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A study on improvement of performance of commercial mosquito trap by using additional bio-attractant factor

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Abstract
A variety of mosquito traps have been developed and used in survey and surveillance programmes that are useful in forecasting of vector and disease prevalence and in planning of vector measures. Though, a number of commercial mosquito traps have been introduced, their direct use in control of mosquito populations has been poorly investigated. Recently findings of a study have demonstrated that one of the portable commercial traps, Terminator-I, is effective in capturing and controlling mosquitoes. In the present study, the possibility of increasing efficiency of terminator-I was explored. Four card board strips impregnated with a number of sweet solutions (sugar, fermented sugar, honey, fermented honey) were fixed in the trap. In control traps non-impregnated strips were fixed. Perusal of findings revealed that the number of mosquitoes caught was higher in all experimental systems. Even the plain water impregnated mats provided slightly better results than the control. The maximum increase (~162%) was noticed in fermenting than non fermenting solutions. Not only the quantity but the quality of the catch was also affected. In control traps, the presence of male and non-gravid female mosquitoes was rare. While in experimental traps, it was slightly higher. The Terminator-I is installed with UV lamp and a photo catalytic plate to generate carbon dioxide, heat and moisture as attractive cues and hence it mimics the human host. Inclusion of fermenting solution is supposed to provide an extra source of carbon dioxide, moisture and food (nectar) mimicking aroma for non-gravid female and male mosquitoes.

Keywords: Insecticides, mosquito traps, attractive sugar bait (ASB), UV light, and CO_2

Introduction
Mosquitoes are blood suckers (Diptera: Culicidae) and a serious biting nuisance as well as vectors of diseases causing high morbidity and mortality, such as malaria, dengue, dengue hemorrhagic fever [1] chikungunya [2, 3] Japanese encephalitis and filariasis [4, 5]. Regarding as a major public health concern, dengue is transmitted by infected female mosquitoes of the genus *Aedes* while blood feeding. Over 2.5 billion people, approximately 40% of the human population, are at risk from dengue. According to current estimates of World Health Organization, there may be 50 - 100 million dengue infections worldwide every year.

For a successful mosquito trapping, traps should have the ability to attract as well as to get mosquitoes inside the traps. Mosquitoes are usually attracted towards different attractive substances like UV-light, CO_2, heat and moisture. Darker colors like blue and black or contrasting colors like black and white are also thought to attract different mosquito species. Concentration of CO_2 gets increased by the air breathed out by humans and other animals. Perspiration includes many chemical compounds including traces of lactic acid, chemicals similar to 1-octen-3-ol and moisture. Adult stage of mosquito is considered to be the most appropriate stage of trapping and at this stage there is a great transmission risk of diseases [6]. Traps are important tools for surveying the abundance of vectors [7, 8] but most traps are relatively ineffective, especially against day biting mosquitoes such as *Aedes aegypti* [9]. To control these day-biting species, new traps such as BG-Sentinal, CDC light, ovitraps and sticky traps may be effective enough [10]. Different types of water containing ovitraps are also used for the control of not only adult mosquitoes but also their aquatic stages *i.e.* larvae and pupae [11]. The development of improved adult traps, such as BG-Sentinel™ (BGS) traps, CDC light traps and mosquito-oviposition traps for *Aedes albopictus, Culex quinquefasciatus* and *Anopheles sinensis* [12] and BG-sentinel™ (BGS) and Zumba™ traps provides an opportunity

for improved entomological surveillance and in control of *Aedes aegypti* [13, 14, 15, 16] and *Aedes albopictus* [17,18]. Different types of traps differ in their sensitivities for detection of particular species and certain mosquito species will display differential attraction to a particular bait and trap types. For example, BG-Sentinal traps are very specific for *Aedes aegypti* and *Culex quinquefasciatus* as compared with CO_2 baited EVS traps, which attracts many general species. Commercial mosquito traps have gained wide acceptance and use different attracting substances like dry ice, CO_2, UV- light, combustible gases like Propane and Butane and some mosquito traps can be baited with attractants such as colored light-emitting diodes (LED's), incandescent light, sound, heat, moist air, odors such as 1-octen-3-ol (octenol) and lactic acid [19]. The concept of using mosquito traps for the surveillance and the study of biting insect populations is at least 70 years old [20, 21, 22, 23]. Use of mosquito traps is one of the several methods that have been developed worldwide for sampling the mosquito population [24]. With the knowledge of new records of mosquitoes, it not only enhances our knowledge about mosquito systematics, but also assesses the risk of associated vector-borne disease agents [25]. Okumu in 2010 evaluate a synthetic mosquito lure that is more attractive than humans [26]. The BG-sentinal traps used by Salazar *et al.* in 2012 [27] consists of lactic acid, ammonia and caproic acid compounds, all of which are components of human sweat. Trap design, location and attractive bait play an important role in mosquito catching [28].

Monitoring mosquito populations represents a key aspect for identifying risks of pathogen transmission to humans and animals. In order to control various mosquito-borne diseases, improved traps should be used for surveillance and effective catching. Grien's All Iinn Mosquito Trap/Killer, model Terminator-I is very effective and efficient trap for surveillance of various mosquito species. In this study we meliorated this Terminator-I trap by attaching 4 impregnated card board strips so that much more mosquito species should be captured with less effort.

Materials and Methods

Study design

This experiment was conducted inside animal house located behind SOS Zoology, Jiwaji University Gwalior (26.22° N, 78.18 °E), Madhya Pradesh (India) over thirty nights from 18 July to 16 August, 2017 and 16th January to 25th January, 2018 over 10 nights. The Gwalior District has a population of 2,030,543 according to the data provided by the Indian census, 2011 and an area of 5,214 km^2. In Madhya Pradesh the monsoon usually starts from the middle of June and continues till the middle of September. Diversity of mosquito species is seen abundant during rainy season.

Trap used

Traps used in both experiments were Grien's All Iinn Mosquito Trap/Killer, model Terminator-I.

Experiment-I: Two Terminator-I traps were used in which one acts as a control and other as experimental one. The experimental one is accompanied with four card board strips impregnated with different attracting substances like sugar, curd, protein powder and honey. Such substances were mixed, and a paste hence formed is applied on card board strips. After the application of attractant these strips were covered with polythene having holes on the attractant surface for emanations of aroma and easy handling. Now, these card board strips are ready for trap attachments.

Experiment-II: A number of sweet solutions (sugar, fermented sugar, honey, fermented honey) were used and applied on different card board strips. Such five strips were prepared in which Ist card board strip is dipped in water for emission of moisture as an attractant cue. The IInd card board is dipped in sugar solution, IIIrd card board strip in fermented sugar, IVth card board strip is accompanied with honey and Vth card board strip with fermented honey. In control traps non-impregnated strips were fixed. As in experiment-I, all these strips were wrapped with polythene cover having a number of pores for evaporation of fumigants and easy handling.

These strips hence formed were fixed with the help of adhesive tape along the four corners of the terminator-I mosquito trap. In this way one trap is accompanied with four attractant card board strips.

Trap placement

In Experiment-I, both controls as well as experimental trap was placed at the same location in one corner of the corridor of animal house at a distance of one meter, for thirty nights. The traps were set between 19:00 and 07:00 and collections were retrieved 3 hours later. In Experiment-II, a set of six traps (Control, water, sugar, fermented sugar, honey and fermented honey accompanied Terminator-I traps) were placed at a distance of 2 ft with one another in the corridor of animal house for ten days from January 16th – January 25th, 2018 between 19:00 and 07:00.

Collected dipterans as well as non-dipterans were placed in glass petridishes for counting and identification inside laboratory. These petridishes were placed in an oven for 12 hours at 60°C to remove moisture and then they were stored in air tight plastic containers for future use. All trapped insects were sorted out according to their orders including Diptera, Lepidoptera, Coleoptera, Hemiptera, Hymenoptera, Orthoptera. The dipterans trapped inside both traps include mosquitoes, sand flies, houseflies, psychodids and chironomids.

Statistical analysis

The data hence collected were put under statistical analysis like Mean, Standard Error and Karl Pearson's correlation coefficient (γ).

Results

Two Terminator-I traps used, were set at the same place where mosquito abundance was maximum. One provided with Additional Bio-attractant strips and other without strips. A significant difference was seen between these control and experiment traps. The experimental trap which is accompanied with strips attracts more mosquitoes and other species such as psychodids, ants and houseflies than the control one. Mosquitoes and ants were seen in large amount than other insects. Total mosquitoes collected during the study were 6909, among them 4999 were captured by the experimental trap *i.e.* accompanied with Additional Bio-attractant strips and remaining 1910 by the control trap as shown in Table-1. The maximum increase (~162%) was noticed in fermenting than non fermenting solutions as depicting in Table-2. Efficacy between control and experimental traps can be easily understood from Fig. 1. This is obvious from the current study that usage of additional bio-

attractants really increases sensory abilities of not only mosquitoes but also other harmful insects by tricking them with features that mimics human skin. Ants shown the maximum increase (~705%) in experimental trap than control trap, followed by beetles (~176%), cockroaches (~143%), chironomos (~123%), houseflies (~105%), psychodids (~92%), sandflies (~47.5%) and moths (~43%). From the overall catch of all mentioned insects, mosquitoes solely constitute 83% and rest by all other insects. Maximum catch about 72.39% is performed by meliorated experimental traps with additional bio-attractants and only 27.61% by control one. These additional bio attractant strips may release some kind of pheromones like (Z)-9-Hexadecenal that acts as an attractant and appetite booster for ants and hence maximum catch is seen in these ASB striped traps.

In experiment-II, only the mosquitoes were put into consideration and remaining insects were ignored. Total number of mosquitoes trapped were identified and distributed according to their sex. During ten nights, total mosquitoes caught were 1582. Among them 668 and 914 were males and females respectively. More females were attracted towards this additional bio-attractant strip fixed traps because a variety of physical and chemical cues are used by mosquitoes to search their hosts, oviposition sites and resting places. Among different types of attractants fermented honey baited trap catches maximum mosquito species followed by fermenting sugar, honey and sugar baited traps as shown in Table-3. Even plain water impregnated strips provided slightly better results than the control as shown in Fig. 2 and Fig. 3. Fermented honey may mimic equally to that of animal skin and hence maximum catch (~191%) was seen in the trap impregnated with fermented honey mats. Total catch as well as their percentage by these ASB stripped traps is shown in Fig. 4.

Table 1: Total number, Average ± Mean of mosquitoes and other insects collected from 17th July to 16th August, 2017.

		Mosquitoes	Psychodids	Chironomos	Housefly	Sand flies	Cockroaches	Moths	Beetles	Ants
Control	Total	1910	146	53	18	40	7	37	21	43
	Mean ±SE	63.67± 5.99	4.87± 0.85	1.77± 0.40	0.6± 0.17	1.33±0.32	0.23± 0.11	1.23±0.37	0.7± 0.20	1.43± 0.35
Experimental	Total	4999	280	118	37	59	17	53	58	346
	Mean ±SE	166.63±12.81	9.33± 1.36	3.93± 0.78	1.23±0.27	1.97±0.62	0.57± 0.18	1.77±0.32	1.93±0.36	11.53±1.58

Table 2: Total number, Average ± Mean of mosquitoes trapped by Terminator-I, from 17th July to 16th August, 2017.

Type of Trap	Range from Min. To Max.	Total Catch	Mean ± SE
Control	15 – 120	1910	63.67 ± 5.99
Experimental	48 – 290	4999	166.63 ± 12.81
Percent Increase	162%		

Table 3: Average total number of male and female mosquitoes caught in terminator-I during December and January- 2018 (10 nights).

S. No.	Types of attractants	Male	Female	Total	Percentage	Mean ± Error
1	Control	56	84	140		70 ± 14.03
2	Experiment-I Water strip	75	106	181	29.28%	90.5 ± 15.54
3	Experiment-II Sugar	107	142	249	77.85%	124.5 ± 17.54
4	Experiment-III Fermented Sugar	141	193	334	138.57%	167 ± 26.07
5	Experiment-IV Honey	117	154	271	93.57%	135.5 ± 18.55
6	Experiment-V Fermented Honey	172	235	407	190.71%	203.5 ± 31.58

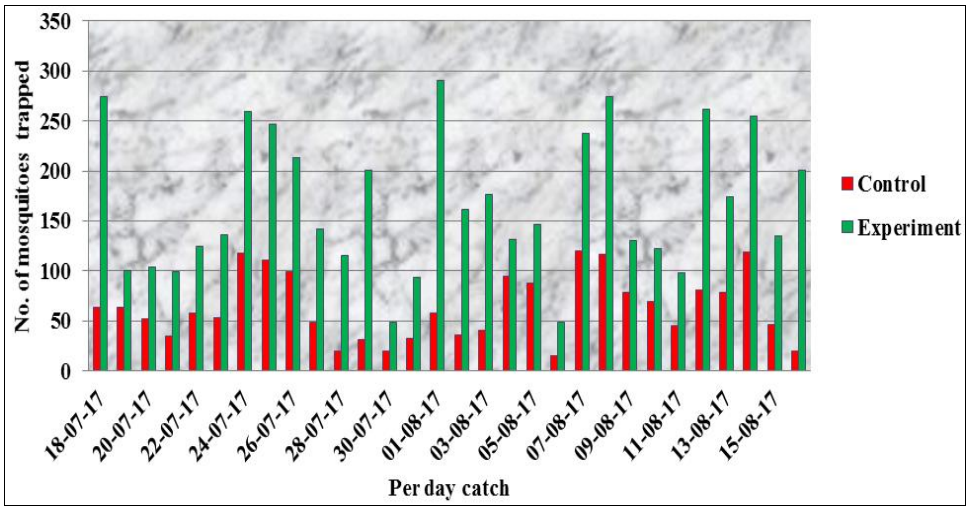

Fig 1: Comparison between total number of mosquitoes trapped in experimental and control Terminator-I.

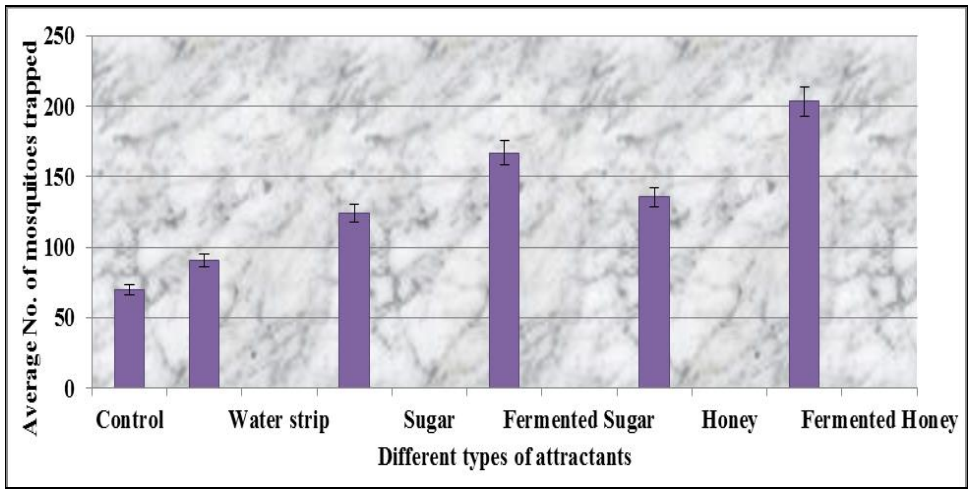

Fig 2: Average number of mosquitoes trapped with different additional bio-attractants.

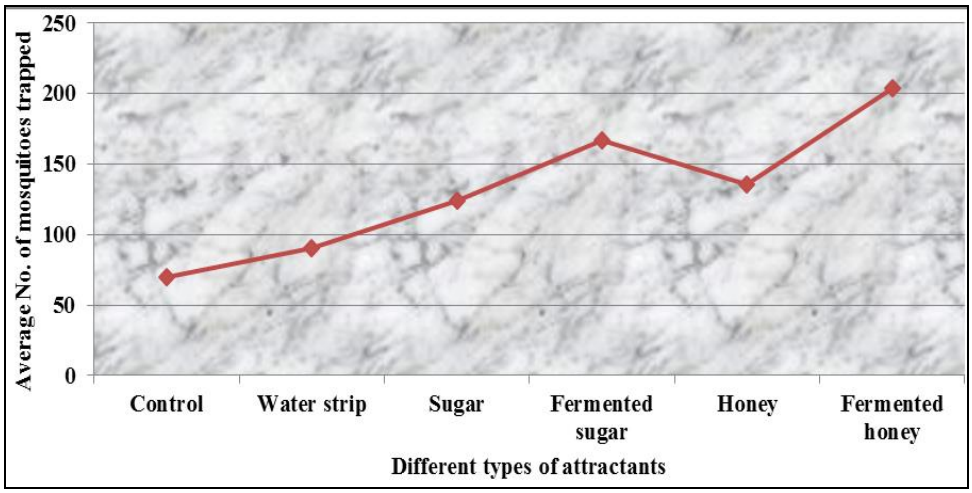

Fig 3: Attractive efficiency of different additional bio-attractants.

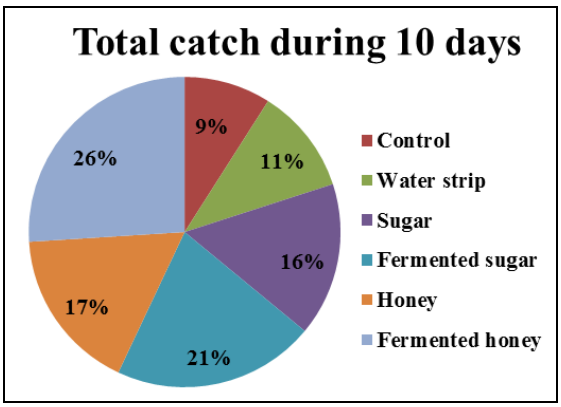

Fig 4: Attractive efficiency of different additional bio-attractants.

Discussion

A variety of physical and chemical factors like light, sound, temperature, perceived movement, exhaled air (CO_2), skin emanations (lactic acid, fatty acids, octenol, *etc*.) are used by mosquitoes to search their hosts, resting places and oviposition sites. Traps are generally used for vector and disease surveillance programmes. Photo catalytic reaction of titanium dioxide takes place in Terminator-1 mosquito trap that generates CO_2, heat and moisture which attract mosquitoes and some other insects to be entrapped and killed by air blowing. In the present study four different strips used were impregnated with different solutions in order to increase the attractive efficiency of these traps. From all these experimental traps the number of mosquitoes trapped was high. Besides, mosquitoes other insects especially ants was also seen in large quantity. From these findings we can say that mosquitoes are not only attracted to a particular source like UV-light but, a number of factors are responsible for attraction like CO_2, Octenol and Lactic acid.

Most national malaria control programmes use long-lasting insecticide-treated nets (LLINs) and/or indoor residual spraying (IRs) and there is a growing interest in environmental management and larval control [29]. However they do not consistently reduce malaria prevalence because even barely detectable low numbers of infective bites per person per year can be associated with malaria prevalence. Despite years of public health efforts and research progress, an effective vaccine against dengue virus is not yet available. For this reason disease prevention remains dependent on vector management and control strategies [30, 31]. The mosquito traps helped a lot to eradicate these noxious mosquito vectors and makes the environment free from diseases. Only adult mosquitoes are captured with these traps and not their aquatic stages like larvae and pupae. So, other additional measures should be employed to control these aquatic stages also. The adults as well as aquatic stages (eggs, larvae and pupae) were controlled to some extent with the help of ovitraps in 2013 by Bhat and Agrawal in the Jiwaji University campus Gwalior, Madya Pradesh.

The lack of viable new methods for vector control is one reason why integrated pest management (IPM) strategies have not been fully embraced and implemented [32]. Clearly, in order to control these vectors a convenient and ecofriendly method based on "attract and kill" principle were used. The experimental traps impregnated with mats or strips increases their efficiency by producing more carbon dioxide as well as moisture and food (nectar), mimicking aroma for both male and female mosquitoes. The results are clearly seen in Table 3. UV-light traps caught more mosquitoes than the traps with incandescent bulbs, but caught many insects other than mosquitoes requiring time-consuming separation and were unpopular with villagers. Encephalitis vector surveillance (EVS) traps has also UV-light source and when hung outdoor and baited with carbon dioxide caught few mosquitoes. CDC traps when placed at the same location baited with CO_2 or lactic acid caught large number of *Culex tritaeniorhynchus.* Three mosquito species *Aedes*, *Anopheles* and *Culex* were seen in these traps. The plain water impregnated mats provided slightly better results than the control. The reason may be maximum production of moisture from these traps. Hence, moisture also acts as an attractive source for mosquito populations and when used with other sources like CO_2 and lactic acid increases the attractive efficiency of mosquito traps.

Various authors performed many experiments from time to time on commercial available traps in which one act as control and others with some modifications as experimental. Hoel *et al.* in 2009 [33] tested two commercial mosquito traps, one as control and the other as experimental trap for the collection of *Aedes* mosquitoes. *Aedes* and *Culex* mosquitoes are also attracted towards octenol and L- lactic acid in a minute quantity [34]. In the present experiment *Aedes*, *Anopheles* and *Culex* mosquitoes are attracted in large amount as well as houseflies, sandflies, moths, beetles, Ants and cockroaches were also attracted and trapped in these ASB stripped traps.

There is seen a fairly high degree of correlation between male and female mosquitoes as the value (0.85) lies between +0.75 to +0.9, as described by Karl Pearson's correlation coefficient (γ) and Sir Francis Galton.

Conclusion

The experiment was not performed under controlled conditions but under natural conditions. The recapture success was higher in experimental traps. Interestingly, large numbers of mosquitoes were captured than other insects and the maximum number were seen during the replacement of old and dried strips. From the present study we can concluded that CO_2, lactic acid, heat and moisture emitting from these additional bio-attractant factor baited Terminator-I traps are responsible for the attraction of maximum number of dipterans and non-dipterans. Inclusion of fermenting solution is supposed to provide an extra source of carbon dioxide, moisture and food (nectar) mimicking aroma for non-gravid female and male mosquitoes. Here we can also say that single attractive source does not show maximum results. Hence in order to increase the attractive efficiency of mosquito traps various types of attractive cues should be used.

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