JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Efficient attractants and simple odor-baited sticky trap for surveillance of *Anopheles arabiensis* Patton mosquito in Ethiopia

Dawit Hawaria[1], Dante R Santiago[2], Delnesaw Yewhalaw[3]

[1] *Department of Social and Population Health, Yirgalem Medical Science College, Yirgalem, Ethiopia*
[2] *Department of Environmental Health Science and Technology, Jimma University, Jimma, Ethiopia*
[3] *Department of biology, Jimma University, Jimma, Ethiopia*

Abstract

Introduction: Many efforts have been made to ease the burden of malaria through vector control, among whichis the development of odor-baited traps and evaluation of efficient attractants that could replace host odor. However, most traps and evaluated attractants are expensive, which poor communities cannot afford. This study was conductedwith the aim to devise asimple and affordable odor-baited trap and to investigate effective but affordable attractants for trapping *Anopheles arabiensis*.

Methodology: First, an odor-baited sticky trap was developed; next, an experimental study with randomized design was conducted to evaluate the efficacy of selected attractants for trapping *Anopheles arabiensis* using the designed trap from June to August 2014. Laboratory strain *Anopheles arabiensis* were obtainedfrom the Adama Malaria Research and Training Center Insectary. Wild *Anopheline* mosquito larvae were collected from a temporary breeding site, reared in Asendabo Vector Biology Laboratory, and tested.

Results: A simple odor-baited sticky trap was designed. Selected attractants were tested for attracting efficiency using the designed trap. Among the evaluated attractants, cow urine, which was kept for four days, attracted significantly more wild population and laboratory strains of the *Anopheles arabiensis*than a worn sock alone and the combination of cow urine and a worn sock.

Conclusions: Although further comparison studies with other standard traps are needed, the designed trap in conjunction with efficient attractant is shown to be effective for mosquito surveillance. Of the tested attractants, cow urine was anefficient attractant both for the wild population and the laboratory strain of *Anohpeles arabiensis*.

Key words: trap; attractant.

J Infect Dev Ctries 2016; 10(1):082-089. doi:10.3855/jidc.6841

(Received 05 March 2015 – Accepted 17 September 2015)

Introduction

Many efforts have been made to control the burden of malaria; however, it still remains among the major causes of illness and death in the world. Globally, an estimated 3.3 billion people are at risk of being infected and developing disease, and 198 million cases occurred in 2013. The burden is heaviest in sub-Saharan African, including Ethiopia [1]. According to Ethiopia's Federal Ministry of Health, around 75% of the country is malarious, and malaria is ranked as the leading communicable disease.*Anopheles arabiensis*is the primary malaria vector in Ethiopia, with secondary vectors of *Anopheles funestus*, *Anopheles pharoensis*, and *Anopheles nili* [2,3].

Currently available broadly applied interventions of malaria vector control are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [3]. In Ethiopia, the control of malaria relies on vector control by IRS and large-scale distribution of LLINs [4]. However, malaria vectors, including *Anopheles arabiensis*,have been developing resistance to insecticides in many areas, including Ethiopia [2,3,5-7].

An alternative to insecticides is the use of odor-baited traps to lure mosquitos.Smallegange and co-workers found that several components of human breath, sweat, and urine, when combined, are attractive to *A.gambiaes* [8]. Building on such experimental results, Okumu *et al*. developed a synthetic odor blend that is more efficient in attracting *A.gambiaes* mosquitoes, which lured roughlythree to five times more mosquitoes than the natural human odor [9]. The odor blend showed the potential to raise trap collections. Therefore, this can provide better surveillance and possible vector control [10,11]. But this odor blend consists of expensive chemicals, mainly carboxylic acids, which are not cost-effective for poor countries like Ethiopia.

Several traps that are applied for surveillance and control of mosquito populations are available commercially. However, they are expensive and require power and skill to operate [10,12]. This could prevent the national malaria control program and community from using them. There have been attempts, such as that of Kwekawhich, to develop cheap traps; however, these traps are less advanced, have a short life span, andare aesthetically unsound [13]. The need, therefore, is to come up with a cheaper but efficient attractant and cheap, locally available sticky traps for mosquitoes.

Thus, the purpose of this study was to develop odor-baited sticky trap for luring and trapping *Anopheles arabiensis*mosquitoes using selected inexpensive attractants and to evaluate the efficacy of these attractants and their combination.

Methodology

Study area

The study was conducted both in laboratory and semi-field conditions. The laboratory study was conducted at the Adama Malaria Training and Research Center from June to July 2014. A semi-field test was conducted at the Asendabo Vector Biology Laboratory compound under a roof and insurrounding temperature from July to August 2014. Asendabo is a district in Jimma zone in the southwestern part of the country, which is 300 km south west of the capital city, Addis Ababa, and 55 km from the town of Jimma.

Study design

After developing an odor-baited sticky trap, an experimental study with randomized design was followed to evaluate the efficacy of selected attractants for trapping *Anopheles arabiensis* adults.

Mosquito rearing

The laboratory strain *Anopheles arabiensis* adults were obtained from the Adama Malaria Research and Training Center Insectary. The strains had originally been obtained from a field population at Arba Minch, in the southern part of the country.The larvae were fed on yeast and maintained at a room temperature of 26°C–28°C in relative humidity of 60%–80%. Adult mosquitoes were kept inside mosquito cages of 50 cm × 50 cm × 50 cm in a separated room with the same temperature and relative humidity as the larvae's room. The adults were fed 10% glucose solution delivered through cotton that wasput on the top of cages. The insectarywas set to a photoperiod of 12 hours darkness and 12 hours light.

For the test of the wild mosquitoes, wild populations of *Anopheles arabiensis* were reared insurrounding temperature at the Asendabo Vector Biology Laboratory to adult stage. Larvae were collected from a temporary breeding site using a dipper. Collected larvae were kept in mosquito rearing trays supplied with yeast until pupation. Then, pupae were collected in beakers and placed inside small cages for adult emergence. Adult mosquitoes were kept in cages for at least three days and provided with 10% sugar solution with cotton as a food source. The test was carried out using adult mosquitoes three to five days of age.

Selected attractants

The chemical attractants used were L-lactic acid (Anhui B&G Lactic Acid Co., Ltd., Tianjin, China), ammonia (Lingu Hengchang chemical Co., Ltd, China), carbon dioxide, acetone (Dhruvika chemicals Trading Pvt. Ltd, Mumbai, Maharashtra,India), and butanol (Jiangus, China). Worn socks and cow urine were also tested as attractants. The basic blend consisted of L-lactic acid (88%), aqueous ammonia solution (2.5%), and carbon dioxide (220 mL/min). The CO_2 gas was generated by adding 35 g of yeast (Aruba brands produced in China) to 250 g of sugar in 2.5 liters of tap water in a five-liter plastic container of the trap and incubating for two hours [14]. Acetone was used at 38 mL/L and butanol at 1ug/L. The complex attractants were cow urine and human sweat collected by worn socks. Cow urine was collected from a female cow using a washbasin. Socks from 25- and 31-year-old males worn for 10–12 hours were used. From plant attractants, fresh ripe guava fruits were tested.

Mosquito trap design description

A trap was designed using that of Facchinelli *et al*. as a model [15]. The trap designed for this study consisted of twoblue five-literbuckets; the top bucket fitted upside down over the bottom bracket (Figure 1). The cover of the top bucket, with a six-centimeter hole covered with screen wire, was inverted over the bottom bucket and served as a barrier separating the upper and lower compartments of the trap. The yeast-sugar solution mixture was held back in the bottom bucket and the CO_2 was emitted through the screen-wire-covered hole of barrier. The cotton cloth strips soaked in attractants were arranged on the barrier. A panel, consisting of two intersecting plastic plates forming four small walls, sat in the middle of the barrier. Transparent acetate plastic sheets coated with castor oil were attached to the panel with paper clips, one sheet to each wall. The oil-coated plastic sheets served as the sticky factor that caught incoming mosquitoes. The top bucket had four rectangular holes that allowed attractant plumes to escape and allowed attracted mosquitoes fly in.

Cotton cloth strips preparation and attractant application

All chemicals used, including cow urine, were separately applied on strips of cotton cloth. Cotton strips were prepared following the method of Okumu*et al*., who found the strips to be a suitable method of dispensing different attractants into mosquito traps [16]. The textile composition was 88% cotton. The cloth was cut into narrow strips, each measuring 20 cm long and 2 cm wide. The terminal of each strip was cut in a unique manner, to identify the attractant applied to it. After attractants were prepared in separate beakers, the cotton cloth strips were separately soaked in solutions of the attractants. Finally, attractants in the strips were dispensed.

Experimental set-up and evaluation procedures

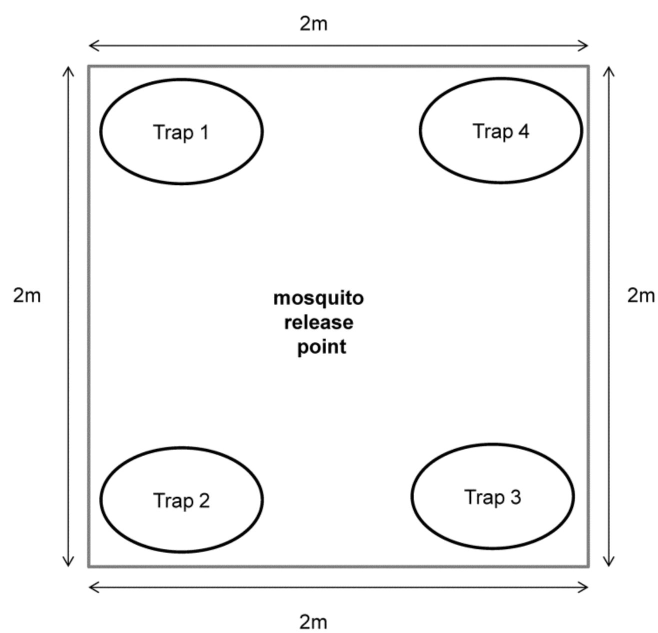
The selected attractants were tested for efficacy under the laboratory and semi-field conditions, patterned after the methods of Okumu*et al*. [9] and Verhulst*et al*. [17]. Untreated mosquito bed nets (2m × 2m × 2m) were employed as field cages. For the laboratory test, the nets were set in the room. The nets were located fivemeters far apart to prevent the attractants in one cage influencing the effects of others. Only one set of treatments was tested in each cage at a time. The treatments in one set were: (1) carbon dioxide only as a positive control; (2) basic blends; (3) basic blend plus the selected attractant; and (4) one selected attractant plus carbon dioxide. For the treatment sets witha combination of worn socks and cow urine, clean air(trap with no attractants inside)was used as a negative control. The treatments were separately kept in traps, *i.e.*, each trap was baited with one treatment. The traps were arranged at each corner of the net as it is shown inthe schematic diagrambelow.

For each test, 100 female mosquitoes three to five days of agethat had been held with males to allow mating but had not received a blood meal were used. They were put in small paper cups, covered with mosquito nettings, and provided 10% sugar solution with cotton.The mosquitoes were released from the center of the net at 18:00 hours. At 6:30 hours the following morning, the traps were retrieved and the mosquitoes within each trap were counted and recorded. Every afternoon, mosquitoes remaining in the cage were captured using a mouth aspirator. Each trial was run with new batches of mosquitoes. The

Figure 1. Odor-baited sticky trap developed and tested in this study.

A: sticky trap assembled as operative; **B:** top part of trap with removable quadrants/panels where adhesive plastic sheet attached; **C:** adhesive plastic sheet with trapped mosquito; **D:** disassembled trap

Figure 2 . Diagrammatic representation of trap arrangement under the net



place of the treatment in the cage was interchanged following every test to minimize potential bias due to location. Each treatment was replicated three times. Surgical gloves were worn by the investigator to avoid contamination of the traps with human volatiles.

The treatment set result with zero values was square root transformed to normalize the distribution and then subjected to analysis of variance. The least significant difference *post hoc* test was used to see the differences between treatments means, and p value< 0.05 was considered as significant during analysis.

Results

Response of laboratory strains of Anopheles arabiensis to various attractant blends

The effects of attractants on the efficacy of the basic blend were evaluated using the designed baited sticky trap against a laboratory population of *Anopheles arabiensis*. Human sweat absorbed in worn socks did not increase the efficacy of the basic blend since both treatments had similar proportions of trapped mosquitoes. Worn socks combined with CO_2 did not differ from CO_2 alone in the efficacy of attracting mosquitoes. These treatments were equally attractive to the mosquitoes when used as odor bait in the sticky trap. In contrast, cow urine increased the attracting efficacy of the basic blend (p = 0.031). However, the combination of cow urine with CO_2 did not result in a better attracting efficacy than CO_2 alone. Fresh guava fruit decreased the attractant properties of the basic blend and CO_2 (Table 1).

Of the two chemicals tested, acetone depressed the efficacy of the basic blend and it did not increase the attracting efficacy of CO_2 (Table 1D). Indeed, the basic blend had a significantly higher percentage of attracting mosquitoes than when acetone was added to it (p = 0.023). Acetone, therefore, hadan antagonistic effect on the basic blend. Butanol, the second chemical tested, had no effect on both basic blend and CO_2, as seen in the similar attracting efficacy of the treatments.

It was noted that when one of the treatments wasmore attractive, the efficacy of CO_2 alone (as the control) was low (Table 1D). If the other treatments had low to moderate attracting potential, then CO_2 mosquito traps were on par with the other treatments.

Since cow urine and worn socks (containing human sweat) are easily obtainable, they were further tested for attracting ability in combination with CO_2. The efficacies of both complex chemicals were not increased by the presence of CO_2 (Table 2).

Table 1. Percentage of *Anopheles arabiensis* laboratory strain caught by sticky traps baited with attractants and their blends

Treatment	Mean ± SE	F value	P value
(A)			
CO_2	8.3 ± 1.20	0.31	0.818
BB	7 ± 2.08		
Worn socks + BB	9.7 ± 2.84		
Worn socks + CO_2	10 ± 3.21		
(B)			
CO_2	5.7a ± 1.76	2.937	0.031
BB	5.3a ± 1.33		
Cow urine + BB	11b ± 1.73		
Cow urine + CO_2	6.7ab ± 1.20		
(C)			
CO_2	12 ± 4.16	1.57	0.271
BB	10 ± 3.05		
BB + Guava fruit	4.7 ± 0.66		
Guava fruit + CO_2	6.7 ± 0.66		
(D)			
CO_2	2a ± 0.57	5.59	0.023
BB	6.7b ± 0.88		
BB + acetone	3a ± 1.15		
Acetone + CO_2	3.7ab ± 0.66		
(E)			
CO_2	6 ± 3.05	0.19	0.900
BB	5.3 ± 1.76		
BB+ Butanol	4.7 ± 1.76		
Butanol + CO_2	4 ± 0.00		

Means followed by the same letter(s) are not significantly different at p < 0.05 using least significant difference; BB: basic blend; SE: standard error.

Comparative response of wild and laboratory strains of Anopheles arabiensis to mixed cow urine and human sweat

Cow urine and worn socks in combination were tested for their attraction to the wild as well as laboratory strains using clean air (empty trap) as a negative control. Cow urine alone attracted significantly more wild population ($p = 0.018$) and laboratory strain ($p = 0.019$) mosquitoes than did worn socks alone and the combination of cow urine and worn socks. In both tests, the attracting efficacyof the cow urine-worn sock combination (6.3% and 4%, respectively) was less than half of that of cow urine alone (19% and 13.3%, respectively). These results indicate antagonism between the two complex chemicals (Table 3).

Discussion

Adult mosquito collectionis one of tactics for vector control, surveillance,and population size reduction [18]. In the study of vector-borne disease epidemiology, the density of blood-host-seeking females is the most relevant information. The current method used to gather data aboutthis is the human landing mosquito collection technique, which is unethical. The alternate method is the use of traps baited with light or attractants and killing agents such as insecticides. However, these traps are expensive and require electricity (from batteries) to work. One Centers for Disease Control and Prevention (CDC) miniature 6 VDC light trap,excluding shipping cost and batteries,costs around US$106. The new standard miniature 6 VDC light trap with photocell-controlled CO_2 release costs around US$ 298.

Efforts have been made to develop attractant-baited traps which do not require power to work, such as the ovitrap [13]. This trap, however, is crude, does not last long, and is aesthetically unappealing. This study was conducted to devise an odor-baited sticky trap using cheap materials that can be easily used by any individual in Ethiopia.

The sticky trap used in this study is cheap, costing less than US$3 (around 50 Ethiopian Birr), and is portable. These characteristics will allow easy usage as a complementary tool in the control of mosquitoes.Though the designed trap follows the trap design of Facchinelli *et al*. as a model [15], modifications have been made to make it more reliable to use. The trap developed by Facchinelli *et al*. is a sticky oviposition trap, whereas the trap here designed

Table 2. Percentage of *Anopheles arabiensis* laboratory strain caught by sticky traps baited with cow urine, CO_2, and worn socks

Treatment	Mean ± SE	F value	P value
(A)			
Cow urine	10 ± 3.51	0.12	0.889
Cow urine + CO_2	9.3 ± 1.76		
CO_2	8.3 ± 1.45		
(B)			
Worn sock	5.3 ± 0.66	1.53	0.291
Worn sock + CO_2	9.3 ± 2.72		
CO_2	14.7 ± 5.92		

SE: standard error.

Table 3. Percentage of *Anopheles arabiensis* wild population and laboratory strains caught by sticky traps baited with cow urine, worn socks, and their combination

Treatment	Mean ± SE	F value	P value
Wild strain			
Worn sock	14.3ab ± 0.69	6.18	0.018
Cow urine	19b ± 0.32		
WS + CU	6.3ab ± 0.51		
Clean air	2a ± 0.60		
Laboratory strain			
Worn sock	7ab ± 0.57	6	0.019
Cow urine	13.3b ± 3.38		
WS + CU	4a ± 0.57		
Clean air	3a ± 1.52		

Data for the wild strain were transformed using the square root of x + 0.5 due to the presence of zero values. Means followed by the same letter(s) are not significantly different at $p < 0.05$ using least significant difference; WS: worn sock; SE: standard error; CU: cow urine is a sticky odor-baited trap. Thus, it is designed in a manner that is suitable to contain attractants. Available odor-baited entry traps require accessories, including a CO_2 tank and a power source [10,14,19,20], whereas in the new designed trap, the bottom compartment for the generation source of CO_2 is an integral part of the trap. The bottom can contain cow urine that can serve as an attractant in place of a sugar solution and yeast mixture (Figure 1).

The comparative study of the odor-baited trap and the standard CDC light trap revealed that the odor-baited trap performed favorably for outdoor trapping [11]. This study was conducted to realize this fact with a simple, cheap, buteffective trap. As shown in the treatment set with the best result (Table 3), the new trap showedpoorer performance (42%) compared with the CDC light trap,which is above 70% [14], but approachedthe performance of the baited MM-X trap, which is around 50% [20]. This doesnot indicate that the maximum performance was achieved. Hence, more efforts have to be madeto decide the overall performance status by conducting a size optimization and a comparison study with standard traps.

After designing the trap, the next steptaken to make the trap workable wastesting attractant blends. This study is behavioral, and attractants were supplied for the choice of the odor *Anopheles arabiensis* like most.Several attractant blends were tested in conjunction with the designed sticky trap for use against adult mosquitoes. The attracting efficacy of acetone, butanol, guava fruit, cow urine, and worn socks and their combinations with the basic blend were evaluated. The addition of a worn sock to basic blend did not significantly increasethe attracting efficacy of basic blend. Similarly, the numbers of mosquitoes caught by worn sock plus CO_2 were not significantly different from the control. In another test conducted to evaluate attracting efficacy of worn socks and CO_2, the combination of a worn sock and CO_2 caught a greater number of mosquitoes than dida worn sock alone.This finding agrees with that of a previous study where thecombination of yeast-produced CO_2 and worn socks caught significantly more mosquitoes than did worn socks alone [14,20]. CO_2 alone attracted 2.8 times more mosquitoes than did the combination of worn socks and CO_2, a finding that opposes that of Njiru, who found that a combination of foot odor and CO_2 increased catches of CO_2 alone by 2.7 times [20]. Another study revealed that traps baited with a blend of NH_3, foot odor, and carbon dioxide collected 91% of released female mosquitoes [21]. Another similar studyshowed that yeast-produced CO_2 caught a significantly higher number of *Anopheles gambiae* mosquitoes than did industrial CO_2, both in the laboratory and semi-field conditions [14].

Preliminary analyses of headspaces of yeast-sugar solutions revealed that two hours post mixing, yeast produced volatile organic compounds such as ethanol, 2-methylpropanal, 2-methyl-1-propanol, 3-methylbutanal, 3-methyl-1-butanol, and others.These compounds were previously found in human emanations, which may therefore play a role in the host-seeking behavior of *Anopheles arabiensis* [14]. This could be the possible reason for the noted attracting efficiency of yeast-produced CO_2 in this particular study.

Because *Anopheles arabiensis*mosquitoes are bothanthropophilicand zoophilic, depending on geographical location and host availability [22,23], cow urine was also tested for its attractancy. Of the attractants tested, cow urine increased the attracting efficiency of thebasic blend. In the test to evaluate worn socks and cow urine, cow urine alone attracted significantly more mosquitoes than did worn socks plus cow urine and clean air both for the laboratory strain and wild population. Worn socks showed an antagonistic effect on the cow urine. This result revealed that cow urine is equally effective as an attractant for both resistant and susceptible *Anopheles arabiensis*. This finding is supported by the results of a study conducted to evaluate cow urine for outdoor sampling, which foundthat significantly more adult *Anopheles arabiensis*were collected in the resting box baited with cow urine than in the unbaited box [24,25]. In another similar study, cow urine was effective as an oviposition attractant for *Anopheles gambiae* in the first four days [13]. In general, when we look at percentage of mosquitoes caught by trap baited with cow urine, itsattracting effectiveness of 20% seems unconvincing. The possible reason for this result may be that the cow urine was supplied with more than two different attractants thatcan share mosquitoes.

Another study identified that carboxylic acid (R-COOH), urea, ammonia, 3- and 4-methyl phenol, 3-ethylphenol, 3-n-propylphenol,and 2-methoxyphenol are some of the chemicals in cow urine. These compounds were found to have electro-antennographical effects on mosquitoes [13]. Thus, a possible reason forthe attracting efficacy of cow urine in this study could be that chemical compounds identified from cow urine were attractive to *Anopheles arabiensis*.

Guava fruit lessened the attracting efficacy of basic blend. The combination of guava fruit with CO_2 caught a smaller number of mosquitoes when compared to CO_2 alone. According to a study in Mali, guava fruit was the most attractive fruit for both female and male *A.gambiaes* [26]. The difference of guava fruits may be the possible reason for the unparalleled efficacy.

The addition of acetone to basic blendreduced the efficacy of basic blend. The number of mosquitoes caught by acetone plus CO_2 showed no difference compared with the control. In a previous study, responses of *A.gambiaes* females to ammonia, L-lactic acid, acetone, and dimethyldisulphide in different combinations indicated that the addition of acetone to ammonia or lactic acid attracted significantly greater numbers of mosquitoes than did clean air. Again,the combination of acetone and lactic acid attracted more mosquitoes than did lactic acid alone, but the number did not differ from that of acetone alone [27]. A similar study on attractiveness of binary blends consisting of L-lactic acid and acetone to *Aedesaegypti* showed that the response to the combination was greater than that to acetone alone [28]. These results are in disagreement with our studies. Butanol also did not increase the attractant effect of basic blend. According to the study of Verhulst*et al*., butanol had significant synergistic effects on catches of the basic blend of ammonia, L-lactic acid, and tetradecanoic acid for *A.gambiaes*mosquitoes [17]. The remarked opposite efficacy of both chemicals in this study may be sensitivity differences among species towards different attractant combinations.

Conclusions

Of all attractants tested, none of them had significantly greater attracting efficacy than the basic blend. Cow urine alone was found to be an efficient attractant both for the laboratory strain and wild population of *Anopheles arabiensis.* Yeast-produced carbon dioxide alone is able to attract *Anopheles arabiensis* as efficiently as the blend. The new trap in conjunction with an attractant was ableto capture adult *Anopheles arabiensis*. Therefore, it canserveas an alternative tool in malaria vector surveillance and monitoring.However, the trap requires a size optimization study followed by a trapping efficiency comparison with standard traps.

Acknowledgements

We thank Adama Malaria Training and Research Center Insectary and Asendabo Vector Biology Laboratory, which facilitated the conditions to rear mosquitoes and conduct an experiment, as well as the staff therein who technically supported us during the mosquito-rearing process.

Authors' contributions

DH designed the study, analyzed and interpreted the data, and drafted the manuscript. DRS co-designed the study, participated in the analysis and interpretation of data, and contributed to the drafting of the manuscript. DY participated in the analysis and contributed to the drafting of the manuscript

References

1. World Health Organization (2014) World Malaria Report. In. Geneva: World Health Organization:142
2. Federal Ministry of Health (2010) Guidelines for malaria vector control in Ethiopia. In. Edited by HEALTH EaACDMO. In. Addis Ababa: FMoH:12
3. World Health Organization (2011) World malaria report. In. Geneva: World Health Organization: 278.
4. Federal Ministry of Health (2006) National five-year strategic plan for malaria prevention & control in Ethiopia 2006 – 2010. In. Addis Ababa: FMoH: 46
5. Balkew M, Ibrahim M, Koekemoer LL, Brooke BD, Engers H, Aseffa A, Gebre-Michael T, Elhassen I (2010) Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of kdr mutation. Parasite Vectors 3:40.
6. Yewhalaw D, Van Bortel W, Denis L, Coosemans M, Duchateau L, Speybroeck N (2010) First evidence of high knockdown resistancefrequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. Am J Trop Med Hyg 83: 122-125.
7. Yewhalaw D, Wassie F, Steurbaut W, Spanoghe P, Van Bortel W, Denis L, Tessema DA, Getachew Y, Coosemans M, Duchateau L, Speybroeck N (2011) Multiple Insecticide Resistance: An impediment to insecticide-based malaria vector control program PLoS One6:e16066. doi: 10.1371/journal.pone.0016066.
8. Smallegange RC, Knols BGJ, Takken W (2010) Effectiveness of synthetic versus natural human volatiles as attractants for *Anopheles gambiae* (Diptera: Culicidae) Sensu stricto. J Med Entomol 47: 338-344.
9. Okumu FO, Killeen GF, Ogoma S, Biswaro L, Smallegange RC, Mbeyela E, Titus E, Munk C, Ngonyani H, Takken W, Mshinda H, Mukabana WR, Moore S (2010) Development and field evaluation of a synthetic mosquito lure that is more attractive than humans. PLoS One 5: e8951.
10. Qiu TY, Smallegange RC, ter Braak CJF, Spitzen J, Van Loon JJA, Jawara M, Milligan P, Galimard AM, Van Beek TA, Knols BGJ,Takken W (2007) Attractiveness of mm-x traps baited with human or synthetic odor to mosquitoes (Diptera: Culicidae) in The Gambia. J Med Entomol 44: 970-983.
11. Jawara M, Awolola TS, Pinder M, Jeffries D, Smallegange RC, Takken W, Conway DJ (2011) Field testing of different chemical combinations as odour baits for trapping wild mosquitoes in The Gambia. PLoS One 6: e19676.
12. Mathenge EM, Misiani GO, Oulo DO, Irungu LW, Ndegwa PN, Smith TA, Killeen GF, Knols BG (2005) Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of *Anopheles arabiensis,* An.

funestus and culicine species in a rice irrigation in western Kenya. Malaria J4: 7.
13. Kweka EJ, Owino EA, Mwang'onde BJ, Mahande AM, Nyindo M, Mosha F (2011) The role of cow urine in the oviposition site preference of culicine and *Anopheles mosquitoes*. Parasit Vectors 4: 184.
14. Smallegange RC, Schmied WH, van Roey KJ, Verhulst NO, Spitzen J, Mukabana WR, Takken W (2010) Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito Anopheles gambiae. Malaria J 9: 292.
15. Facchinelli L, Valerio L, Pombi M, Reiter P, Costantini C, Dellatorre A (2007) Development of a novel sticky trap for containerbreeding mosquitoes and evaluation of its sampling properties to monitor urban populations of Aedes albopictus. Med Vet Entomol 21: 183-195.
16. Okumu F, Biswaro L, Mbeleyela E, Killeen GF, Mukabana R, Moore SJ (2010) Using nylon strips to dispense mosquito attractants for sampling the malaria vector *Anopheles gambiae s.s.* J Med Entomol 47: 274-282.
17. Verhulst NO, Mbadi PA, Kiss GB, Mukabana WR, van Loon JJ, Takken W, Smallegange RC (2011) Improvement of a synthetic lure for Anopheles gambiae using compounds produced by human skin microbiota. Malaria J 10: 28.
18. Kline DL (2006) Traps and trapping techniques for adult mosquito control. J Am Mosquito Control Assoc 22: 490-496.
19. Mahande A, Mosha F, Mahande J, Kweka E (2007) Feeding and resting behaviour of malaria vector, Anopheles arabiensis with reference to zooprophylaxis. Malaria J 6: 100.
20. Njiru BN, Mukabana WR, Takken W, Knols BG (2006) Trapping of the malaria vector Anopheles gambiae with odour-baited MM-X traps in semi-field conditions in western Kenya. Malaria J 5: 39.
21. Jawara M, Smallegange RC, Jeffries D, Nwakanma DC, Awolola TS, Knols BGJ, Takken W, Conway DJ (2009) Optimizing odor-baited trap methods for collecting mosquitoes during the Malaria season in The Gambia. PLoS One 4: 12.
22. Kweka EJ, Mwang'onde BJ, Lyaruu L, Tenu F, Mahande AM (2010) Effect of different hosts on feeding patterns and mortality of mosquitoes (Diptera: Culicidae) and their implications on parasite transmission. J Glob Infect Dis2:121-123.
23. Coetzee M, Craig M, le Sueur D (2000) Distribution of African malaria mosquitoes belonging to the A*nopheles gambiae* complex. Parasitol Today16:74-77.
24. Kweka EJ, Mwang'onde BJ, Kimaro E, Msangi S, Massenga CP, Mahande AM (2009) A resting box for outdoor sampling of adult *Anopheles arabiensis* in rice irrigation schemes of lower Moshi, northern Tanzania. Malaria J 8: 82.
25. Mahande AM, Mwang'onde BJ, Msangi S, Kimaro E, Mnyone LL, Mazigo HD, Mahande MJ, Kweka EJ (2010) Is aging raw cattle urine efficient for sampling *Anopheles arabiensis* Patton? BMCInfect Dis10: 172.
26. Müller GC, Beier JC, Traore SF, Toure MB, Traore MM, Bah S, Doumbia S, Schlein Y (2010) Field experiments of *Anopheles gambiae* attraction to local fruits/seedpods and flowering plants in Mali to optimize strategies for malaria vector control in Africa using attractive toxic sugar bait methods. Malaria J 9: 262.
27. Qiu YT, Smallegange RC, Vanloon JJA, Takken W (2011) Behavioural responses of Anopheles gambiae sensu stricto to components of human breath, sweat and urine depend on mixture composition and concentration. Med Vet Entomol 25: 247-255.
28. Bernier UR, Kline DL, Posey KH, Booth MM, Yost RA, Barnard DR (2003) Synergistic attraction of *Aedes aegypti* (L.) to binary blends of lactic acid and acetone, dichloromethane, or dimethyl disulfide. J Med Entomol 40: 653-656.

Corresponding author

DawitHawariaLogita
Yirgalem Hospital Medical Science College
Department of Public Health
184, Yrgalem, Ethiopia
Phone: +251-916-044-383
Fax: +251-46-225-0996
Email: hawaria.dawit@gmail.com

Conflict of interests: No conflict of interests is declared.