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# A review on use of attractants and traps for host seeking *Aedes aegypti* mosquitoes

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Arthropod vectors like mosquitoes and other blood sucking insects transmit diseases such as malaria, dengue, chikungunya, zika vius. Among the vectors, *Aedes aegypti* and *Ae. albopictus* are medically important since they cause huge numbers of morbidity and mortality to human beings. Many blood feeding arthropod vectors locate their hosts using vision and primarily through olfactory cues including carbon dioxide ($CO_2$), lactic acid, ammonia, and carboxylic acids. Release of $CO_2$ from commercial cylinders and dry ice enhances efficiency of the traps. Different traps have been developed to trap mosquitoes and other vectors. They were found effective when attractants were added to $CO_2$. Understanding the cues and synergism of attractants on peripheral olfactory system of mosquitoes, may lead to identification of newer molecules with improved efficiency for host seeking mosquitoes and will be useful for natural control of vectors transmitting dengue and other diseases.

**Keywords:** *Aedes*, Attractants, Surveillance, Traps, Virus, Zika.

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## Introduction

Mosquitoes are biting nuisance and they transmit dreadful diseases like malaria, dengue fever, chikungunya fever, and Japanese encephalitis. Mosquito borne diseases continue to cause significant human health problems largely in the tropics and subtropics[1]. Vector borne diseases affect nearly two-third of the human population in the world, mostly in developing countries especially near sub-Saharan region and kill millions annually. Insect borne diseases cause about 1.5 million human deaths every year[2]. Dependence on chemical insecticides for mosquito control has resulted in increased reports of mosquito species developing resistance to a larger number of chemical insecticides that are approved for vector control programmes. Exposure of public to insecticides through food chain and greater environmental issues has led to an increased interest among researchers to develop integrated pest management (IPM) programs using better surveillance tools, source reduction, appropriate use of larvicides, and biological control in addition to public awareness and education[3]. Use of newer technologies like application of semiochemical baited traps[4] and targets for mass trapping or killing of adult mosquitoes under IPM programs targeting mosquitoes have been encouraged[5].

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### Medical importance of *Aedes* mosquitoes

*Aedes aegypti* is one of the most important disease vectors worldwide known as principal vector of dengue virus (DENV)[6,7], chikungunya virus (CHIKV)[8], and Yellow fever viruses (YFV)[9]. Among arboviral diseases, dengue fever has been reported to cause more human morbidity and mortality than any other arthropod-borne viral disease[10,11]. It is estimated that each year, 50–100 million dengue infections and several hundred thousand cases of dengue hemorrhagic fever (DHF) occur, depending upon epidemic activity[12,13]. Dengue fever is a threat to more than 2.5 billion people with approximately 24,000 deaths occurring worldwide per year[14,15].

The recent outbreak of zika virus (ZIKV) in Brazil and American countries transmitted by *Aedes* mosquitoes[16-18] is a big threat and challenge. Zika virus, previously reported in Africa and Asia[19], and now in Brazil[20,21], causes dengue fever-like symptoms and is transmitted by *Aedes* (*Stegomyia*) mosquitoes. ZIKV, a mosquito-borne flavivirus is a member of the Spondweni serocomplex, whose natural transmission cycle involves mainly vectors from the *Aedes* genus (*Ae. furcifer, Ae. taylori, Ae. luteocephalus*, and *Ae. africanus*) and monkeys[19], while humans are occasional hosts, direct contact is also considered a potential route of transmission among humans, probably during sexual intercourse[22,23].

The first isolation of ZIKV was in 1947 from the blood of a sentinel Rhesus monkey No. 766, stationed in the zika forest, near the Lake Victoria in Uganda and in 1948, isolated in the same forest from a pool of *Ae. africanus* mosquitoes[24]. However, Hayes[19] reported that ZIKV has been isolated from different species of *Aedes* mosquitoes including *Ae. africanus, Ae. apicoargenteus, Ae. luteocephalus, Ae. aegypti, Ae. vitattus, and Ae. furcifer*, where *Ae. hensilii* was the predominant mosquito species present in the Yap region during the ZIKV disease outbreak in 2007. For half a century, the virus was described as causing sporadic human infections in Africa and Asia until 2007, when a zika fever epidemic took place in the Yap Island, Micronesia[25]. In 2013, a large epidemic was reported in French Polynesia, concomitant with a dengue epidemic caused by serotypes 1 and 3. Since 2007, ZIKV has been considered as emergent[26,27].

In Brazil, *Ae. aegypti* transmitted ten important emergent arboviruses including DENV, YFV, CHIKV[28-29], Venezuelan equine encephalitis virus (VEEV)[30], and Mayaro virus (MAYV)[31]. In addition, *Ae. albopictus* can also transmit several arboviruses like DENV, MAYV, YFV, Rocio virus, Saint Louis virus, and Oroupoche virus that are responsible for over 95 % of cases of arbovirus infections in Brazil[32,33]. MAYV was recently identified at Mato Grosso, Brazil in the sera of patients with fever, nausea, vomit, myalgia, arthralgia, and ocular and abdominal pain, who had been previously diagnosed with dengue fever[34].

## Attractants involved in host seeking mosquitoes

Host-finding by mosquitoes is a critical component of survival for most species and much research is focused on the cues used for host location. Many insect vectors of diseases find their human hosts primarily through olfactory cues like various compounds emanated by humans such as $CO_2$, lactic acid, ammonia, and carboxylic acids; some of these acts synergically to promote an attractive response. Of the many cues involved, “host odor” is generally considered the most important. Although mosquitoes feed on a broad range of hosts, humans have received the most attention. Variations in the body odour of individual humans determine the differences in their attractiveness to mosquitoes. Considerable research has been conducted on odors produced by humans that elicit attraction in mosquitoes with emphasis on compounds from sweat and skin[35-44]. Responses to these odors appear to be enhanced by $CO_2$, which is a universally present emission from vertebrates. $CO_2$ is responsible for increased flight activity, attraction in some species, and sensitization of mosquitoes to host odors[45-47]. The attractant activity of human sweat was initially associated with lactic acid[48]. Smith *et al*. reported the enhanced attraction of mosquitoes to sweat in conjunction with $CO_2$ and concluded that other components of human odor may be involved as well[35]. Subsequently, the role of lactic acid as a component of host attraction for *Ae. aegypti* and *Anopheles gambiae* has been examined in detail[39,48-52] and appears to be responsible for synergism with other compounds[53]. Mosquitoes can discriminate different animals due to their ability to recognize host-specific odors for blood feeding[43,54]. An enormous attention has been paid to mosquito attractants and repellents as alternatives to pesticides by the researchers around the world in managing mosquitoes, in which many synthetic and natural volatile organic compounds possess the ability to either attract or repel adult mosquitoes[55]. The effectiveness of various attractants and other volatile organic compounds that may serve as spatial repellents has been tested extensively against *Ae. aegypti* mosquitoes[37,52,56] to protect humans from mosquito bites. It has been reported that human sweat and skin residues are highly attractive to *Ae. aegypti*, *An. gambiae*, and *Culex quinquefasciatus* mosquitoes[48,57-60]. A number of host skin emanation chemicals like 1-octen-3-ol, acetone, short-chain carboxylic acids, ammonia, and L-lactic acid have been found to be attractive to *Ae. aegypti*[54,57,61]. It has been demonstrated that ammonia, lactic acid and many carboxylic acids[36,41,53,57,62,63], acetone, and dimethyl sulfide[64] act as potential attractant for *Aedes* and *Anopheles* mosquitoes. Attractant chemicals from host skin emanations appear to provide the most immediate promise for use in traps. Nowadays, researchers are more focused on eco-friendly approaches using semiochemicals (pheromones or parapheromones) of natural and synthetic origin with multiple strategies to control hematophagous insects[65,66].

### *Ae. aegypti* mosquitoes attraction towards host

*Ae. aeypti* mosquitoes show a stronger attraction to and preference for human odor which was true when humans were pitted against diverse hosts as guinea pigs, rats, and chickens[67-69]. The $CO_2$ exhaled from vertebrate host activates the mosquito host seeking behavior and it instantly sensitizes *Ae. aegypti* females to other host-derived stimuli[47]. However, when given the option, the *Ae. aegypti* females navigated up turbulent plumes of human odor instead of $CO_2$[70]. Lactic acid is 10–100 times more abundant in the human skin residues than those of other animals, including other primates[51]. In a pioneering study, Acree and colleagues identified lactic acid as the active component in a fraction of human arm washings that attracted *Ae. aegypti aegypti*[48]. Females responded to lactic acid in substantial numbers when $CO_2$ was present. Subsequent work replicated the original finding and further suggested that lactic acid is a signature human odorant for this mosquito. Attractive human odor extracts can be rendered unattractive by enzymatic removal of lactic acid[37], while unattractive animal-odor extracts can become attractive by its addition[50]. Ammonia is also abundant in human sweat and may be as important to *An. gambiae/coluzzii* as lactic acid is to *Ae. aegypti aegypti*. Ammonia attracts the former species, but not the latter. Apart from lactic acid and ammonia, no other human odorants have proven consistently attractive to human-preferring mosquitoes when presented singly or with $CO_2$. Olfaction is highly contextual; however, a compound that is neutral or repellent when presented alone may be attractive when mixed with other compounds. This type of synergism is critical for specialist mosquitoes like *Ae. aegypti aegypti* and *An. gambiae/coluzzii.* Ammonia enhances the attractiveness of lactic acid to *Ae. aegypti aegypti* and *vice versa*, lactic acid enhances the attractiveness of ammonia to *An. coluzzii*[61]. Acetone and an array of carboxylic acids could attract these two species when added to blends[52,57,64]. The mix of carboxylic acids given off by Limburger cheese — a food item that shares both its pungent aroma and characteristic bacteria with human feet attracted *Ae. aegypti* mosquitoes. Recognizing the importance of synergism among human odorants, several groups have worked to develop synthetic blends containing between three and fifteen compounds mixed in precise proportions that attract as many or sometimes even more mosquitoes than human odor itself[44,64,71]. Finally, research to date has focused almost exclusively on attraction in no-choice assays, rather than preference in a choice context. There are conceptual and empirical reasons to believe that the chemical bases of these behavioral responses are not identical. A mosquito may be attracted to an odor blend, yet still discriminate against it when presented with an alternative option. Indeed, the synthetic blends that attract as many or more mosquitoes than human odor in no-choice trials perform poorly when pitted directly against human odor in a choice setting. Further work by McBride and colleagues identified important evolutionary changes in the OR family as a whole and in a specific OR named *AaegOr4*. Among the two, *Ae. aegypti* subspecies and their hybrids, preference for humans was tightly correlated with naturally occurring *AaegOr4* variants that were highly expressed and more sensitive to a component of human odor called sulcatone. The pattern suggests that an increase in sensitivity to this human-enriched compound sulcatone may be contributing in preference for humans, whereas, the previous work identified sulcatone as a repellent that may steer away mosquitoes from individual humans whose odor contains naturally high levels of sulcatone[59,72].

### Use of attractants and traps for arthropod vectors surveillance and control

#### Attractants

Optimism that attractant-based technology for adult mosquito control could be developed based largely on the success of tsetse fly control. Nowadays, vector control workers have completely replaced aerial drift-spraying of tsetse-infested bush with insecticides (endosulfan or deltamethrin) or by spraying the resting sites of tsetse in Zimbabwe and elsewhere in Africa since both techniques were expensive and logistically complex and achieved success with attractant-baited, insecticide-impregnated targets and traps[73-79]. Much of the success of tsetse fly removal trapping programs was attributed to both the biological peculiarities of tsetse flies and the research programs that were funded to gain a better understanding of tsetse behavior, which resulted in the development of effective targets that combine visual and olfactory attractants used by tsetse flies to locate their hosts[75,80]. Since 1969, international symposium on developing and evaluating attractant-based technology and strategies for adult mosquito control has progressed a lot. Earlier traps were used as components in mosquito management programs where their role has been restricted to surveillance[81]. Such trapping data have been generally used to make decisions on the initiation or termination of control measures as well as to assess efficacy of control approaches. Therefore, at the time, interest in investigating trapping-out technology for mosquito management began, only 2 basic types of traps were available, namely the New Jersey (NJ) light trap[82] and the Centers for Disease Control (CDC) trap[83]. These traps were designed and intended for routine surveillance but not for mosquito control. Light and $CO_2$ were basically the only attractants available for use with these traps[84-86]. Thus, the consensus of the participants who attended the series of symposia and workshops that followed on this emerging technology was that to provide greatest priority for this technology and to develop more efficient and economical traps, targets, and attractants to succeed against mosquitoes.

#### Traps

In addition to conventional insecticides, other methods including commercially available mosquito traps like Mega-Catch, Mosquito Magnet**,** Lentek, Dragonfly**,** and Biogents Sentinel trap are also employed for surveillance as well as control of mosquitoes[87-89]. In most of the traps, attractants like octenol, lactic acid, heat, and $CO_2$ are used to attract mosquitoes[45,46,90]. $CO_2$ is combined with other attractants for mosquitoes like human bait or human derived odors with a suction mechanism in mosquito traps[91-93]. $CO_2$ baited traps have the added advantage that most moths and beetles are not attracted by $CO_2$, so the yield is usually considerably "cleaner"[94-98]. $CO_2$ is a major constituent of vertebrate breath that plays an important role in the host seeking behaviour of mosquitoes. Moreover, addition of $CO_2$ to trap increases the catch of mosquitoes and other blood-sucking insects like mosquitoes[99-103], bedbugs[104], and sandflies[105]. $CO_2$ and carbon-monoxide fumes were generated by an engine adapted to operate on liquid propane gas for mosquito traps[106]. Some trap models burn propane to create $CO_2$ while most of them rely either on $CO_2$ cylinder or dry ice as a source of attractant to mosquitoes[4,5,107,108]. Dry ice is cheap and light, but difficult to obtain everywhere. Moreover, transportation of $CO_2$ cylinders or generators has limitations due to their heaviness and being expensive when trying to cover a wide area for mosquito surveillance. Other sources of $CO_2$ may be from microbial production of different sources like bacteria (Chemolithotrophs), enteric bacteria (*Enterobacter aerogenes*), sulfur reducing bacteria, carboxydotrophic bacteria, methylotrophic bacteria, yeast, and fungi. Among them, yeast (*Saccharomyces cerevisiae* ) which is easily available[109] has been used to produce $CO_2$ in lesser time for application in traps to attract mosquitoes. Sukumaran and coworkers[110] have optimized the production of biogenic $CO_2$ with different carbon sources including sugar, jaggery, glucose and using baker's yeast along with or without YPD media in the laboratory and applied in field condition in two different adult mosquito traps; Mosquito Killing System evaluated during night hours for *Culex* mosquitoes and Biogents sentinel trap in the day time aimed for *Aedes* mosquitoes.

### Newer areas for future research in olfaction of mosquitoes

Human odor has a significant amount of sulcatone. If it is added in small quantities to a blend lacking sulcatone, it would enhance attraction of mosquitoes. On the other hand, *Ae. aegypti* genome may contain multiple sulcatone-sensitive receptors mediating distinct behavioral effects. An increase in the sensitivity of *AaegOr4* could help counteract repellent effects mediated by other ORs. This interesting controversy highlights the potential complexity of the push–pull context from which preference emerges[111]. In another situation, adding lactic acid to an animal odor blend could rescue attraction by human-preferring mosquitoes[50]. But this may or may not confuse mosquitoes into choosing the host. Still, research has to be done to fully understand the nature of preference to mosquitoes for humans at the chemical level. Some attempts on electrophysiological studies suggest that specialization in *Anopheles* might have involved changes in the peripheral olfactory system. Because recordings from 20–50 antennal sensory neurons in each of three related species, showed excitement of more neurons towards human-associated carboxylic acids in *An. coluzzii* than in the animal-preferring *An. quadriannulatus* or even the opportunistic *An. arabiensis*[112]. The receptors and/or accessory proteins that mediate these responses are still not known. Recent large-scale deorphanization studies have identified ligands for many *An. gambiae/coluzzii* ORs[113,114]. But less is known about ionotropic receptors (IRs), gustatory receptors (GRs), and odour binding proteins (OBPs) in *Anopheles* and these genes in families of *Aedes*. So far, no receptors or accessory proteins have been mapped out to specific sensory neurons or hairs on the antennae of mosquitoes in either genus. Several recent studies suggested that changes in the tuning and expression of peripheral receptors have sensitized the antennae of *Ae. aegypti* and *An. gambiae/coluzzii* to human odorants. The OR family clearly plays a role in this process, despite the fact that most known attractants in human odor are not OR ligands. This finding suggests that many of the compounds mosquitoes use to discriminate humans from other animals have yet to be appreciated as such. It also highlights the distinction between attraction and preference - two properties of mosquito host-seeking behavior that likely have different, if overlapping, genetic and chemical bases. Future studies may also reveal important changes in IRs or other peripheral olfactory genes. Beyond the peripheral olfactory system, the critical role of synergism in mosquito host-seeking behavior makes central olfactory circuits a potentially fruitful and fascinating area for future work. Responsible for integrating signals mediated by different receptors, these circuits may have experienced changes conferring preference for the specific blend of compounds that define the way we smell and filling these gaps would greatly facilitate further study of the peripheral changes underlying preference for human odor[68,69].

## Conclusion

Dengue is endemic in over 125 countries and outbreak in Europe and Americas where vector control programmes face many challenges like insecticide resistance among vectors, lack of trained man power, poorly equipped facilities, and weak entomological surveillance system. Hence, every country is expected to strengthen their disease surveillance, health information system, and preparedness to implement integrated vector control programme, so that the arthropod vectors can be controlled[115,116]. At this juncture, understanding attraction of vectors toward host is very important because newer and more efficient eco friendly tools like attractants/repellents and traps can be employed with less or no insecticide for controlling the dengue and other arthropod vectors.

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