Parasites
&Vectors

The role of cow urine in the oviposition site preference of culicine and *Anopheles* mosquitoes

Eliningaya J Kweka[1,2*], Eunice A Owino[3], Beda J Mwang'onde[1], Aneth M Mahande[1], Mramba Nyindo[2] and Franklin Mosha[2]

Abstract

Background: Chemical and behavioural ecology of mosquitoes plays an important role in the development of chemical cue based vector control. To date, studies available have focused on evaluating mosquito attractants and repellents of synthetic and human origins. This study, however, was aimed at seasonal evaluation of the efficiency of cow urine in producing oviposition cues to *Anopheles gambiae* s.l. and *Culex quinquefasciatus* in both laboratory and field conditions.

Methods: Oviposition response evaluation in laboratory conditions was carried out in mosquito rearing cages. The oviposition substrates were located in parallel or in diagonal positions inside the cage. Urine evaluation against gravid females of *An. arabiensis* and *Cx. quinquefasciatus* was carried out at Day 1, Day 3 and Day 7. Five millilitres (mls) of cow urine was added to oviposition substrate while de-chlorinated water was used as a control. In field experiments, 500 mls of cow urine was added in artificial habitats with 2500 mls of de-chlorinated water and 2 kgs of soil. The experiment was monitored for thirty consecutive days, eggs were collected daily from the habitats at 7.00 hrs. Data analysis was performed using parametric and non-parametric tests for treatments and controls while attraction of the oviposition substrate in each species was presented using Oviposition Activity Index (OAI).

Results: The OAI was positive with ageing of cattle urine in culicine species in both laboratory and field experiments. The OAI for anopheline species was positive with fresh urine. The OAI during the rainy season was positive for all species tested while in the dry season the OAI for culicine spp and *Anopheles gambiae* s.l., changed with time from positive to negative values.
Based on linear model analysis, seasons and treatments had a significant effect on the number of eggs laid in habitats, even though the number of days had no effect.

Conclusion: Oviposition substrates treated with cow urine in both laboratory and field conditions have shown that cow urine left to age from 1-7 days has an influence on oviposition behavioural response in mosquitoes. The analysis of microbial colonies for decaying urine should be investigated along with its associated by-products.

Background

Oviposition site selection by gravid mosquitoes is one of the crucial events in non-parental organisms [1-6]. In mosquitoes, the reproductive success depends on the ability of a mosquito to search for the suitable oviposition site that will ensure survival for the progeny [7,8]. Habitat related chemical cues have been shown to play an important role in the oviposition site selection by gravid female mosquitoes [9,10]. *Anopheles gambiae* s.s. and *Culex quinquefasciatus* mosquitoes are anthropophilic, thus, highly associated with habitats close to human dwellings [7,11,12], but *An. arabiensis* mosquitoes in particular are anthropophilic or zoophilic depending on geographical location and host availability [13,14]. In different areas, *Cx. quinquefasciatus* have been found laying

* Correspondence: pat.kweka@gmail.com
[1]Tropical Pesticides Research Institute, Division of Livestock and Human disease vectors control, Mosquitoes Section, P. O. Box 3024, Arusha, Tanzania
Full list of author information is available at the end of the article

BioMed Central

egg rafts in habitats with higher levels of organic pollutants [15,16], while *An. gambiae* s.s. and *An. arabiensis* have been found to lay eggs in turbid small water pools with short vegetation or without vegetation [17,18]. Micro-organisms inhabiting mosquito breeding sites have been found to play a major role in the decomposition of detritus and other nutrients present in these habitats, leading to the production of several metabolites emanating different chemical cues [19]. Gravid mosquitoes are often attracted to water in spite of the fact that the decision to lay eggs may depend on additional olfactory signals or other stimuli received when the insect lands on the water surface [5,20-22]. Therefore, interruption of oviposition site selection for mosquitoes may lead to population growth reduction and have a positive impact in control programmes.

Using resting boxes baited with cow urine [23,24] and cow as bait in an odour-baited trap, high densities of female *An. gambiae* s.l. were sampled [25,26]. It is further known that most of the sampled females were either semi-gravid or fully gravid [23,24]. These results gave rise to the hypothesis that, cow urine-polluted habitats influence the attraction of gravid female mosquitoes for oviposition. Other arthropods have been shown to be attracted to the contents of cow urine i.e., (4-methylphenol, 3-n-propylphenol) during host seeking experiments [27]. Sampling of *Hybomitra* horseflies increased by 1.5-1.9 times when cow urine was used as bait in the traps [27]. To build up on the foundation that has been laid by these observations, it would be necessary to set up experiments that assess the responses of gravid mosquitoes to artificial habitats baited with different stages of ageing cow urine.

Therefore, the aim of this study was to assess the oviposition response of *An. gambiae* s.l. and *Cx. quinquefasciatus* mosquitoes in laboratory and field conditions baited with cow urine.

Materials and methods

Laboratory experiments

Laboratory experiments were carried out at the Tropical Pesticides Research Institute field station, located at lower Moshi rice irrigation schemes (37°20' E, 3°21' S; 750 M above sea level). *An. arabiensis* and *Cx. quinquefasciatus* mosquitoes (Mabogini strain, colonised in insectaries since 2006) were used. Mosquitoes were blood fed using shaved restrained rabbits at the age of 3 days after emergence. Both *An. arabiensis* and *Cx. quinquefasciatus* were allowed to feed on the rabbit for duration of one hour in the laboratory. Both species were monitored for 48 to 96 hrs post blood meal until they were fully gravid. The insectary

temperature was maintained at 27.4 ± 1°C with a relative humidity of 70 to 80%. The light regime in the insectary was 12L:12D [28]. Gravid females of both *An. arabiensis* and *Cx. quinquefasciatus* were provided with sugar solution (10% sucrose) during oviposition experiments.

Oviposition choices

One fully gravid female mosquito of *An. arabiensis* or *Cx. quinquefasciatus* (48th to 96th hr after full blood meal) was placed in a cage (30 × 30 × 30 cm), made from iron rods and covered with fine netting material. Experiments were set up using the oviposition requirements and substrates specific for each species (i.e. a wet cotton wool covered by filter paper on a Petri dish for *An. arabiensis* and a three quarters water filled plastic cup for *Cx. quinquefasciatus*). The composition of the solution used in oviposition substrate was in the ratio of 1:5 for cow urine and water respectively. Each experiment used 1, 3 and 7 day old cow urine and had twenty replicates for each species. A total of 60 gravid female mosquitoes of each species were used for evaluations with the three ageing urine sets. Oviposition choice experiments were made either in parallel or diagonal arrangements. In parallel oviposition choice experiments, the oviposition substrates were placed 20 cm apart in the centre of the cage. In diagonal oviposition experiments, the choice experiment was set diagonally (two oviposition substrates were placed in cage corners diagonally to each other).

In both settings the treatments were made up using urine collected from a 2 year old and zero grazed female Zebu cow to avoid variations [23,29]. The urine was collected between 6 to 7 am daily by the cow owner in a plastic basin. On day one, fresh urine was used directly in laboratory experiments in a ratio of 1:5. Urine for experiments on days 3 and 7 was aged in open plastic basins to allow microbial decomposition. The oviposition substrates were wetted with three treatments of either 5 mls of fresh urine (Day 1), three day old (stored for 3 days) or seven day old urine (stored for 7 days) diluted in 25 mls of de-chlorinated water. De-chlorinated water was used as a control in all experiments. Each experiment had ten replicates; the numbers of eggs laid were counted at 7:30 am the next day. The protocol for laboratory experiments was adapted from Sumba *et al.* [22] with some modifications, under the assumption that mosquitoes lay their eggs randomly.

Design of Field experiments

Field experiments were carried out outside human dwellings at lower Moshi rice irrigation schemes, located 19 km south of Moshi town, on the lower slopes of Mount Kilimanjaro. The study area is

described in detail elsewhere by Kweka *et al.* [23,29]. The experiments were carried out in two seasons: during the dry season (January to February) and the rainy season (mid March to May). The treatment and the control were placed five metres apart. A pair comprising of a treatment and control were placed ten metres apart from each other.

The experimental set up design was the same for both the dry and wet season, since oviposition site selection for *Aedes aegypti* is more affected by seasons [30,31]. Munga *et al.,* [32] found that in the dry season, *An. gambiae* significantly deposited more eggs in water from farmlands compared to water from forests and natural wetlands. However, the differences were statistically insignificant during the wet season. Furthermore, substrate composition and concentration have been found to influence the oviposition site selection for *An. gambiae* [5]. *An. gambiae* s.l. eggs were collected carefully using a magnifying hand lens to ensure accuracy.

Dry season oviposition choice in small scale field experiments

About twenty artificial habitats were created by mixing 2 kilograms of soil, 2500 mls of chlorine free water and 500 mls of cow urine (a similar ratio of 1:5 for cow urine and water was used as in the laboratory experiments). These were placed in washing basins measuring 35 cm in diameter and 14 cm in depth. The inner side of each basin was lined up with white paper. The control habitats were made with the same composition of resources but excluded the cow urine. Mosquito eggs were counted daily for thirty days. Water with respective aged urine levels in habitats was monitored and when decreased/evaporated was replenished with similar ratios.

Rainy season oviposition choice in field experiments

Experimental and artificial habitats were set up, egg laying and monitoring schedules were similar to the ones used in the dry season.

Data Analysis

In laboratory experiments, data were recorded at 7:30 hrs while in field experiments set up was daily every morning at 7:30 hrs. Due to high variations in the number of eggs laid in each treatment and between replicates, the number of eggs laid was log (n + 1) transformed. Following log transformation, the normally distributed data were analysed using the parametric test while a non-parametric test was used for data that were not normally distributed. For field data, the comparison between treatments and controls were carried out using the Kruskal Wallis test. The comparisons of different factors associated with oviposition choice of gravid mosquitoes in different habitats were analysed using the general linear model multivariate analysis (MANOVA). Number of eggs laid was used as the dependent variable, while seasons, treatments and species were used as covariates and days were used as random factor. Data analysis was performed using PASW statistics version 18 (SPSS Inc., Chicago, IL).

The oviposition attraction of cow urine was scored by oviposition activity index (OAI), which was calculated using the protocol used by Hwang *et al.* [33]. OAI = $(N_T-N_S)/(N_T + N_S)$ where N_T denotes the mean number of eggs laid in the treated substrate and N_S denotes the mean number of eggs laid in the control substrate. All the index values ranged from -1 to +1. Positive OAI means that more eggs were laid in the treatment substrate than in control while, for the negative OAI, more eggs were laid in the control than in treatment substrate.

Results
Laboratory experiments

For the diagonal oviposition choice setup, the mean number of eggs laid in oviposition substrates treated with fresh urine (1 day) was significantly higher compared to eggs laid in the control substrates, for *An. arabiensis* (t = 12.71, d.f. = 1, *P* = 0.010) and *Cx. quinquefasciatus* (t = 12.71, d.f. = 1, *P* = 0.030). For the oviposition substrates treated with 3 day old cow urine, more eggs were laid in the control than in treatment substrates, for *An. arabiensis* (t = 12.71, d.f. = 1, *P* = 0.010) while for *Cx. quinquefasciatus*, more eggs were laid in the treatment than in control substrates (t = 12.71, d.f. = 1, *P* = 0.050). For oviposition substrates treated with 7 day old cow urine, the number of *An. arabiensis* eggs laid were significantly higher in control than in treatment substrates (t = 12.71, d.f = 1, *P* = 0.030); for *Cx. quinquefasciatus*, the number of eggs laid in treatment substrates were significantly higher than those in the control substrates (t = 12.71, d.f = 1, P = 0.030).

In parallel choice experiments, the number of eggs laid in fresh urine treated substrates by *An. arabiensis* was significantly higher than in control substrates (t = 12.71, d.f. = 1, *P* = 0.010). In *Cx. quinquefasciatus* eggs laid in treatments and control were statistically similar (t = 12.71, d.f. = 1, *P* = 0.350). In results from the 3 day old cow urine in the parallel set up, *An. arabiensis* laid more eggs in the control than in treatment group (t = 12.71, d.f. = 1, *P* = 0.020) and *Cx. quinquefasciatus* laid more eggs in the treatments than in the control group (t = 12.71, d.f. = 1, *P* = 0.010). In 7 day old cow urine in parallel oviposition choice, *An. arabiensis* significantly oviposited more eggs in the control than in the treatment groups (t = 12.71, d.f. = 1,

$P = 0.010$) while *Cx. quinquefasciatus* significantly oviposited more eggs in the treatment substrates than in control substrates (t = 12.71, d.f. = 1, $P = 0.010$).

OAI for both *An. arabiensis* and *Cx. quinquefasciatus* ranged from -1 to + 1 in treatments throughout all experimental days. In one day-old urine, both diagonal and parallel experimental settings recorded more eggs than in the control groups for both species (Table 1). For the 3 day old urine, *An. arabiensis* laid more eggs in the control for both parallel and diagonal settings resulting in a negative OAI. However, *Cx. quinquefasciatus* laid more eggs in treatments that resulted in positive OAI values (Table 1). For the 7 day old urine, *An. arabiensis* treatment replicates in both the parallel and diagonal settings recorded fewer eggs than in the control groups, which resulted in negative OAI while *Cx. quinquefasciatus* recorded more eggs in treatment replicates resulting in a positive OAI value (Table 1).

Field experiments

In the dry season, the thirty days records for *An. gambiae* s. l. eggs in treatment replicates were significantly higher than those from controls (t = 2.26, d.f. = 29, $P <0.010$); a similar trend was observed for culicine spp (t = 2.26, d.f. = 29, $P < 0.010$). Nevertheless, the number of eggs of *An. gambiae* s.l. decreased with the increasing number of days while for culicine spp, increased with days.

In the rainy season, the number of eggs laid in urine treated oviposition substrates were statistically similar from control substrates for both *An. gambiae* s.l. (t = 2.26, d.f. = 29, $P = 0.790$) and culicine spp (t = 2.26, d.f. = 29, P = 0.790).

General linear model analysis revealed that treatments, species, and seasons significantly influenced oviposition; days had no influence on oviposition substrates selection by gravid mosquitoes (Table 2).

The OAI were found to shift from positive to negative values and vice-versa. The OAI values shifted from negative (-0.25) to positive (0.25) for culicine spp and changed from positive (0.48) to negative (-0.28) for *An. gambiae* s.l. throughout the study and from Day 1 to Day 30, respectively.

Discussion

The results of this study suggest that *An. gambiae* s.l. are attracted to ovipositing in habitats with fewer

Table 2 Analysis of the factors associated with the influence of gravid mosquitoes oviposition in artificial habitats for thirty days of field evaluations.

Source of variation	F-test	P-values
Season	$16_{1, 238}$	<0.001
Days	$0.32_{29, 210}$	0.99
Treatments	$7.07_{1, 238}$	<0.01
Species	$19.4_{1, 238}$	<0.001

* (The source of variation was considered significant at $P <0.05$).

organic pollutants in both laboratory and field conditions while culicines have been shown to be attracted to oviposit in habitats with decomposed organic contents. These findings are consistent with our hypothesis that in culicine mosquito species oviposition cues come from the decomposed organic substrates while *An. gambiae* s. l. respond to cues from habitats without decomposing matter. This response was clearly observed in field experiments, which resulted in an oviposition shift between *An. gambiae* s.l. and culicine spp. Laboratory findings showed that experimental design had no influence on oviposition. Yet, treatments were found to influence oviposition. The microbial activities on urine decomposition have been assumed to produce different cues and hence attract mosquito species differently as bacterial colonies in habitats have an influence on chemical cues released from these habitats. Different chemical cues have been found to influence species for oviposition in different natural habitats [7,8,34,35].

In previous studies, it was observed that the oviposition of *An. arabiensis* in rice fields increased with first application of organic fertilizer while subsequent applications increased *Cx. quinquefasciatus* mosquitoes [21,36-38]. Additions of manure and environmental manipulation in farms have been shown to decrease the population of anopheline species and increase the population of culicine species [36,37,39].

Four compounds have been identified from fresh cow urine; these are 3- and 4-methyl phenol, 3-ethylphenol, 3-n-propylphenol and 2-methoxyphenol [40,41]. These compounds have electro-antennographical effects on mosquitoes and *Glossina spp* [40,41]. The attractant and/or repellent effect of cow urine to *An. arabiensis* and *Cx. quinquefasciatus* in our experiments could be due to the fact that the four chemical compounds

Table 1 Oviposition activity index of mosquito species during the insectary experiments in different substrates and settings.

	Fresh urine (immediately collected		3 days old		7 days old	
	Diagonal	**Parallel**	**Diagonal**	**Parallel**	**Diagonal**	**Parallel**
An. arabiensis	0.93	0.70	-0.90	-0.99	-1.00	-1.00
Cx. quinquefasciatus	0.92	0.39	0.98	1.00	1.00	1.00

formerly identified from cow urine were active. Due to microbial activities, the chemical compounds could have been produced as by-products that might influence oviposition attraction and/or deterrence cues for each mosquito species. Furthermore, the presence of chemicals and continued decomposition of cow urine increases microbial colonies, which might have influenced the observed results. The increase in microbial colonies in habitats generates more volatile compounds that attract gravid mosquitoes' to oviposit [5,7,21,38].

Addition of cow urine to habitats attracted oviposition by anopheline species in the early day intervals while in the later ones culicines were attracted more [16,36,37,42]. The trend in decreased oviposition observed in *An. gambiae* s.l. during the dry season might have been due to the hot weather which, increased evaporation rate of water and hence increased the concentration of the contents in the habitats and subsequently the microbial activity outputs. Consequently, in the rainy season, oviposition was observed to be low due to the effect of frequent dilution of habitat substrate by rain and the presence of alternative natural habitats. This variation caused a change in the number of eggs laid by each species per habitat and treatment. Oviposition studies previously carried out in Tanzania showed that, the residue of skatole and synthetic oviposition pheromone in the field against culicine spp were strong for one week (7 days) [43]. In this study, decaying cow urine was effective in attracting gravid culicine spp from Day 4 to Day 30. The oviposition trap evaluation in rural Tanzania captured both gravid *An. gambiae* s.l. and *Cx. quinquefasciatus* in natural habitats [44]. The density of mosquitoes collected could have been higher if cow urine was incorporated in those devices. This would increase cues emanated and influence collection as trends showed that both species share habitats.

According to our experiments, cow urine was effective as an oviposition attractant for *An. gambiae* s.l. in the first four days while for *Cx. quinquefasciatus* attractivity increased from day four to thirty in the dry season. This is worthwhile information that could be used to lure-and-kill gravid mosquitoes and hatched larvae. Even though there is a growing interest in using bio-pesticides such as fungal spores for mosquito larvae control [45], the main focus biases to the control of adult mosquitoes [46,47]. Therefore, a need to incorporate oviposition chemical cues for gravid mosquitoes and apply it in larval habitat control arises.

Oviposition attractants, which are locally available, low cost and reliable, should be deployed in aggregating mosquitoes' larval habitats for use in the planning and management of effective control programmes for mosquitoes. The impact of larval control is significant in reducing adult mosquitoes and malaria cases [48-51].

The bacterial colonies associated with the decomposition of cow urine in intervals of days, should be isolated to understand the species identity and chemical cue products responsible.

Conclusions

The findings of this study suggest that cow urine evidently influences choice of oviposition site, which vary between species, and aged urine induces markedly different responses to fresh urine.

Author details
[1]Tropical Pesticides Research Institute, Division of Livestock and Human disease vectors control, Mosquitoes Section, P. O. Box 3024, Arusha, Tanzania. [2]Kilimanjaro Christian Medical College of Tumaini University, P. O. Box 2240, Moshi Tanzania. [3]School of Biological Sciences, University of Nairobi, P. O. Box 30197 - 00100, Nairobi, Kenya.

Authors' contributions
EJK conceived and designed experiments. AMM and BJM conducted all the experimental work. EJK and BJM did data analysis and interpretation. EJK wrote the manuscript. EAO, MN, FM, BJM and AMM edited the manuscript. All authors approved the final version for submission.

Competing interests
The authors declare that they have no competing interests.

References
1. Arav D, Blaustein L: **Effects of pool depth and risk of predation on oviposition habitat selection by temporary pool dipterans.** *J Med Entomol* 2006, **43**:493-497.
2. Blaustein L, Kiflawi M, Eitam A, Mangel M, Cohen JE: **Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue.** *Oecologia* 2004, **138**:300-305.
3. Egan RS, Paton PWC: **Within pond parameters affecting oviposition by wood frogs and spotted salamanders.** *WETLANDS* 2004, **24**:1-13.
4. Howard RD: **The influence of male-defended oviposition sites in early embryo mortality in bullfrogs.** *Ecology* 1978, **59**:789-798.
5. Huang J, Walker ED, Giroux PY, Vulule J, Miller JR: **Ovipositional site selection by *Anopheles gambiae*: influences of substrate moisture and texture.** *Med Vet Entomol* 2005, **19**:442-450.
6. Touchon JC, Warkentin KM: **Reproductive mode plasticity: aquatic and terrestrial oviposition in a tree frog.** *Proc Natl Acad Sci USA* 2008, **105**:7495-7499.
7. Bentley MD, Day JF: **Chemical ecology and behavioral aspects of mosquito oviposition.** *Annu Rev Entomol* 1989, **34**:401-421.
8. Bentley MD, McDaniel TN, Lee h, stiehl B, Yatagai M: **Studies of *Aedes triseriatus* oviposition attractants producted by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae).** *J Med Entomol* 1976, **13**:112-115.
9. Braks MA, Leal WS, Carde RT: **Oviposition responses of gravid female *Culex quinquefasciatus* to egg rafts and low doses of oviposition pheromone under semifield conditions.** *J Chem Ecol* 2007, **33**:567-578.
10. Burkett-Cadena ND, Mullen GR: **Field comparison of Bermuda-hay infusion to infusions of emergent aquatic vegetation for collecting female mosquitoes.** *J Am Mosq Control Assoc* 2007, **23**:117-123.

11. Mutuku FM, Alaii JA, Bayoh MN, Gimnig JE, Vulule JM, Walker ED, Kabiru E, Hawley WA: Distribution, description, and local knowledge of larval habitats of Anopheles gambiae s.l. in a village in western Kenya. *Am J Trop Med Hyg* 2006, **74**:44-53.

12. Minakawa N, Mutero CM, Githure JI, Beier JC, Yan G: Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *Am J Trop Med Hyg* 1999, **61**:1010-1016.

13. Coetzee M, Craig M, le Sueur D: Distribution of African malaria mosquitoes belonging to the Anopheles gambiae complex. *Parasitol Today* 2000, **16**:74-77.

14. Kweka EJ, Mwang'onde BJ, Lyaruu L, Tenu F, Mahande AM: Effect of Different Hosts on Feeding Patterns and Mortality of Mosquitoes (Diptera: Culicidae) and their Implications on Parasite Transmission. *J Glob Infect Dis* 2010, **2**:121-123.

15. Chaves LF, Keogh CL, Vazquez-Prokopec GM, Kitron UD: Combined sewage overflow enhances oviposition of Culex quinquefasciatus (Diptera: Culicidae) in urban areas. *J Med Entomol* 2009, **46**:220-226.

16. Walton WE, Van Dam AR, Popko DA: Ovipositional responses of two Culex (Diptera: Culicidae) species to larvivorous fish. *J Med Entomol* 2009, **46**:1338-1343.

17. Muirhead-Thomson RC: Studies on the breeding places and control of Anopheles gambiae and Anopheles gambiae Var.melas in the coastal district of Sierra Leone. *Bull Entomol Res* 1945, **36**:185-252.

18. Kweka EJ, Zhou G, Lee MC, Gilbreath TM, Mosha F, Munga S, Githeko AK, Yan G: Evaluation of two methods of estimating larval habitat productivity in western Kenya highlands. *Parasit Vectors* 2011, **4**:110.

19. Androsov GK, Skupchenko NL, Orlovskaia NV: Microflora of blood-sucking mosquitoes in the taiga zone of the European part of northeastern USSR. *Med Parazitol* 1986, 21-24.

20. Lindh JM, Kannaste A, Knols BG, Faye I, Borg-Karlson AK: Oviposition responses of Anopheles gambiae s.s. (Diptera: Culicidae) and identification of volatiles from bacteria-containing solutions. *J Med Entomol* 2008, **45**:1039-1049.

21. McCrae AW: Oviposition by African malaria vector mosquitoes. II. Effects of site tone, water type and conspecific immatures on target selection by freshwater Anopheles gambiae Giles, sensu lato. *Ann Trop Med Parasitol* 1984, **78**:307-318.

22. Sumba LA, Guda TO, Denga LA, A H, JC B, BGJ K: Mediation of oviposition site selection in the African malaria mosquito Anopheles gambiae (Diptera: Culicidae) by semiochemicals of microbial origin. *Int J Trop Insec Sci* 2004, **24**:260-265.

23. Kweka EJ, Mwang'onde BJ, Kimaro E, Msangi S, Massenga CP, Mahande AM: A resting box for outdoor sampling of adult Anopheles arabiensis in rice irrigation schemes of lower Moshi, northern Tanzania. *Malar J* 2009, **8**:82.

24. Mahande AM, Mwang'onde BJ, Msangi S, Kimaro E, Mnyone LL, Mazigo HD, Mahande MJ, Kweka EJ: Is aging raw cattle urine efficient for sampling Anopheles arabiensis Patton? *BMC Infect Dis* 2010, **10**:172.

25. Kweka EJ, Mahande AM: Comparative evaluation of four mosquitoes sampling methods in rice irrigation schemes of lower Moshi, northern Tanzania. *Malar J* 2009, **8**:149.

26. Mahande A, Mosha F, Mahande J, Kweka E: Feeding and resting behaviour of malaria vector, Anopheles arabiensis with reference to zooprophylaxis. *Malar J* 2007, **6**:100.

27. Mihok S, Mulye H: Responses of tabanids to Nzi traps baited with octenol, cow urine and phenols in Canada. *Med Vet Entomol* 2010, **24**:266-272.

28. Das S, Garver L, Dimopoulos G: Protocol for mosquito rearing (*A. gambiae*). *J Vis Exp* 2007, 221.

29. Kweka EJ, Mwang'onde BJ, Mahande AM: Optimization of odour-baited resting boxes for sampling malaria vector, Anopheles arabiensis Patton, in arid and highland areas of Africa. *Parasit Vectors* 2010, **3**:75.

30. Edman JD, Scott TW, Costero A, Morrison AC, Harrington LC, Clark GG: Aedes aegypti (Diptera: Culicidae) movement influenced by availability of oviposition sites. *J Med Entomol* 1998, **35**:578-583.

31. Harrington LC, Ponlawat A, Edman JD, Scott TW, Vermeylen F: Influence of container size, location, and time of day on oviposition patterns of the dengue vector, Aedes aegypti, in Thailand. *Vector Borne Zoonotic Dis* 2008, **8**:415-423.

32. Munga S, Minakawa N, Zhou G, Barrack OO, Githeko AK, Yan G: Oviposition site preference and egg hatchability of Anopheles gambiae: effects of land cover types. *J Med Entomol* 2005, **42**:993-997.

33. Hwang YS, Schultz GW, Axelord H, Kramer WL, Mulla MS: Ovipositional repellency of fatty acids and their derivatives against Culex and Aedes mosquitoes. *Environ Entomol* 1982, **11**:223-226.

34. Collins LE, Blackwell A: Colour cues for oviposition behaviour in Toxorhynchites moctezuma and Toxorhynchites amboinensis mosquitoes. *J Vector Ecol* 2000, **25**:127-135.

35. Collins LE, Blackwell A: Olfactory cues for oviposition behavior in Toxorhynchites moctezuma and Toxorhynchites amboinensis (Diptera: Culicidae). *J Med Entomol* 2002, **39**:121-126.

36. Lacey LA, Lacey CM: The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. *J Am Mosq Control Assoc Suppl* 1990, **2**:1-93.

37. Mutero CM, Ng'ang'a PN, Wekoyela P, Githure J, Konradsen F: Ammonium sulphate fertiliser increases larval populations of Anopheles arabiensis and culicine mosquitoes in rice fields. *Acta Trop* 2004, **89**:187-192.

38. McCrae AW: Oviposition by African malaria vector mosquitoes. I. Temporal activity patterns of caged, wild-caught, freshwater Anopheles gambiae Giles sensu lato. *Ann Trop Med Parasitol* 1983, **77**:615-625.

39. Imbahale SS, Mweresa CK, Takken W, Mukabana WR: Development of environmental tools for anopheline larval control. *Parasit Vectors* 2011, **4**:130.

40. Bernier UR, Kline DL, Barnard DR, Schreck CE, Yost RA: Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants for the yellow fever mosquito (Aedes aegypti). *Anal Chem* 2000, **72**:747-756.

41. Spath J: Olfactory attractants for West African tsetse flies, glossina spp. (Diptera: Glossinidae). *Trop Med Parasitol* 1995, **46**:253-257.

42. Haddow AJ, Ssenkubuge Y: Laboratory observations on the oviposition-cycle in the mosquito Anopheles (Cellia) gambiae Giles. *Ann Trop Med Parasitol* 1962, **56**:352-355.

43. Mboera LE, Takken W, Mdira KY, Pickett JA: Sampling gravid Culex quinquefasciatus (Diptera: Culicidae) in Tanzania with traps baited with synthetic oviposition pheromone and grass infusions. *J Med Entomol* 2000, **37**:172-176.

44. Harris C, Kihonda J, Lwetoijera D, Dongus S, Devine G, Majambere S: A simple and efficient tool for trapping gravid Anopheles at breeding sites. *Parasit Vectors* 2011, **4**:125.

45. Bukhari T, Takken W, Koenraadt CJ: Development of Metarhizium anisopliae and Beauveria bassiana formulations for control of malaria mosquito larvae. *Parasit Vectors* 2011, **4**:23.

46. Howard AF, N'Guessan R, Koenraadt CJ, Asidi A, Farenhorst M, Akogbeto M, Thomas MB, Knols BG, Takken W: The entomopathogenic fungus Beauveria bassiana reduces instantaneous blood feeding in wild multi-insecticide-resistant Culex quinquefasciatus mosquitoes in Benin, West Africa. *Parasit Vectors* 2010, **3**:87.

47. Mnyone LL, Koenraadt CJ, Lyimo IN, Mpingwa MW, Takken W, Russell TL: Anopheline and culicine mosquitoes are not repelled by surfaces treated with the entomopathogenic fungi Metarhizium anisopliae and Beauveria bassiana. *Parasit Vectors* 2010, **3**:80.

48. Fillinger U, Lindsay SW: Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Trop Med Int Health* 2006, **11**:1629-1642.

49. Fillinger U, Knols BG, Becker N: Efficacy and efficiency of new Bacillus thuringiensis var israelensis and Bacillus sphaericus formulations against Afrotropical anophelines in Western Kenya. *Trop Med Int Health* 2003, **8**:37-47.

50. Fillinger U, Ndenga B, Githeko A, Lindsay SW: Integrated malaria vector control with microbial larvicides and insecticide-treated nets in western Kenya: a controlled trial. *Bull World Health Organ* 2009, **87**:655-665.

51. Fillinger U, Sonye G, Killeen GF, Knols BG, Becker N: The practical importance of permanent and semipermanent habitats for controlling aquatic stages of Anopheles gambiae sensu lato mosquitoes: operational observations from a rural town in western Kenya. *Trop Med Int Health* 2004, **9**:1274-1289.