A Laboratory Investigation into Oviposition Responses of *Aedes aegypti* to Some Common Household Substances and Water from Conspecific Larvae

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Abstract

Background: The surveillance of Dengue vector - *Aedes aegypti*, is routinely done by using ovitraps (a dark container filled with water and a wooden paddle/towel cloth provided as a oviposition substrate), which indicates the extent of *Aedes* activity in an area. **Method:** The study evaluated the oviposition responses of laboratory bred gravid *Aedes aegypti* to commonly available household substances by use of ovitrap method.

Results: Oviposition avoidance or deterrence was exhibited by gravid *Aedes* to ovitraps baited with salt, fenugreek, vinegar, lime juice, hibiscus leaves, radish and curry leaves, whereas positive oviposition response was noticed to waters from conspecific (of the same species) larvae and cumin seeds baited ovitraps.

Conclusion: The study findings may be utilized by researchers for further chemical analysis of the active ingredients of the substances found promising with a view to explore the possibility of their use by community and individuals for *Aedes* surveillance and control.

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Key Words : *Aedes*; Dengue; Oviposition deterrence/ attraction

Introduction

Dengue is transmitted by the bite of an infective mosquito vector, *Aedes aegypti*, which breeds in domestic and peridomestic water containers. This container inhabiting mosquito species is known to follow visual or olfactory cues to appropriate water containers and then use both chemical and physical factors in the waters for selecting it for oviposition [1]. Some of the commonly used cues are colour and optical density of water, oviposition substrate, temperature, olfactory cues and chemical cues provided by mosquito larvae [2]. Ovitrap (usually a dark coloured container filled with water and provided with a towel cloth/ wooden paddle for oviposition) are the preferred surveillance tool for monitoring *Aedes* activity; which can be made more attractive to the gravid females by the addition of an attractant. These ovitraps can also be provided with an insecticide like the Insect Growth Regulators for effectively controlling the breeding. Alternatively, addition of oviposition deterrents in stored water containers would prevent domestic breeding; however, it needs to be implemented by the entire community for prevention of *Aedes* borne diseases.

The larvae of *Aedes* are known to release substances in the aquatic environment, which render the water attractive to gravid conspecific females [3,4]. However, high larval density deters gravid mosquitoes from ovipositing in these habitats [5]. The presence of bacteria is also known to be attractive to the gravid mosquitoes. The oviposition responses of *Aedes aegypti* to oak leaf infusion and *Plagiorchis elegans* infected larvae have been extensively studied [6,7]. Conflicting findings regarding oviposition responses of gravid *Aedes* to predacious copepod *Mesocyclops longisetus* have been reported [8,9]. However, there has been no study on the oviposition responses of gravid *Aedes aegypti* to commonly available household substances. The aim of this study was to evolve a simple ovitrap using the commonly available household substances for monitoring *Aedes aegypti* with a view to provide an early warning for initiation of vector control activities to prevent Dengue outbreaks. Once these substances are identified as oviposition attractants or deterrents (avoidance), they can be used for monitoring and management of *Aedes*.

The current work reports oviposition attraction/ deterrence of common household substances to gravid

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Aedes aegypti and the oviposition responses to water from conspecific normal larvae.

Material and Methods

Mosquitoes - *Aedes aegypti* were reared in the laboratory by standard rearing procedures. Larvae @ 600 were reared in enamel trays (40 x 30 x 5 cm) at $27 \pm 1^\circ\text{C}$, and were fed on yeast tablets. Adults were kept in cages. Males were fed on 10% glucose soaked cotton pads while females were fed on rabbits and allowed to develop eggs prior to testing.

Treatment - Various household substances (1 gm or 1 ml) in 100 ml of distilled water were offered to gravid females in 250 ml enamel bowls with a strip of filter paper for oviposition. The various substances included in the study were: (i) salt (ii) fenugreek seeds, soaked and crushed (iii) garlic crushed (iv) ginger crushed (v) green chilly crushed (vi) asafetida (vii) sugar (viii) turmeric powder (ix) red chilly powder (x) cumin soaked and crushed (xi) vinegar (acetic acid) (xii) lime juice (xiii) *Hibiscus* leaves crushed (xiv) *Lantana* leaves crushed (xv) curry leaves crushed (xvi) beetroot crushed (xvii) radish crushed (xviii) Potato 1cumm (xix) Onion 1cumm and 100 ml of water from *Aedes* larval rearing trays with larvae (10 larvae) and without larvae. Distilled water was kept as control to check the oviposition response.

Laboratory bioassay - The experiment was conducted during the months of August and September. About 50 gravid females held in cages (77 by 60 by 60 cm) were offered ovitraps with various household substances for 24 hours. The ovitraps were thereafter examined and the eggs counted on the filter paper. All the ovitraps were placed in the centre of the cage and rotated for removing positional bias. The experiments were replicated three times with fresh ovitraps and separate batches of gravid females to ensure no bias. Ovitrap with oviposition were graded in relation to ovitrap with maximum number of eggs. Those ovitraps where no oviposition took place were considered exhibiting oviposition avoidance/deterrence. The various ovitraps were also subjected to pH analysis to ascertain whether pH could possibly play a role in influencing gravid females in choice of ovitraps.

Results

The results presented in Table 1 indicate oviposition avoidance/deterrence when ovitraps were baited with salt, fenugreek, vinegar, limejuice, hibiscus, radish and curry leaves. These ovitraps had pH ranging from 3 to 6.73. However, other ovitraps with same or higher pH did not elicit similar response from the gravid females. The positive oviposition response to distilled water (150 eggs) further strengthens the assumption that the above mentioned substances offered true avoidance/deterrence to the gravid *Aedes*. The results presented in Table 2 indicate that ovitraps having larval rearing water with 10 larvae (conspecific) was the most preferred ovitrap (850 eggs), as compared to the same water without larvae; the pH remaining same (7.22) for both these waters. Cumin seeds also rendered the water fairly attractive for oviposition (600 eggs). Other ovitraps offered low to moderate attraction to the ovipositing female vis-à-vis *Aedes* rearing water with 10 larvae.

Table 1

Treated ovitraps eliciting oviposition deterrence responses from gravid *Aedes aegypti*

Ovitrap	Mean no. of eggs	pH
Distilled water (Control)	150	5.70
Salt	Nil	6.14
Fenugreek seeds	Nil	5.94
Hibiscus (Shoe flower) leaves	Nil	6.73
Curry leaves	Nil	6.00
Radish	Nil	5.63
Lime juice	Nil	3.03
Vinegar	Nil	4.00

Table 2

Treated ovitraps eliciting positive oviposition responses from gravid *Aedes aegypti*

Ovitrap	Mean no. of eggs	% oviposition	pH
Distilled water (Control)	150	18	5.70
Asafetida	15	2	4.92
Beet root	45	5	6.28
Lantana (Common hedge plant)	60	7	7.43
Sugar	70	8	6.79
Green chilly	80	9	7.02
Ginger	110	13	6.76
Ajwain	110	13	6.76
Potato 1 cumm	211	25	6.93
Onion 1 cumm	240	28	2.73
<i>Aedes</i> rearing water without larvae	325	38	7.22
Red chilly powder	340	40	5.09
Cumin seeds	600	76	6.63
<i>Aedes</i> rearing water with larvae (10)	850	100	7.22

Discussion and Conclusion

The choice of an oviposition site by gravid females is the principal factor responsible for the distribution of the mosquitoes in breeding sites and their subsequent dispersion in different geographical areas. Monitoring of *Aedes* abundance and its distribution is critical in predicting Dengue epidemics or its risk in an *Aedes* active area. Many workers have studied grass and hay infusions as an oviposition attractant for monitoring *Aedes* activity. This study examines the oviposition attraction/deterrence of common household substances for their use in ovitraps to monitor *Aedes* activity as well as form an effective and simple control tool.

The pH of the various ovitraps was not found to influence the oviposition responses of the gravid females, indicating thereby, the role of some other factor released by various substances used in the ovitraps which influenced the oviposition responses. The attractiveness of *Aedes* larval rearing waters to the ovipositing female is well documented [6]. The study findings indicate that presence of larvae in rearing water rendered the water

more attractive to the gravid mosquitoes as compared to the same water without larvae; indicating presence of certain other chemical responses offered by the larvae and perceived by the female as an encouraging signal for oviposition. The same has been corroborated by other workers [10,11]. The positive oviposition response of gravid *Aedes* to ovitrap baited with Cumin seeds (76% oviposition) necessitates a closer look at this household substance. There is a need to chemically analyze the substance with a view to study the effect of its active ingredients on the oviposition responses of *Aedes*.

The total avoidance/ deterrence exhibited by gravid *Aedes* to ovitraps baited with fenugreek seeds, hibiscus, radish and curry leaves is very interesting and calls for further study and response evaluation. It will also be pertinent to check whether the females of other *Aedes* species share these responses. The oviposition deterrent effect of salt is a well-known fact and is widely recommended for prevention of *Aedes* breeding in vases and other small containers.

The findings of the study have implications on the monitoring of *Aedes* breeding wherein these household substances (oviposition attractants) may be utilized in ovitraps instead of plain water or hay infusion. However, the community based application of these laboratory generated findings needs to be established by appropriate field studies. Researchers working in the field of screening plants and their products for anti-insect activity may undertake chemical analyses of the active ingredients of the substances found to have oviposition avoidance/ attraction as brought out by the study. Purification of the active ingredient in a form or preparation that can be used for *Aedes* surveillance by Institutes or Organizations where large number of water containers/ breeding may be present needs to be undertaken as crude extract preparation may become cumbersome for large scale use.

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