; F=27.436; $p<0.001$), no net (df=1; F=6.669; $p<0.012$), three nets] irrespective of species. Most anophelines (2.32±0.09) and Culicines (3.73±0.26) were found in houses with open and closed eaves respectively. It is concluded that effectiveness of crude ethanol Endod extracts on indoor resting mosquitoes is dependent on house characteristics, that crude extracts of Endod repels mosquitoes and that it could be used as an alternative insecticide to reduce the risk and burden of mosquito borne infections.

Keywords *Phytolacca dodecantra*; Anopheline; Culicine; Crude extracts; House characteristics; Resting boxes

Background

Malaria parasitemia levels were reported to have declined from 11% in 2010 to 8% in 2015 (WHO, 2018) probably due to increased use of insecticide treated nets (ITNs), indoor residual spraying (IRS), early diagnosis and prompt treatment (Bhatt et al., 2015). However, malaria transmission has continued to be observed even in areas with high coverage of these interventions (Killeen, 2014). This has been due to among other factors house characteristics. House characteristics in the context of this study is a description of all composite components that include structural design, modification, accessories, activities and residency. These features are important and have been reported to influence mosquito distribution (Mburu et al., 2018; Kaindoa et al., 2018; Jatta et al., 2018; Ngadjeu et al., 2020) in different ways. House characteristics complemented with indoor activities such as cooking, and indoor tethering of livestock provides not only optimal temperature and microclimate (Paaijmans and Thomas, 2011; Afrane et al., 2006) for survival but also gives the mosquito an opportunity to access blood meals.

Increased presence of household openings, such as windows, eaves and occupancy (Ricci, 2012; Roberts and Matthews, 2016; Bannister-Tyrrell et al., 2017; Mugwagwa et al., 2017), have been associated with increased entry (Lwetoijera et al., 2013; Haque et al., 2013) and buildup of indoor resting mosquitoes that have increased parasite prevalence (Konradsen et al., 2003) and malaria burden (Lwetoijera et al., 2013; Bradley et al., 2013). The elephant in the house in most Kenyan household is the challenge in accessing quality housing (Arvantis, 2013; Mbaka, 2013; Ochieng et al., 2017). This exposes most Kenyans to the mercies of indoor resting or visiting mosquitoes and mitigating against this would require managing the numbers by capturing and killing or simply making the house impenetrable.

Barricading the houses to make it impenetrable to mosquitoes may be a tall order but trapping and killing may be easy to achieve. A number of traps have been designed to this end and used for entomological monitoring (Mburu et al., 2019), retaining (Pombi et al., 2014; Kreppel et al., 2015) and poisoning (Chaiphongpachara et al., 2018) recovered mosquitoes. These traps have not only proven effective in model and efficiency (WHO, 2018) but have also provided surveillance data (Becker et al., 2013), that has helped in the management of mosquito numbers and by extension mosquito-borne diseases (Jöst et al., 2011) and nuisance (Jackson et al., 2012).

Though the traps and improved house quality have done well, synchronous effect of both especially insecticide treated traps has not been evaluated. This study reports on the complementary effect of crude ethanol fruit extracts of *Phytolacca dodecandra* (hereafter Endod) applied in simple mosquito resting boxes with house characteristics (eave, presences or absence and use of various types of nets for protection, number of individuals sleeping under or without nets and number of persons resident in a house) on densities of indoor resting mosquitoes and by extension their impacts on human health (Osório et al., 2014).

1 Results

More Culicine than Anopheline mosquitoes were recovered from the simple resting boxes and though the numbers were small, they were significantly different (p<0.001) regardless of species (Table 1). A higher number of mosquitoes were found in houses without insecticide treated nets. The number however, reduced with increased number of nets and differed significantly (p<0.02) irrespective of mosquito species or net type (Table 2). In all cases regardless of eve, wall type, fumigant (Mortein Doom®) used or otherwise, the density of captured culicine differed significantly (p<0.02) and exceeded those of Anopheline (Table 3). Interestingly, though the densities of captured mosquitoes reduced with increased number of individuals sleeping under nets, the numbers differed significantly (p<0.05), irrespective of species (Table 4). High densities of mosquitoes were recovered from houses with the highest number of individuals sleeping under no net and though the numbers didn't follow any particular trend they differed significantly (p<0.02), mosquito species not withstanding (Table 5).

Table 1 Mosquito groups sampled by resting boxes. Numbers are represented as means and standard error of means (SEM)]

Mosquito type	Mean ± SEM	t	df	*P*	95% Confidence Interval of the Difference	
					Lower	Upper
Anopheline	2.65 ± 0.04[a]	70.051	643	0.000	2.57	2.72
Culicine	4.41 ± 0.03[a]	128.192	643	0.000	4.3421	4.4772

Note: df: Degree of freedom; t: t statistical factor for student t test; *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 difference in the densities of mosquito species sampled by the resting boxes

Table 2 Impact of quantity and net type on density of indoor resting mosquitoes; Effects represented as means and standard error of means (SEM)]

Parameter	Status	Mosquito Type		df	F	P
		Anopheline	Culicine			
Number of mosquito nets within the house	Zero	3.05 ± 0.14[a]	5.03 ± 0.35[a]	1	27.436	0.000
	One	3.08 ± 0.15[a]	4.74 ± 0.29[a]	1	25.554	0.000
	Two	3.01 ± 0.12[a]	4.18 ± 0.31[a]	1	12.219	0.001
	Three	2.83 ± 0.14[a]	3.48 ± 0.21[a]	1	6.669	0.011
Treated insecticide Net (ITN) used	Plain net	2.88 ± 0.14[a]	5.14 ± 0.36[a]	1	35.438	0.000
	Olyset	2.46 ± 0.09[a]	3.75 ± 0.35[a]	1	13.600	0.000
	Permanet	3.38 ± 0.16[a]	4.50 ± 0.35[a]	1	6.247	0.014
	Olyset+Parmanet	3.01 ± 0.12[a]	3.69 ± 0.20[a]	1	8.327	0.005
	Other ITN	2.32 ± 0.09[a]	3.08 ± 0.26[a]	1	7.563	0.007

Note: df: Degree of freedom; F: F statistical factor ANOVA test; *P*: Probability for the level of significance; P was taken as significant at p<0.05; Rows having mean and standard error of mean (SEM) superscripted with the same letter "a" indicate significance difference in the influence of the status of parameters on the densities of mosquito species sampled

Table 3 Impact of house characteristics on the densities of indoor resting mosquitoes. Impact represented as means and standard error of means (SEM)]

Parameter	Status	Mosquito Type		df	F	P
		Anopheline	Culicine			
Eave type	Closed	2.32 ± 0.09[a]	3.08 ± 0.26[a]	1	7.563	0.007
	Open	2.15 ± 0.05[a]	3.73 ± 0.26[a]	1	34.947	0.000
Wall type	Mud	2.16 ± 0.05[a]	3.41 ± 0.23[a]	1	28.099	0.000
	Plastered	2.73 ± 0.17[a]	4.47 ± 0.24[a]	1	35.983	0.000
Fumigant used	None	3.50 ± 0.17[a]	4.28 ± 0.26[a]	1	6.504	0.012
	Doom	2.51 ± 0.10[a]	4.02 ± 0.40[a]	1	13.657	0.000

Note: df: Degree of freedom; F: F statistical factor ANOVA test; *P: P*robability 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 difference in the influence of the status of parameters on the densities of mosquito species sampled

Table 4 Impact of people sleeping under nets on densities of indoor resting mosquitoes. Effects represented as means and standard error of means (SEM)]

Number of individuals sleeping under nets	Mosquito species		df	F	P
	Anopheline	Culicine			
Two	2.94 ± 0.10[a]	4.43 ± 0.26[a]	1	37.236	0.000
Three	2.80 ± 0.13[a]	4.37 ± 0.21[a]	1	32.222	0.000
Four	2.67 ± 0.09[a]	3.68 ± 0.16[a]	1	31.173	0.000
Five	2.64 ± 0.09[a]	3.63 ± 0.19[a]	1	22.211	0.000
Six	2.46 ± 0.09[a]	2.97 ± 0.16[a]	1	7.844	0.006

Note: df: Degree of freedom; F: F statistical factor ANOVA test; *P: P*robability 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 difference in the influence of the status of parameters on the densities of mosquito species sampled

Table 5 Impact of number of residencies on densities of indoor resting mosquitoes; Effects represented as means and standard error of means (SEM)]

Number of residents per experimental house	Mosquito type		df	F	P
	Anopheline	Culicine			
One	2.88 ± 0.12[a]	4.17 ± 0.27[a]	1	26.928	0.000
Two	2.82 ± 0.14[a]	4.16 ± 0.29[a]	1	17.188	0.000
Three	2.81 ± 0.10[a]	4.09 ± 0.32[a]	1	12.711	0.000
Four	2.68 ± 0.11[a]	3.89 ± 0.22[a]	1	19.179	0.000
Five	2.66 ± 0.11[a]	3.88 ± 0.23[a]	1	23.220	0.000
Six	2.55 ± 0.11[a]	3.28 ± 0.20[a]	1	10.264	0.002

Notes: df: Degree of freedom; F: F statistical factor ANOVA test; *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 difference in the influence of the status of parameters on the densities of mosquito species sampled

2 Discussion

In this study, complementary effect of crude ethanol extracts of Endod applied in simple mosquito resting boxes and four major aspects of house characteristics on density of indoor resting mosquitoes were examined: Structure of house wall, presence and use of ITNs, number of individuals sleeping under and without ITNs and number of occupants per house. The results were a recovery of two important species of mosquitoes (Anopheline and Culicine) involved in the transmission of mosquito borne diseases such as malaria (Ndenga et al., 2006; Tchouassi et al., 2012; Dia et al., 2013) and viruses (Ochieng et al., 2013) in Western Kenya (Karungu et al., 2019).

In this study, a high number of culicine compared to Anopheline mosquitoes were found resting indoors. This being a natural setting inhabited by both wild, domesticated as well as human beings, it was natural for the numbers of culicines to surpass anophelines as culicines are not only found in all zoogeographical areas (Bhattacharya et al., 2016) but are also dominant in human settlements (Ciota and Kramer, 2013).

By default, closed eaves should result in fewer indoor resting mosquitoes and if the mosquitoes are to gain entry, then the number will depend on the degree of eave closure. This however, is only true for Anopheles and not culicine (Mburu et al., 2018). In the current study closed eaves were more restrictive to Culicine than Anopheline mosquitoes though the difference was not significant. This was contrary to an observation of houses with fully closed eaves that were observed to have reduced rates of house entry by anopheline mosquitoes compared to houses with fully open eaves (Kirby et al., 2009; Ogoma et al., 2009 Menger et al., 2016; Mburu et al., 2018).

A relatively higher density of indoor resting mosquitoes was found resting in cement plastered walls regardless of species, an observation that was consistent with that made earlier on the influence of house characteristics on mosquito distribution (Mburu et al., 2018; Kaindoa et al., 2018; Lwetoijera et al., 2013). The findings herein, however, was contrary to Ngadjeu et al. (2020) observation that noted that houses constructed with cement walls or walls with mixed materials demonstrated a lower density of mosquitoes than houses with mud or plank (Ngadjeu et al., 2020) walls. Plastered walls as per the observation made here was more attractive to mosquitoes and therefore would be less protective against mosquito bite and mosquito borne diseases contrary to an observation by Tusting et al. (2015) that envisioned increased protection with house improvement.

A high number of Anopheline and Culicine mosquitoes were observed resting indoors despite availability and use of ITNs. This observation was similar to that made by Machani et al. (2020) for *An. gambiae* and *An. funestus* in the presence of long lasting insecticidal nets (LLINs) but contrary to the expectation of Mathenge et al. (2001) and Malima et al. (2008) who believed that using ITNs in houses would have led to reduced rate of entry or increased exit of adult mosquitoes respectively. The logical explanation of this phenomenon would probably be due to the unpredictable use of ITNs by different individuals within households that would result in some people being unprotected (Korenromp et al., 2003; Eisele et al., 2009; Macintyre et al., 2012). These people would not only be fair targets but would enhance the attractiveness of the houses leading to high numbers of visiting mosquitoes despite substantial increase in the number of households owning ITNs (WHO, 2012). This is consistent with an earlier observation that houses in which only some residents used LLINs the previous night had more *An. arabiensis* and *An. gambiae s.s*. than houses in which all residents used LLINs the previous night (Gimnig et al., 2003). This is suggestive that people not sleeping under LLINs, even in houses with LLINs served as baits for the blood seeking mosquitoes. Another explanation is that either the strain of mosquitoes that dared entre house with such lethal provisions were from a breed resistant to pyrethroid insecticide as earlier observed in western Kenya (Ochomo et al., 2014; Wanjala et al., 2015), or their survival of onslaught or repellence was due to mounted behavioural (Siegert et al., 2009) and or physiological (Ranson and Lissenden, 2016) defenses.

In the current study a higher number of mosquitoes were captured in houses with least occupants and where the least number of inhabitants slept under nets. This was contrary to popular observation that as the numbers of individuals increased in a house so did hosts' properties such as odorants, body heat and body mass that would naturally make the houses attractive to the mosquitoes (Lyimo and Ferguson, 2009; Takken and Verhulst, 2013). Naturally mosquitoes are lured into human habitats by odorants such as CO_2 and exuded heat pinpoint the exact position of the individual. It was therefore expected that the presence of increased residents would equal high numbers of mosquitoes in the residential houses (Lwetoijera et al., 2013; Kaindoa et al., 2016). This is because when numbers of residents increase so is the source of blood meal. However, for some reason it's either that the mosquitoes did not find the houses attractive and if they entered, they must have hurriedly exited leaving very few of them behind. If this was the case, then this is beneficial as it meant that Endod plant extracts applied in the boxes did repulse or repel the mosquitoes and made them exit the buildings. This would imply that the otherwise increased risk that would have been enhanced by increased densities of mosquitoes (Harbison et al., 2006; Odiere et al., 2007) would be reverted. In the event that all the factors are actualize and optimally operationalized then it is expected that fewer mosquitoes will be entering (Kirby et al., 2009;Atieliet al., 2009; Kampango et al., 2013) and roaming within the homes resulting in reduce mosquito bites (Atieliet al., 2009; Ogoma et al., 2009; Kirby et al., 2010; von Seidlein et al., 2017) and reduced anaemia among the inhabitants (Kirby et al., 2009).

3 Materials and Methods

3.1 Study site

The current study was conducted in Korando “B” Sub-location in Western Kenya. The site is about 11.4 km from the City of Kisumu along Kisumu-Busia/Mumias road. Populace here are mainly Luo ethnic community who engage in a myriad of economic activities to eke a living.

3.2 Mosquito Resting boxes

Simple resting boxes for the capture and sampling mosquitoes were made using galvanized wire frame measuring 30 cm × 30 cm × 30 cm and designed in a similar way as one described by Crans (1989). Bright blue and black pieces of cloth were sewn out and inside respectively and square pieces of cartons placed between them for reinforcement (Figure 1).

3.3 Endod plant materials acquisition, extraction and preparation

Mature green fruits of Endod plant were sourced, identified and voucher specimen deposited as earlier described (Yugi et al., 2015). The plant parts were shade dried at room temperature, grounded and extracted using ethanol following Das et al. (2010) procedure. Preparation of 80 mg stokes crude ethanol Endod extract was made by dissolving the same in 100 ml of rain water and the resulting solution used to spray the inner fibric of test resting boxes.

3.4 Selection of houses for experimentation

A completely randomized block design (Kothari, 2004) was used to select houses for study. Four blocks of a km^2 apart were identified and homesteads within sampled randomly. Seven houses per block were purposively selected in view of characteristics such as wall, eve and insecticide treated net and fumigant type used within.

3.5 Sampling Indoor resting mosquitoes

A four by seven (4×7) factorial research design (Kothari, 2004) was used to shuffle resting boxes used in trapping and sampling of indoor resting mosquitoes from experimental houses. To each experimental house, a simple resting box baited with a dirty sock with strong human foot scent was placed. The trap was placed on the floor at a corner in no particular fashion (Mboera, 2005). The boxes were left to stay in the houses undisturbed for two days before shuffling. Shuffling was done in four phases. In phase one, boxes from one block was shuffled with those of the other block and in phase two, boxes were shuffled between rooms within the same block. The shuffling was continued until a box had a chance to be in every block and house in the set up respectively.

Trapped mosquitoes were aspirated from the boxes every morning by 6.30 hours. This was done using a battery powered aspirator (procopak) with the aid of torch light for illumination. The recovered mosquitoes were put in plastic mosquito holding cups (Figure 2) and transported to the laboratory for processing. Gillies and Coetzee (1987), identification key was used to identify the mosquitoes to species level.

Figure 1 Assembling a simple mosquito resting boxes (modified after Harbison et al., 2006) for indoor resting mosquito sampling


Bilge blower 4" inline model 240


Plastic holding cups

Figure 2 Aspiration equipment for collecting mosquitoes from resting boxes

3.6 Data analysis

Data was organized in excel and tested for conformation to the assumptions of normality. Descriptive statistics was used to report on the densities of captured mosquitoes. Student T test was used to assess the level of comparative attractiveness of the experimental houses to the indoor resting mosquitoes. One-way analysis of variance (ANOVA) was used to analyze and report on the effects of house characteristics on densities of captured mosquitoes. All statistical analysis was performed using Statistical Package for Social Scientists (SPSS) version 22.

4 Conclusion

In the foregoing discussion, its apparent that house characteristics influence resident mosquito density and by extension burden of mosquito borne infections. It is also apparent that the use of Endod extracts in the treatment of resting boxes impacted densities of indoor residual mosquitoes. A combination of Endod in resting boxes together with improved house characteristics can be used as an alternative tool against mosquito borne diseases.

Acknowledgements

The author thanks Harnnel Owiti and Richard Amito, for culturing all mosquitoes used in this study, Centre for Global Health Research/Kenya Medical Research Institute (CGHR/KEMRI) for mosquitoes, laboratory space and equipment. National Commission for Science, Technology and Innovation (NACOSTI), Kenya is also thanked for funding the project.

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