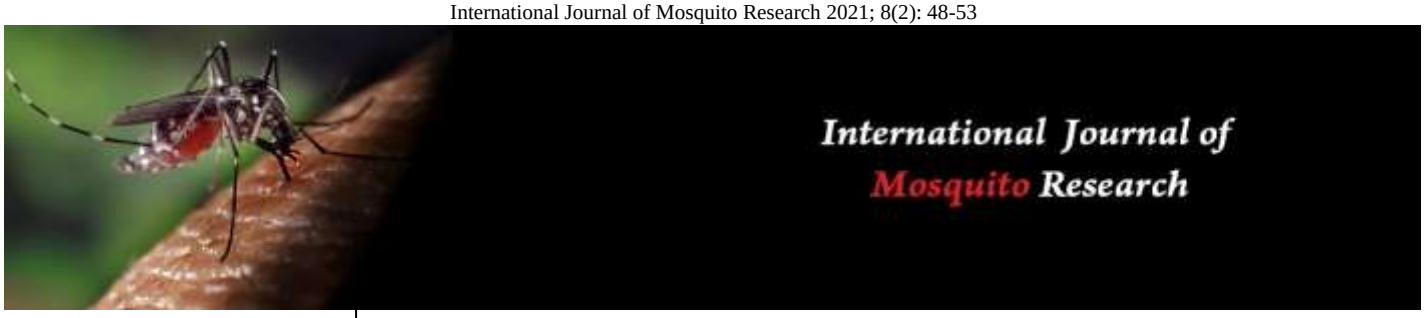

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Ecologically sustainable mosquito repellence using live *Artemisia annua* Linnaeus plants in boarding schools of MWEA rice irrigation scheme, Kenya

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Abstract
Mosquitoes continue to transmit malaria despite the use of several methods of control such as treated bed nets, aerosols and repellent creams. Mosquito biting cause stress and medical problems to school going children. An experimental ecological study was undertaken to evaluate the effectiveness of two months old potted *Artemisia annua* plants for mosquito repellence in boarding schools' dormitories of Mwea Rice Irrigation Scheme, Kenya. Two months old potted live *A.annua* plants were hung next to doors and windows of dormitories and mosquito collection for four months as per WHO protocol was done. The study found significant differences in the mean mosquito catches in treatment dormitories in both girls and boys boarding schools p-values of 0.005, 0.001, 0.001, and 0.001 for the 2nd, 3rd, 4th and 5th months respectively. The study concluded that live *A.annua* reduced mosquito density in dormitories of boarding schools. Further there was reduction of mosquito density in neighbouring dormitories, as far as 50metres where *A. annua* was not introduced. The study recommends that *A.annua* would be very important for implementing vector control strategies to prevent man-vector contact for the prevention of vector borne diseases.
The study recommends use of *A.annua* as vector control strategy to prevent man-vector contact for the prevention of vector borne diseases.

Keywords: *Artemisia annua,* Anophelines, repellency, boarding schools

1. Introduction
Mosquitoes have become a major public health concern due to the blood feeding females of different species transmitting malaria causing protozoa, elephantiasis causing filarial worms, and dengue fever, yellow fever, Rift valley fever, West Nile fever causing viruses [1]. Mosquitoes continue to transmit malaria despite the use of several methods of control such as treated bed nets, aerosols and repellent creams [2]. Continuous mosquito bites have health hazard ranging from loss of sleep, severe stress to serious infections [3]. *Anopheline* mosquitoes transmit malaria, a disease that is the main cause of school absenteeism, high dropout rates, child labour [4], affecting academic performance [5], class repetition [6], and even death of school age children [7].
Transmission of disease can be interrupted by controlling the vector using various methods including botanical phytochemicals with mosquitocidal potential and repellent activities [8]. Various products with plant based insecticidal and repellent properties are widely in use in control of mosquitoes and protection from mosquito bites [9]. Stored product insects, *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (Linnaeus) have been shown to be repelled and inhibited by *A. annua* L. essential oil [10]; and also having a fumigant effect against *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae) [11]. Essential oil extract from the leaves of *A. annua* have been also demonstrated to have repulsive activities against *Anopheles gambiae* [12]. Repellence activities against *Anopheles gambiae s.s.* have been demonstrated by essential oil extracts of *Suregada zanzibariensis* leaves [13].
Tagetes lucida, Lippia alba, Lippia origanoides, Eucalyptus citriodora, Cymbopogon citratus, Cymbopogon flexuosus, Citrus sinensis, Swinglea glutinosa and *Cananga odorata* essential

oils and of a mixture of *L. alba* and *L. origanoides* essential oils have shown repellence to *Aedes* (*Stegomyia*) *aegypti* Rockefeller larvae [14]. *Ervatamia coronaria* and *Caesalpinia pulcherrima* essential oils have also demonstrated repulsive activities against mosquito larvae [15]. Repulsive efficacy of LC50<10 ppm essential oils from *Blumea densiflora, Auxemma glazioviana, Callitris glaucophylla, Cinnamomum microphyllum, Cinnamomum mollissimum, Cinnamomum rhyncophyllum, Zanthoxylum oxyphyllum* have been considered significant efficient [16]. A significant higher repulsive activity against human vector mosquitoes *Anopheles stephensi, Aedes aegypti* and *Culex tritaeniorhynchus* has been shown by *Gnetum ula and Spermacoce hispida* plants essential oils [17].

Ethiopian ethno botanical survey on insect repellent plants usage have demonstrated majority of people use plants as repellents to reduce or interrupt the biting activity of insects [18]. *Ocimum suave* and *Ocimum kilimandscharicum* when used as whole plants as well as insect repellents in village communities in north-eastern Tanzania have been beneficial in reducing vector biting [19]. Quantification of potted plants repellency against *Anopheles gambiae sensu stricto* Giles in experimental huts under semi-field conditions have demonstrated reduction in domestic exposure to malaria vector mosquitoes. This represent a new sustainable and readily applicable malaria vector control tool for incorporation into integrated vector management programs [20]. Repulsion demonstration of live potted plants and thermal expulsion of plant materials against African malaria vectors; *Anopheles gambiae sensu lato* Giles (Diptera: Culicidae), *An. arabiensis* Patton and *An. gambiae sensu stricto* Giles), in traditional houses in western Kenya showed that both methods of application could be effective alternatives to protect houses and complement to bed nets [21]. When live *Lantana camara* was planted as a hedge around houses significant reduction in mosquitoes *A. gambiae* s.s. and *A. funestus* s.s., was reported [22]. This study investigated whether potted live *A. annua* L. plants hung on the windows and doors of the dormitories would reduce mosquito densities and hence reduced mosquito bites.

2. Materials and Methods

2.1 Study area

Mwea Rice Irrigation Scheme (MRIS) (Longitudes 37° 17 and 37° 26 East and Latitudes 0° 37 and 0°. 45 South), a scheme in Kirinyaga County, Kenya with a population of about 221,343 and 45 secondary schools [23], was selected for this study (Figure 1). The Mwea Rice Irrigation Scheme situated in Mwea Sub-County of Kirinyaga covers 13,640 hectares and is 100 km north east of Nairobi. The study area is situated almost exactly at the point of transition between the well watered, high potential upland zone and the surrounding belt of drier marginal lands, where some shortage of (rain) water is experienced almost every year. Water related economic activities in MRIS, have intensified mosquito borne malaria problem due to increased number of larval habitats and extension of transmission season duration [24]. Major malaria vectors; *Anopheles arabiensis, An. funestus, An. sensu stricto* have been recorded in this study area [25]. Recent studies in Mwea have reported susceptibility of *An. gambiae* to the conventional insecticides, especially permethrin and Deltamethrin [26]. Therefore attempts were undertaken to understand the effect of potted live *A. annua* to the vector mosquito and the present communication reports the results of potted live *A. annua* on mosquito in the study area.

A baseline survey on mosquito density for a month before introduction of potted live *A. annua* plants followed by four months post introduction of potted live *A. annua* was done. Two months old potted live *A. annua* plants from Kenyatta University Medicinal Plants Research Gardens were hung next to the doors and windows of dormitories, and mosquito collection for four months as per WHO [27] protocol was done. Potted live *A. annua* was suspended next to the doors and windows of dormitories in one boys' and one girls' boarding school to act as treatment dormitories while another one boys' and one girls' dormitories in the same school had plastic containers filled with soil only suspended next to doors and windows to act as controls. The treatment and control dormitories in the two schools were 50m afar.

Collections of indoor–resting mosquitoes in the baseline treatment and control dormitories and; post introduction treatment and control dormitories were conducted biweekly in the study area in the mornings between 8.30 am and 10.30am. Baseline mosquito collection was done for only month while post potted live *A. annua* introduction mosquito collection was done for four months. The collection of indoor–resting mosquitoes was done using the sucking tube method [27].

The investigators sought authority to conduct the study from Kenyatta University Graduate School and approval from Kenyatta University Ethics Review Committee. Research permit was obtained from the National Commission for Science, Technology and Innovation. At the county level, the County Commissioner and the County Medical Health Officer for Kirinyaga County as well as the County Director of Education were consulted and they gave their written permission before the research started. Signed consent was sought from the schools administrators after explaining and describing to them pertinent information about the study.

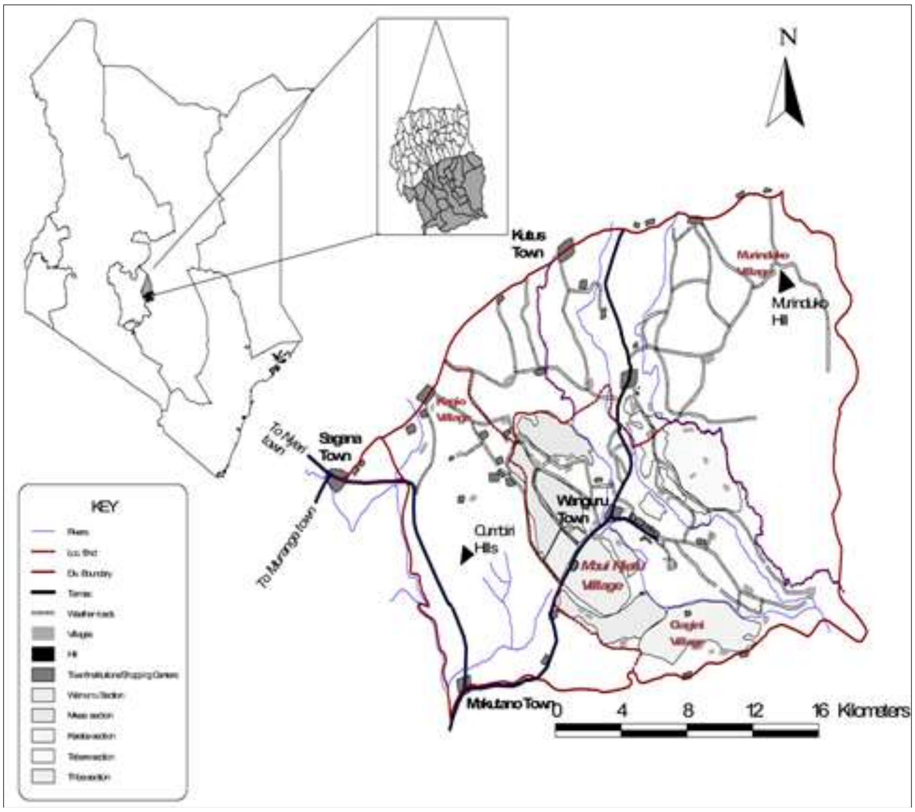

Fig 1: Map of Kenya showing Mwea Rice Irrigation Scheme, Kirinyaga County

2.2 Sampling procedure: Purposive and simple random sampling was used for this study. Mwea Rice Irrigation Scheme was purposively selected. The two boarding secondary schools in MRIS were selected using simple random sampling. Data was collected within five months from February to June 2014.

2.3 Data analysis: Data was entered; cleaned, analyzed and stored using SPSS. Data was analyzed using descriptive statistics to obtain means. Student unpaired t test was used to test for the difference between the means of the variables.

3. Results and Discussion

The baseline mean mosquito catches in the boys dormitories were 39±2.8 and 43±2.0 for the control and treatment dormitories respectively. While baseline mean mosquito catches in the girls dormitories were 48±2.2 and 48±1.9 for the control and treatment dormitories respectively (Tables 1and 2). The study established that there was decreased mosquito density in the 2nd, 3rd, 4th and 5th months respectively when potted live *A. annua* plants were hung next to the windows and doors of the dormitories, compared to the baseline in 1st month when the *A. annua* plant was not hung next to the windows and doors of the dormitories. In the boys control dromitory mean mosquito catches were 39±2.8, 29±2.5, 32±2.8, 39±2.9 and 44±1.1 for the 1st, 2nd, 3rd, 4th and 5th months respectively, while the treatment dormitory mean mosquito catches were 43±2.0, 14±1.3, 15±1.1, 19±1.8 and 21±1.1for the 1st, 2nd, 3rd, 4th and 5th months respectively. In the girls control dormitory mean mosquito catches were 48±2.2, 36±3.9, 38.25±2.6, 38±2.6 and 36±2.00 for the 1st, 2nd, 3rd, 4th and 5th months respectively, while the treatment dormitory mean mosquito catches were 48±1.9, 20±3.3, 15±2.6, 20±1.5 and 18±1.5 for the 1st, 2nd, 3rd, 4th and 5th months respectively (Tables 1and 2).

Before *A. annua* introduction the difference in the mean mosquito catches in both the boys and girls control and treatment dormitories was insignificant (boys dormitory with *p*-value (0.268), while girls dormitory had *p*–value (0.776). After *A. anua* introduction the differences in the mean mosquito catches in both the control and treatment dormitories in boys boarding school were significant with *p*-values 0.001, 0.001, 0.001, and 0.001 for the 2nd, 3rd, 4th and 5th month respectively, while the difference in mean mosquito catches in both the control and treatment dormitories in girls boarding school were significant with *p*-values 0.005, 0.001, 0.001, and 0.001 for the 2nd, 3rd, 4th and 5th month respectively (Tables 1and 2).

Table 1: Biweekly mosquito catches before and after introduction of *Artemisia annua* L. in boys' control and treatment dormitories

Biweekly Boys' Boarding School Mosquito catches											
		Before *A. annua*		**After *Artemisia annua* introduction**							
Dormitory		**1st Month**		**2nd Month**		**3rd Month**		**4th Month**		**5th Month**	
		Dorm C	**Dorm T**	**Dorm C**	**Dorm T**	**Dorm C**	**Dorm T**	**Dorm C**	**Dorm T**	**Dorm C**	**Dorm T**
Week 1	Day 1	30	44	24	15	24	15	40	24	48	22
	Day 2	28	52	21	12	22	18	45	20	43	20
Week 2	Day 1	45	35	21	18	28	13	33	12	40	24
	Day 2	52	36	28	10	32	10	22	16	46	26

Week 3	Day 1	35	48	30	10	38	16	30	12	44	22
	Day 2	36	40	38	16	27	14	39	22	40	18
Week 4	Day 1	39	43	27	14	39	20	46	18	48	20
	Day 2	43	42	39	20	44	18	40	26	45	16
M ± SE		39±2.8	43±2.0	29 ±2.5	14±1.3	32±2.8	16±1.1	39±2.9	19±1.8	44±1.1	21±1.1
		p = 0.776		p = 0.005		p = 0.001		p = 0.001		p = 0.001	

Dorm C- Control dormitory, Dorm T – Treatment dormitory

Table 2: Biweekly mosquito catches before and after introduction of *Artemisia annua* L. in a girls' control and treatment dormitory

Biweekly Girls' Boarding School Mosquito catches											
		Before *A. annua*		**After *Artemisia annua* introduction**							
Dormitory		**1st Month**		**2nd Month**		**3rd Month**		**4th Month**		**5th Month**	
		Dorm C	**Dorm T**	**Dorm C**	**Dorm T**	**Dorm C**	**Dorm T**	**Dorm C**	**Dorm T**	**Dorm C**	**Dorm T**
Week 1	Day 1	35	35	24	12	31	13	35	18	38	24
	Day 2	43	31	23	10	51	26	30	19	30	16
Week 2	Day 1	51	52	37	10	30	15	46	21	35	14
	Day 2	53	50	45	26	31	8	35	28	30	17
Week 3	Day 1	52	49	45	30	38	10	30	18	40	22
	Day 2	50	51	36	32	40	11	37	15	30	13
Week 4	Day 1	45	49	54	25	45	20	40	25	40	24
	Day 2	52	47	29	14	40	22	50	20	45	15
M ± SE		48±2.2	48±1.9	36 ±3.9	20±3.3	38±2.6	16±2.6	39±2.5	20±1.5	36±2.0	18±1.5
		p = 0.268		p = 0.001		p = 0.001		p = 0.001		p = 0.001	

Dorm C- Control dormitory, Dorm T – Treatment dormitory

The study found there was decreased mosquito density in the months of March to June when potted live *A. annua* plants were hung on the windows and doors of the dormitories However, the density of the mosquito was higher in the baseline survey (in the month of February when the plant was not hung). Other studies done on other plants have shown similar findings. Screening of mosquito house entry points by planting tall densely foliated repellant plant *L. camara* L. around houses in Kagera, Tanzania demonstrated reduction in mosquito densities [22]. Mosquitos being sensitive to environmental change; reduction in their densities may be due to the chemicals produced by the plants in the surrounding environment affecting their survival, density and distribution dramatically [28]. The mosquitos' environmental conditions would be affected by plant chemicals, for example growth regulators, toxins, repellents and feeding deterrents hence reducing their population density [29]. This study has demonstrated that a potted live intact *A. annua* plant can repel mosquitoes to reduce human –vector contact in a field trial experiment. The results of this study demonstrate *A. annua* has effect on mosquito population density when planted as a whole plant ($p < 0.05$). Similar studies on the plants *L. camara, Ocimum americanum* and *Lippia uckambensis* reported mosquito repulsion when used as hedges around huts in numerous villages in L. Victoria because of their citronella-like scent [20]. *Ocimum basilicum* (*Lamiaceae*) branches have been used traditionally to keep mosquitoes away in East African houses.

In recent years, studies on oil extracts from the plant families *Lamiaceae, Nepeta cataria, Ocimum basilicum* and *O. americanum* have shown to provide 2-8 hours protection against mosquito species [30]. The plant *Rosmarinus officinalis* has been demonstrated to be repulsive against Anopheles, *Aedes, and Culex* mosquito species [30]. Excellent repulsion against *Aedes albopictus*, *Anopheles dirus* and *Culex quinquefasciatus* by *Vitex trifolia* plant with very short interval against *Aedes aegypti* [30]. The plant family of *E. citriodora*. *Eucalyptus, Melaleuca leucadendron, Melaleuca quinquenervia* and *Psidium guajava* have shown excellent repulsion against *Culex* mosquitoes with modest protection against *Anopheles* and *Aedes* [31]. Field studies have also shown plant repellent effects on mosquitoes by several plants; *Corymbia citriodora, Eucalyptus spp, E. camaldulensis, L. uckambensis, Lantana camara, Ocimum spp. O. americanum, O. basilicum, Hyptis spp. Hyptis suaveolens, Mentha spp. M. piperata, C. citratus* [32].

4. Conclusion

Artemisia annua L. significantly reduced the mosquito population density in the dormitories where it was suspended on the windows and doors of the dormitories

Competing interests

The authors declare no competing interest.

Authors' contributions

This study was done from paper conception to publication by Mbulo Thomas Mutua for the requirement of Master of Public Health in Epidemiology and Disease Control at Kenyatta University with Ephantus Wanjohi Kabiru and Nicholas Kamindu Gikonyo as University supervisors. We have read and agreed to the last form of this manuscript.

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