

How *Anopheles Gambiae* Locate Their Resting or Breeding Places?

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Abstract:

Mosquitoes are emerging problems in different parts of the earth, especially in Asia and Africa. They are not only annoying at night but few types of those mosquito species are also involved in spreading different types of diseases. Different studies have conducted to explore the behavioural ecology of the mosquitoes, to minimize the interaction between mosquitoes and human hosts. This study was

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designed to measure the effect of different changes on Anopheles gambiae to locate the resting or breeding places. Different changes like colour change, change in entry point, change in temperature and change in odour. All these factors cause no serious problem to Anopheles gambiae for location except change in Odour. Results of this study in ascending orders are Change in entry point, change in colour, change in temperature and change in odour. Output of this study provide clear message to the scientists who study behaviour of mosquitoes i.e. Odour of breeding and resting places provide orientation to him. Furthermore this study may also trigger efforts to health department to control the mosquitoes.

Key words: Changes, change in colour, change in entry point, change in temperature, change in odour, *Anopheles gambiae*.

Introduction

Mosquitoes are looked attentively as an irritating insect due to their hemophilic characters that effect badly both human and animals (Lehane, 2005). Only female mosquitoes of all species are responsible for the blood sucking because blood proteins is necessary for their egg laying (Clements, 1999). Mosquitoes are also responsible for transmitting pathogens of some fatal diseases e.g. malaria, dengue fever, west Nile fever, and lymphatic filariasis. These diseases have severe adverse effects on human society (Otranto et al., 2013); (Guzman et al., 2010); (Snow, Guerra, Noor, Myint, & Hay, 2005). More than 50% population of human beings is affected very badly due to mosquito borne diseases globally and also responsible for the rise of morbidity and mortalities in human beings (WHO, 2012). There is no permanent cure of mosquito borne infectious diseases available around the world (Wilder-Smith, Ooi, Vasudevan, & Gubler, 2010); WHO, 2011; (Van Ooij, 2009). Due to unavailability of permanent cure, different vector control measures are used i.e. insecticide treated nets, insecticide

sprays. Among these control measures, indoor residual spraying is effective to some extent against the transmission of these diseases (Okumu, Moore, Okumu, & Moore, 2011). Unfortunately mosquitoes have developed insecticide resistance against most commonly available commercial insecticides. So, researchers realized that use of insecticide as a single control method is not the way to control the mosquitoes. So different integrated pest management programs are being run around the world to control the mosquitoes and also their affiliated vector diseases (Tolle, 2009); (Sutherst, 2004).

Different studies have been conducted to explore the behaviour and ecology of the mosquitoes to minimize the interaction between mosquitoes and their human hosts. To understand the mechanism of regulating the host species choice in mosquitoes and how these could be manipulated and identified as novel diseases transmission control depends on the changing of behaviour of vector. Chemical ecology is one of the most important parts of ecology; chemical ecology of mosquitoes is the very important area of study on which different studies has been conducted for the successful control of vector because mosquitoes depend very highly on different chemical cues used for the host recognition (Takken & Verhulst, 2013). In modern time we have got the better indulgent of the behaviour of mosquitoes due to increase in knowledge about physiological and molecular study yet we still lack the knowledge of ecology.

Material and Methods

Study was conducted at University College of Agriculture, University of Sargodha to investigate how *Anopheles gambiae* mosquitoes find their resting and egg laying places after every flight at night.

Collection of Mosquitoes

In this study collection of adult *Anopheles gambiae* mosquitoes were done with the help of aspirator from humid places. Collected mosquitoes were kept separated and tagged with the name of collected place. Fifty mosquitoes were collected from each place with the help of aspirator. After collection, Mosquitoes were not shifted to laboratory because concept behind this study was the loss of orientation just like the honey bees on shifting.

Test of Colours on mosquito body

Different types of colors (paint, water colour, poster color, glitter colour) were purchased from market. Five colours were selected for the study i.e. white, red, yellow, orange, and blue. All the colours were tested against collected mosquitoes and checked the effects. Colour with high vision and can be seen from the distance were selected for the proposed study. One minute drop of all the colors were dropped on the abdomen of each mosquito and viewed it after drying. After study yellow colour of “poster colour” was selected because it was suitable and best for the body of *Anopheles gambiae* mosquitoes.

Tagging of mosquitoes

After collection of mosquitoes from each place, the female were selected for the next process while the males were discarded. These mosquitoes were sucked out one by one into long plastic transparent tube made-up of flexible plastic. At one end of tube muslin cloth was attached to avoid accidental entry in mouth. Very small needle syringe was used as a coloring tool. Yellow color was added into the syringe after sucking the mosquitoes into the tube when the mosquito trapped into the tube syringe was perked into the tube but not touches the body of mosquitoes because it may damage its body. After coloring, mosquitoes were removed from the tube and kept into the box having muslin cloth at the two sides. All the mosquitoes were

tagged with the help of colour one by one and after drying the mosquitoes undamaged mosquitoes were released into the same collected place like washrooms. But during the release of these mosquitoes doors of the washrooms were closed and entry was restricted. Doors were opened after the mosquitoes were settled down.

Changes in the Resting places

Different changes were made in the resting sites of mosquitoes after every late night when they went outside for searching the host. Four different changes (colour, temperature, odour, and entry points) were made in the resting and egg laying places.

1. Change in Colour

At 1st night after coloring and releasing into the wash rooms at 11 pm when all the mosquitoes were outside for the search of host charts were pasted. For this purpose same colour charts were purchased from the market. These charts were pasted with the help of sketch tap on the walls of the wash rooms. Next day early in the morning numbers of tagged mosquitoes were counted. The Same process was repeated in next night with changed colour of chart and mosquitoes were counted again.

2. Change in entry point

Next day the charts were not removed because the removing of charts might have caused the disturbance. On the third night when the mosquitoes were outside for host searching some charts were pasted outside walls and doors of the washrooms. Walls of the attached rooms were also changed with the help of charts of different colours to cause the disturbance in orientation of mosquitoes. Data was collected after each day morning and the same process was repeated for the next day but with different charts.

3. Change in temperature

During the fifth night large amount of ice was placed at 4^o clock in the washrooms. Data was collected next morning. The same process was repeated for the next day.

4. Change the odour

In this process perfume was sprayed after each night when the mosquitoes were outside. Strong perfume was selected for this purpose and spray was done at 3 o' clock and 4 o' clock two times. Spray of perfume was done in and outside the wash rooms. The same process was repeated again during the next day and data was collected.

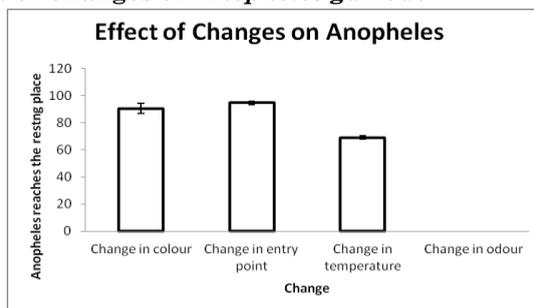
So after the release of fifty mosquitoes in each selected place at 1st day if 45 mosquitoes came back to their resting place then the results were considered as 100% and considered as zero percent affect and so on.

RESULTS

Results of all three data collection sites showed that there is no effect of change in colour and change in entry points on the count of mosquitoes after each night decrease in five mosquitoes are considered as negligible because they may be killed by the some factors during host searching. About 10%, (10% is the percentage of mosquitoes from 100% which were does not locate their resting places) (90.533 ± 1.861) percent results were obtained in case of change in colour of inside walls of the washrooms. On the other hand about 5%, (94.666 ± 0.600) percent results were obtained in case of change in entry point. But keeping in mind 5 mosquitoes were deducted after every result. The 3rd change caused the disruption in mosquitoes location to some extant but with little effect. About 31 %, (69.1666 ± 0.666) results were achieved due to change in temperature but it was not so effect to cause disruption. Temperature changes did not play very effective role but it was

effective than the change in colour and change in entry point or orientation. Last one method used for the study was Change in odour of the resting and breeding place of the mosquitoes. This method was very effective against the mosquito set orientation. In this case 100% (0 ± 0.000) results were achieved because no one mosquito locate their resting place in all three location or washrooms. In other words no one mosquitoes return in to breeding or resting places.

Graph: Effect of Changes on *Anopheles gambiae*



Discussion

A study was conducted investigate the effect of sweaty skin on the attractiveness of the mosquitoes. According to this study *Anopheles gambiae* and *Aedes aegypti* have more relative preference to human blood. Different volatile chemicals are emitted by the human bodies that are the key to locate the human host. Sweat secreted by the human has different chemicals that attract the blood sucking mosquitoes. These chemicals are different in different persons so the attractiveness of human beings to mosquitoes varies greatly from person to person (C, Smallegange, NielsO, Verhulst, & WillemTakken, 2011).

Female mosquitoes also require blood meal for the nourishment of their eggs. Mosquitoes mostly use olfaction sense to detect the host these chemicals are very important for

the human body. From which CO₂ is considered as the activator for the mosquitoes to fly and find the human host. That compound is released during the breathing process along side these different other chemicals also play important role i.e. lactic acid, 1- octen-3- ol and ammonia (J.G & . 2007).

In present study different factors were tested against the *Anopheles gambiae* mosquitoes. Results showed that change in colour was not very important because mosquitoes may be just like the honey bees which are not affected by the change of colour of resting or breeding places. While the second important factor considered for the study was change in the entry point. It was also not important as the reason is unknown. The concept behind this factor was orientation in honey bee. Different studies showed that honey bees set orientation with the help of surrounded things like trees, and buildings and some scientist also say that honey bees also set direction with the help of sun before leave the hive. But the last two factors i.e. Change in Temperature and Change in odour of the resting or breeding places because mosquitoes use these two factors to locate the host. In case of change in Temperature some amount of results was appeared but not very effective. Temperature is the very important factor that is helpful for the mosquitoes to locate the host, resting and breeding places but if the changes in set temperature occurred mosquitoes that may cause the disruption. Change in odour was the last and very effective factor used in the study. Different studies conducted by the scientists showed that odour play very effective role to locate the host by the smell of CO₂ and lactic acid are the very important chemicals emitted by the human skin that helpful to locate the host. In present study 100% results were appeared when different perfumes were sprayed in and around the breeding and resting places. This showed that change in the odour emitted by the location disrupt the locating ability of the mosquitoes.

REFERENCE

- C, Renate, Smallegange, Niels O, Verhulst, & Willem Takken. (2011). Sweaty skin: an invitation to bite? *Trends in Parasitology*, 1–6.
- Clements, A. N. (1999). The biology of mosquitoes, sensory reception and behaviour. *CAB International, Wallingford, United Kingdom, vol. 2.*
- Guzman, M.G, Halstead, S.B, Artsob, H, Buchy, P, Farrar, J, Gubler, D.J, Martínez, E. (2010). Dengue: a continuing global threat. *Nature Reviews Microbiology*, 8, S7-S16.
- J.G, Logan, & ., M. A. Birkett. (2007). Semiochemicals for biting fly control: their identification and exploitation. *Pest Management Sci. Bull*, 63, 647-657.
- Lehane, M.J. (2005). The biology of blood-sucking in insects. *Cambridge Univ Pr. ISBN 0521543959.*
- Okumu, F.O, Moore, S.J, Okumu, F, & Moore, S. (2011). Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malar J* 10, 208.
- Otranto, D, Dantas-Torres, F, Brianti, E, Traversa, D, Petrić, D, Genchi, C, & Capelli, G. (2013). Vector-borne helminths of dogs and humans in Europe. . *Parasites & Vectors* 6(1), 16.
- Snow, R.W, Guerra, C.A, Noor, A.M, Myint, H.Y, & Hay, S.I (2005). The global distribution of clinical episodes of Plasmodium falciparum malaria. *Nature* 434(7030), 214-217.
- Sutherst, R.W. (2004). Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Reviews*, 17(1), 136-173.
- Takken, W, & Verhulst, N.O. (2013). Host Preferences of Blood-Feeding Mosquitoes. . *Annual Review of Entomology*, 58(1), null.

- Tolle, M.A. (2009). Mosquito-borne diseases. *Current Problems in Pediatric and Adolescent Health Care*, 39(4), 97-140.
- Van Ooij, C. (2009). Innate immunity: Not-so-lucky 7 for West Nile virus. *Nature Reviews Immunology*, 9(4), 224-225.
- Wilder-Smith, A, Ooi, E.E, Vasudevan, S.G, & Gubler, D.J (2010). Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development. . *Current infectious disease reports*, 12(3), 157-164.