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Research Article

Efficiency and Persistence of Isotronic Repellent Devices in *Culex pipiens* and *Aedes aegypti* Vectors of Mosquito Borne Diseases

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Abstract

The repellency of ultrasonic devices against two species of mosquitoes, *Culex pipiens* and *Aedes aegypti* was evaluated in laboratory and open conditions. Observations with respect to sitting, biting, and blood feeding behavior *viz-a-viz* synthetic repellent was made in normal, fertilized and starved conditions in multiple replicates on two lines: with or without isotronic devices. The devices found least effective, no deterrent or decline could be observed by isotronic devices in biting behavior. The experiments extended to larger and open area corroborated the laboratory observations in this regard. In contrast, synthetic repellent registered a desired effect and protection for reasonable time was obtained.

Conclusion: The present devices are not a promising tool for repelling mosquitoes.

Keywords: Biting Behavior; Isotronic Devices; Mosquitoes; Persistence Repellent Efficiency; Swarming

Introduction

Mosquitoes are known vectors of the diseases transmitted by their bites, so their control is continued to be important as never before, and worst – number of mosquito-borne diseases are not on decline. Chemical control, mechanical control and even integrated control have not produced desired results. The result: commercially available devices or products are appearing intermittently in a variety of formats claiming repellency effects. Despite this, insect repellents are being tried simultaneously; conveniently categorized into – synthetic and plant derived repellents, whose volatility necessitate repeated reapplications, inviting thereby danger of overdosing. Essential oil impregnated candles as repellent even suggested [1]. Alternately, the use of diffuser devices for intermittent release of the active ingredients is proposed [2]. whatever the approach – cost, safety concern, and efficiency posed a constrained success, observed long before [3]. These reasons forces to look for electronic devices.

Seen species wise, more established chemical repellents can have lower efficacy against several *Anophelines*, and further, short persistence contributed to status quo [4,5] and then to another menace – the insecticide resistance [6]. Case in point; well-known insect repellents; DEET, IR3535 and KBR 3023 [7,8], apparently promising against *Anopheline*, but faced with the problem of persistent resistance. The KBR3023, advocated as a prospective insect repellent has another hazard of inflicting dermal toxicity [9]. The development led to a situation for trying insect repellent alternative devices such as topical repellents and electronic devices. These approaches are generally aimed at the average home owner and seriously lack scientific data to support the claim. Nonetheless, it is taken for granted that an electronic repellent device is a safe alternative compared to a toxic repellent. Could have been so, had it really been working. These techniques claim the electromagnetic or ultra-sound waves to repel mosquitoes. Effectiveness of such devices initially remain un-established, stemming from lack of scientific data on their efficiency. The

evaluation necessarily need not to be restricted to repellent activity – an acoustic evaluation and analysis and output extending into the ultrasonic range is also vital [10].

In this study we determine the effectiveness of three versions of isotronic mosquito repellent devices against some species of prevalent mosquitoes in different imaginable conditions. These species are implicated in many mosquito borne diseases. Observations are mainly aimed to determine whether the devices in scrutiny could provide the reliable and prolonged protection against mosquito bites. The approach is encouraged largely since, the American Mosquito Control Association is keenly interested in seeking the enquiries about such devices [10]. Tests on effectiveness were made on three electronic devices abbreviated as BW, MM and MI. Their potential is discussed in relation to claims for their mode of action.

Materials and Methods

Study Area and Collection Procedure

The experiments were conducted on two main species of mosquitoes. The larvae of *Culex* were collected – comprising all stages – from drains around the university using strainers the adults of *Aedes* collected from lawns by aspirators. The collections were brought to the mosquito rearing lab and used for raising the stock. An expert taxonomist identified the mosquitoes as two most frequently prevalent species; the *Culex pipiens* and *Aedes ageypti*. All stocks were kept in a control room under identical conditions (25 ± 2°C; RH 80 ± 5%; equal L/D). All the necessary rearing techniques have been followed as per earlier observations [11].

Larval stages in the population cages were provided with *Baker's yeast* while adults were fed on sucrose solution, 5%, soaked in cotton pads. These stocks were used for experimentation in the lab as: unstarved, starved and fertilized forms and each tested along two lines comprising without and with isotronic devices. The observations further grouped into: (1), lab conditions using small cages (1' x 1' x 1' size), and population cages (3' x 3' x 3' size); (2), large area using bathroom and hostel rooms; and (3), open areas. Four replicates were carried out for each condition spread at defined intervals segregated by a defined gap. Blood feeding was provided by hand exposure in the experimental conditions and through anesthetized albino using nembutal injection.

The stock independently raised to be employed in above experiments. This ensured homogenous response. The mosquitoes of *Culex* and *Aedes* were used in a stratified manner in the experiments at the rate of 200 and 500 of either species in small and large cages in captive situation. And, a constant 1000 mosquitoes were used for open space for each replicate. The number of mosquitoes landing on demarcated area on sidewalls, during swarming and on collector's hand were segregated in starved, fertilized, unstarved and biting conditions. As a case of natural condition, observation was repeated in room; where the bed was laid at a distance of 1.5m from window. Observations were made with the windows open. The devices were operated depending upon the requirement. Separate experiments were conducted using standard repellent (*goodnight*) available locally and the broad protection in each case was recorded. Each observation was repeated with electronic devices. This helped in a comprehensive comparison and objective analysis.

Detail of Devices

The detail of devices is summarized in (Table 1). All three were commercial sound generators, each with a range of frequency outputs and one even have a dual function i.e. provided with ultrasonic and UV provision. The products used for repellency were: L2 te Mobile Isotronic (MI); Electronic Flying Insect Exterminator (MM) and Black and White Isotronic (BW).

Device brand		Size (Cm)	Range (m)	Battery Required	Frequency (KHz)
 BW	Black and White Isotronic (HV)	L: 76mm, W: 70mm H: 94mm W: 99g	30	4 X AA Micro batteries	23 ± 10%
 MM	M + M (HV + UV)	L: 124mm, W: 76mm, H: 43mm, W: 135g	30	3 X AAA Micro 850 mAH rechargable	7-23 ± 10%

 MI	L2 te mobile isotronic	L: 72mm, W: 67mm, H: 28mm, W: 66g	Jun-30	2 X AAA Micro	7000 ±10%
*Truly Pest Solution Pvt. Ltd., West Bengal, India					

Table 1: Technical details and characteristics of the three isotronic anti-mosquito devices evaluated*.

Statistical Analysis

Each observation was made in four replicates. The results were analyzed using $\bar{X}$ ± SE.

Ethical Considerations

The volunteers were recruited after obtaining informed consent. Before the start of the experiments, they received proper vaccination against yellow fever. During the tests, each followed a prophylactic regime against malaria, and were under regular and strict clinical surveillance. No case was detected during the course of the study. The protocol received formal approval from the ethical committee of the University.

Results

Initial observation started on swarming behavior and landing tendency of the mosquitoes spread over for four prime times during peak hours of evening (6-9 PM) with a gap of 10 mins after every observation. The separate sets were made to see the biting behavior of mosquitoes. Number of mosquitoes biting/blood feeding were counted as the devices were on and repeated without devices. Later, it was extended to open field in the lawns. Here also all experiments involved sound devices and for comparison, separate observations without devices were made: The results are summarized in (Table 2).

Species	Space type	Type of condition	Number of Mosquitos			
			Total	demarcated area* ($\bar{X}$ ± SE)	Sidewalls* ($\bar{X}$± SE)	Swarming* ($\bar{X}$± SE)
Culex	small[1]	nd	800	62.50 ± 9.03	119.75 ± 14.10	17.75 ±7.79
		BW/MM	800	64.75 ± 12.83	112.25 ± 10.99	23.00 ±9.72
	large[2]	nd	2000	154.00 ± 13.68	279 ± 27.68	67.00±10.27
		BW/MM	2000	157.00±14.44	297.75 ± 15.53	45.25 ± 11.23
Anopheles	small[1]	nd	800	142.00±16.05	22.75 ± 6.21	35.25±11.08
		BW/MM	800	131.75±23.74	34.00 ± 7.79	34.25 ± 10.11
	large[2]	nd	2000	267 ± 17.06	194.50±20.06	38.50±10.16
		BW/MM	2000	245.75±10.97	197.25±16.56	57.00 ± 9.75
	Open field[3]	nd	1000	no response		
		BW	1000	no response		
		MM	1000	no response		

Table 2: Observations on swarming, landing and biting behavior of mosquitoes in different conditions (with no device, nd) and with devices (BW, MM).

The idea was in getting the effectiveness of the protecting ability of the devices. This data is separately shown in (Table 3). Independent studies were undertaken using the standard repellent and the relevant information are summarized in (Table 4).

Species	Space type	Condition type	Number of Mosquitoes		
			total	Biting* ($\bar{X}$± SE)	non biting* ($\bar{X}$± SE)
Culex	small[1]	nd	800	170.50 ± 8.21	29.50 ± 6.84
		BW/MM	800	172.25 ± 9.33	27.75 ± 7.69
	large[2]	nd	2000	422.50 ± 12.95	77.50 ± 7.84
		BW/MM	2000	450.50 ± 20.38	49.50 ± 8.71
	Open[3](Hostel)	nd	4000	660.75 ± 30.10	339.25 ± 9.39
		BW/MM	4000	678.25 ± 26.85	321.75 ± 7.45
		Goodnight	4000	286.50 ± 30.42	713.50 ± 8.01
Aedes	small[1]	nd	800	174.00 ± 19.71	26.00 ± 8.00
		BW/MM	800	185.25 ± 28.35	14.75 ± 9.35
	large[2]	nd	2000	433.00 ± 17.71	67.00 ± 8.77
		BW/MM	200	417.75 ± 12.37	82.25 ± 7.39
	Open[3] (Hostel)	nd	4000	852.5 ± 21.71	147.50 ± 9.01
		BW/MM	4000	818.25 ± 19.98	181.75 ± 9.24
		Goodnight	4000	718.25 ± 19.78	281.75 ± 7.65
	Open[3] (Garden)	BW	1000	591.00 ± 11.20	409.00 ± 6.02
		MM	1000	699.00 ± 21.10	301.00 ± 8.21
		MI	1000	920.00 ± 21.37	80.00 ± 8.73
	Open[3] (Canteen Backyard)	nd	4000	730.25 ± 22.53	269.75 ± 8.21
*four replicates; [1]200/observations; [2]500/observations; [3]1000/observations					

Table 3: Biting behaviour of female mosquitoes in no device (nd) and with devices (BW, MM) using different space types in starved, unstarved, fertilized conditions.

Devices	General Observation On Mosquitoes		
	biting	escaped	unaffected
BW[1]	severe	none	all
MM[1]	severe	none	all
Repellent (goodnight)[1]	none	all	none
[1]1000/observation			

Table 4: Comparative efficiency of devices *viz-a-viz* an established chemical repellent (*goodnight*).

Observations

The swarming and flight behavior, and the landing pattern showed that the mosquitoes which settled on demarcated area in cages was a random phenomenon and clearly remain undisturbed even as the isotronic devices were in use. The distance also seemed not to be mattered. Hence, when comparing observation in captivity or open space such as of lawn observations, make no change in behavior or attitude apparently. The claim of protection against mosquitoes up to 30 meter was found totally incompatible as either species continued to be a source of irritation. The swarming and settling behavior in the normal conditions, or when devices were in use made no difference in the desired behavior. Observation on the choice of backgrounds – distinguished as white and black, also bore no effect. In each case almost equal number of mosquitoes were seen in captive conditions.

Biting behavior was observed in the females during unstarved, starved on fertilized or unfertilized females was tested when the instruments on and observed again when instruments off gave no clear-cut response on biting pattern. No decline was found in biting activity of mosquitoes with instruments functioning in all cases of captivity or in lawn with either of species. The claim of protection of bites using devices could not be ascertained. Thus no significant difference in blood feeding activity. A positive control using a chemical repellent, *goodnight* made marked effect in protecting bites.

Discussion

The EMRs emit a high frequency buzz inaudible to the human ear, but with claim to repel the mosquitoes [12]. These devices, are reported to mimic the sounds of male mosquito wing beats or the sound of predatory dragonflies thereby keep the biting females away. The alleged repellent action is based of sound against mosquitoes mimic the flight sound of males, hence females repelled, once inseminated [13-15]. On the contrary, it has been reported that male mosquitoes are attracted by the female flight and females have a very weak sensitivity for such sounds [16-19]. Not very late, a detailed work by Fumihiro [20] has taken an elaborate view of acoustic signals for subjective evaluation. However, the basic fact remains that – most EMRs claim effective by mimicking the sound waves produced by the beating of male mosquito's wings, against females biting humans – is even now a matter of debate. The marketing companies present no scientific data to substantiate the fact. The high frequency of mosquito signal nearing 17 kHz, barely audible to young people not to older, and mosquito signals with audibility range 15 kHz – 17 kHz – is made out to be the basis of acoustic instruments [20]. The frequency analysis decides the characteristic of uncomfortable signals [21,22] and useful to develop and assess the speech quality [23].

Notwithstanding the technical details, none of the devices have any mosquito repellent ability in the present observations, and thus failed to reduce the biting behavior of mosquitoes not to talk about any potential to eliminate mosquitoes. On the contrary biting even elevated despite the devices. With reference to availability of space, observations in captivity or larger area also have no bearing on many a behavior when isotronic devices on. Rather, in occasional cases the repeated biting was experienced by the test subjects inspite of the devices operated. In condition where mosquito repellent used, for comparison sake, biting declined drastically, almost to a nonexistent level providing an effective protection observed for a considerable time. The previous studies on Electronic Mosquito Repellents (EMR) bear testimony to the conclusion drawn from present study [10,12]. The repellent hypothesis based on the hearing in females is itself debatable. Some authors do reported a rather weak hearing ability of the females [17-19]. On the contrary, the hearing system of males is relatively strong, since, the plumose antennae enables males to detect the vibration in the environment and the sound of female mosquitoes more efficiently [18]. No wonder then the present outcome of results found the EMRs, a poor repellent. In the present case there is another side effect of serious concern. The device (MM) combining ultrasonic and UV light induced headache if the person standing in its range, including irritation and pain to the eyes. An uneasy sensation due to sound with BW device was also experienced.

The studies on field based situation in natural setting and even laboratory based observation – the conclusion is unanimous: there is no difference in the number of mosquitoes landed on the bare body parts of human subjects with or without an EMR. A few review articles too have arrived at similar observation and opined that: the EMR, are mostly ineffective in repelling mosquitoes [24,25]. In all fairness thus, the acoustic devices despite claims continued to be ineffective. In this situation, the initiative appears to be shifting back to the natural plant extracts of late [26-33]. However, it has done little to deter the manufacturers for marketing EMRs. This may likely lead the consumers not using other protective measures and the trend could result in an increased risk of infection with mosquito-borne diseases [34]. Irrespective of the scientific view and research findings, EMRs are still widely promoted and used by the public.

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