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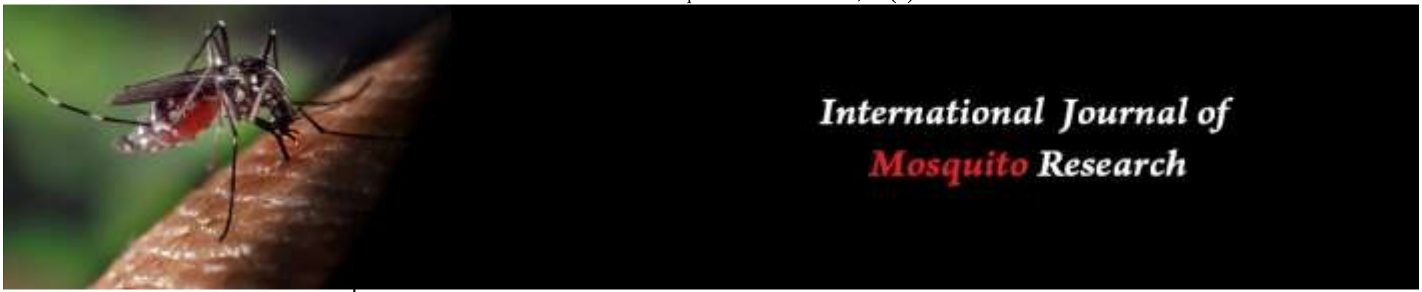

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Susceptibility status of dengue vector *Aedes aegypti* against the pyrethroids and organophosphate insecticides in Jaipur (Rajasthan) India

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Abstract
The threat of mosquito-borne diseases has increased, which makes mosquito control a key concern worldwide. *Aedes aegypti* control is now more important than ever due to the outbreaks of Zika, Dengue, and Chikungunya. Furthermore, the excessive use of synthetic insecticides in mosquito control operations has led to significant levels of insecticide resistance, which has negatively impacted both human health and the environment. This research was intended to assess *Ae. Aegypti* susceptibility to the two different kinds of adulticides because dengue has grown prevalent in the Rajasthan. According to the CDC procedure, adult bioassays were carried out using lambda-cyhalothrin, Malathion, permethrin, deltamethrin and observed complete mortality to all insecticides from the Jaipur district of Rajasthan. Thus, at CDC recommended diagnostic doses, the current findings show 100% susceptibility to deltamethrin, permethrin, lambda-cyhalothrin, and Malathion. As a result, additional research should be planned to examine the regional diagnostic concentrations for several insecticides for the state of Rajasthan.

Keywords: *Aedes*, dengue, insecticides, resistance, susceptibility

Introduction

More than 100 nations around the world are prone to mosquito-borne diseases, which annually affect millions of people worldwide. *Aedes aegypti* is regarded as the most dangerous Arbovirus vector in the tropics, wreaking havoc with a variety of diseases like Yellow fever, Dengue, Zika, and Chikungunya [1]. One of the most pervasive *Aedes* borne viral illnesses affecting people in the tropics is dengue infection. Dengue fever has recently become a serious public health issue in India, as it is rapidly spreading to new locations too. According to National Vector Borne Disease Control Programme there were 1, 93,245 dengue cases in total in the year 2021, with 346 fatalities in India. With increase cases in 2022, there were 2, 33,251 dengue cases reported from India, with 303 fatalities. *Aedes aegypti* has also drawn a lot of interest from researchers due of the recent suspected cases of Chikungunya in India, with a projected total of 1, 48,587 cases in 2022 [2].

Transmission of the dengue virus has significantly grown in urban and semi-urban areas and has even spread to rural areas, raising serious public health concerns. Despite the lack of an effective vaccine against these diseases, the only option is to reduce the mosquito vector through widespread larval mortality [3, 4]. The most effective strategy for reducing mosquito populations below critical levels and breaking the cycle of disease transmission is the main focus of this strategy. The removal of their breeding grounds, the employment of different biological agents, the release of sterile insects, etc., are just a few of the control strategies that have been developed and used to date [5].

For the past few decades, India's vector control programs have used a variety of insecticides from various groups extensively. But the sporadic and ongoing use of insecticides has led to the emergence of resistance in mosquito vectors [6].

The most recent studies on that species susceptibility to the various insecticides in use are necessary for developing an effective mosquito management program. Since, it is common knowledge that improper insecticide uses against insect pests can result in environmental contamination and adverse effects on humans and non-target species, it is essential to assess the toxicities of various insecticides when developing control tactics.

It is necessary to evaluate the present insecticide susceptibility status for the larvae of *Ae. aegypti* due to a lack of baseline susceptibility information, the ongoing upsurge in *Aedes*-borne diseases, and rise in insecticide resistance in this species. The current research thus makes an effort to obtain a complete depiction of the susceptibility in larvae of the Dengue disease vector against two different classes of insecticides pyrethroids and organophosphate. The investigation will provide advance knowledge and more precise picture of *Ae. aegypti* vulnerability. It will assist in developing an effective mosquito control plan, which is crucial today.

Material and Methods

Study area

The research was carried out in Jaipur city of state Rajasthan during the year 2021 and 2022. The study area was chosen based on the dengue cases that the NCVBDC, Rajasthan, India, reported during the previous five years. The study area, which is the tenth most populated city in the nation, has a hot, semi-arid environment that is affected by the monsoon, with long, extremely hot summers and short mild to cool winters.

Mosquito sampling methods

Throughout the research period, *Aedes* mosquito were collected with all stages of life cycle. Larval samples were randomly obtained from several sites of study area including residences, markets, nurseries, buildings, and parks. This study area is cover approximately 180 KM^2 between 26°48' N to 26°57' N and 75°41' E to 75°51' E (Fig. 1).The larval habitats of the *Aedes* species has chosen for sampling i.e. earthen receptacles, cement tanks, tree holes, plastic containers, clay pots, plastic tanks, metal drums, cement tanks and used tires used to hold water, in or out of houses or nearby them. A number of sample techniques, including pipetting, dipping, and flash lighting (based on the kind of container and site) were used for collection (Fig. 2).

Fig 1: Study site location map from Jaipur

Fig 2: Pictures depicting the Collection, Identification, and susceptibility bioassay processing

Mosquito rearing and identification
Aquatic stages (larvae and pupa) of *Aedes* mosquito breeding areas in the study area were sampled as part of field and laboratory studies. The juvenile stages (F^0 -generation) were obtained from a variety of breeding locations, including drains, storage tanks, tires, tree holes, chopped bamboo, building sites, and flower pots. Larvae or pupae from every available breeding location were collected from each sample site and taken to the laboratory, Department of Zoology, University of Rajasthan, Jaipur. Some of these fourth instars were moved into cages and raised up to the adult stage under conventional conditions (Temperature 25 ± 2 °C; Relative humidity 70-80%). By referring to the common morphological characteristics, the adults were recognized [7].

CDC Bottle Bioassay
The CDC bottle bioassay was carried out in accordance with the prescribed procedure of CDC at recommended diagnostic doses of insecticides [8]. The identified *Ae. aegypti* adults were subsequently employed for insecticide susceptibility bioassay. Permethrin, malathion, lambda-cyhalothrin, and deltamethrin stock and working solutions were created in accordance with CDC recommendations [8]. The glass containers of 250 ml in volume were all coated with the appropriate insecticide preparations. Three bottles were treated with each insecticide for the bioassay (Deltamethrin: 10ug/ml, Lambda-cyhalothrin: 10ug/ml, Permethrin: 15ug/ml, Malathion: 50 ug/ml), and one served as the control coated with acetone. In each covered bottle, 20-25 mature female mosquitoes were placed for one hour. Up to 120 minutes, the mortality was noted every 15 minutes. If the mosquito was unable to stand or fly, it was considered as dead (Fig-3).

Data Analysis
Mortality rates recorded during bioassays were analysed according to the CDC bottle bioassay criteria [8]. The populations of *Ae. aegypti* were classified as “resistant” if less than 80% mortality was observed, as “suspected resistant” if mortality were found between 80% to 97% and “susceptible” for more than 97% mortality rate. The bioassay that resulted in higher than 10% mosquito fatality in control bottles revealed that the mosquitoes were improperly chosen, therefore they were discarded and repeated. However, if 3 to 10% mortality occurred in control experiments, Abbott's method was used to correct it. [8].

$$\%\ \text{Corrected mortality} = \frac{\%\ \text{Test mortality} - \%\ \text{Control mortality}}{100 - \%\ \text{Control mortality}} * 100$$

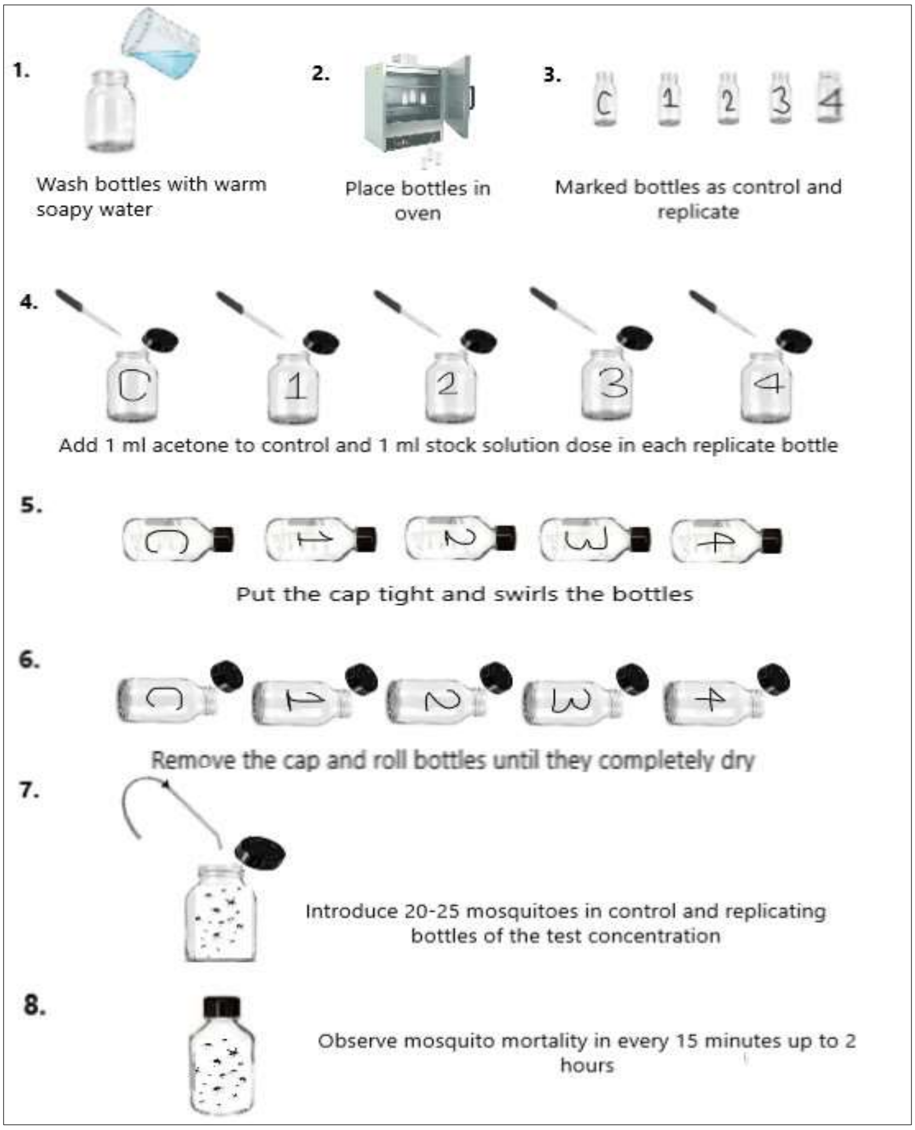

Fig 3: Schematic representation of CDC Bottle bioassay

Result

The susceptibility test results for *Ae. aegypti* mosquitoes to the different diagnostic dose and diagnostic time against two groups of insecticides are given in Table 1. The mortality rate of F_0 adult mosquito strain in control bottles was less than 3% and no correction of the mortality rate was then required. Tests performed with pyrethroids (deltamethrin, permethrin, lambda-cyhalothrin) and organophosphate (malathion) in 2021 and 2022 on *Ae. aegypti* populations collected from Jaipur, found susceptible to all four selected insecticides suggesting a resistance.

Table 1: Susceptibility of *Ae. aegypti* to distinct insecticides from Jaipur, Rajasthan

Insecticide	Insecticide concentration (ug/bottle)	Diagnostic time (minutes)	No. of mosquitoes exposed		No. of mosquitoes dead		Status
			Test	Control	Test	Control	
Deltamethrin	10	30	75	25	75	0	Susceptible
Permethrin	15	30	75	25	75	0	Susceptible
Lambda-cyhalothrin	10	30	75	25	75	0	Susceptible
Malathion	50	30	75	25	75	0	Susceptible

Discussion

The use of various insecticide classes, including pyrethroids, organophosphates, organochlorines, and carbamates, to control mosquitoes has been extending over many years. The issue of resistance in mosquito vectors is a result of the widespread usage of these insecticides. In order to develop an efficient approach for controlling mosquitoes, it is crucial to assess the susceptibility of several synthetic insecticides before choosing the most effective ingredient.

In the current study, an effort was made to determine the level of susceptibility of *Ae. aegypti* in the Jaipur District of Rajasthan, India, against four different insecticides that belong to two different classes: organophosphates (Malathion), and pyrethroids (Lambda-Cyhalothrin, Deltamethrin, and Permethrin). All four insecticides tested in this bioassay for insecticide susceptibility had 100% susceptibility results at the CDC-recommended diagnostic doses. Similar to this study, reports of *Aedes* mosquito susceptibility to pyrethroids and organophosphates have reported from Delhi, Punjab, Assam, and Jharkhand in India [3, 4, 6, 9, 10]. All major classes of insecticides, including malathion and deltamethrin, were found to be toxic to *Ae. aegypti* in Maharashtra, Puducherry, Kolkata, and Gujarat [11, 12, 13, 14]. *Ae. aegypti* was discovered to be sensitive to lambda-cyhalothrin, propoxur, fenitrothion, malathion, deltamethrin, and permethrin in southern India [15]. Research on the relative susceptibility of *Ae. aegypti* to synthetic pyrethroids and organophosphates in the arid region of Rajasthan (Jodhpur) also showed that the *Aedes* vector population was more sensitive to deltamethrin and more resistant to malathion [16]. *Aedes* species were discovered in another investigation from Andhra Pradesh to be resistant to malathion. Studies from West Bengal, Bengaluru, Kerala, Delhi, Tamil Nadu, and Andaman and Nicobar also discovered resistance to pyrethroid insecticides (deltamethrin, lambda-cyhalothrin, and permethrin) [17, 18, 19, 20, 21].

Rajasthan still lacks information on the local diagnostic concentrations of several insecticides. Therefore, additional research is advised on the mapping of the susceptibility status of dengue vectors to the insecticides utilized by the NVBDCP in the vector control program on a regional basis.

Conclusion

This study assessed the *Aedes aegypti* insecticide susceptibility status in the city of Jaipur, Rajasthan. It demonstrated that, *Ae. aegypti* is completely susceptible to lambda-cyhalothrin, malathion, permethrin, and deltamethrin. These findings might be useful in directing insecticide-based control methods. Although there is a paucity of understanding regarding the regional diagnostic concentrations for several insecticides in Rajasthan, there is an essential necessity for wider surveillance of insecticide resistance in *Aedes* mosquitoes. Understanding the mechanism of resistance and creating adequate diagnostic tools would enable effective control and the sustainable application of insecticides.

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