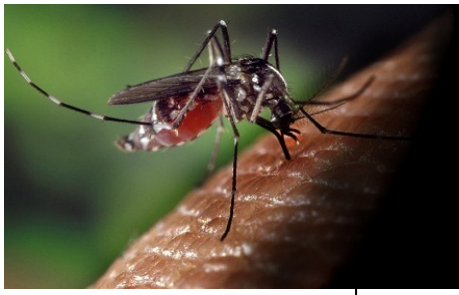

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Efficacy of various larvicides on ecologically different populations of *Aedes albopictus* from Kota and Barmer regions of Rajasthan

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Abstract
Aedes albopictus, also known as the Asian tiger mosquito, is a vector of several arboviruses that pose significant public health threats globally. To control these disease vectors, various chemical and biological methods have been employed, with reports indicating the development of resistance to conventional insecticides. In this context, this study aimed to assess the susceptibility of *Aedes albopictus* populations from the Barmer and Kota regions in Rajasthan, India, to three distinct larvicidal agents: temephos, neem oil, and *Bacillus thuringiensis* var. israelensis (*Bti*). The study utilized third instar larvae and conducted bioassays with different larvicides. The results revealed that the Barmer strain of *Aedes albopictus* displayed greater susceptibility to temephos, neem oil, and *Bti* compared to the Kota strain. Temephos, a conventional larvicide, exhibited comparable efficacy in both populations. Neem oil, derived from *Azadirachta indica*, demonstrated broad-spectrum larvicidal activity but with a notable 1.27-fold greater resistance observed in the Kota strain. In contrast, *Bti* displayed high efficacy in both populations, with the Barmer strain showing 1.6-fold greater susceptibility. The research emphasizes the importance of environmentally friendly larvicides and community-driven vector control strategies in mitigating the impact of mosquito-borne diseases, highlighting the need for region-specific approaches. Understanding resistance mechanisms and optimizing eco-friendly interventions are crucial in the fight against vector-borne diseases.

Keywords: *Aedes albopictus*, susceptibility, temephos, neem oil, *Bacillus thuringiensis*

1. Introduction
The *Aedes albopictus* mosquito, commonly known as the Asian tiger mosquito, is a vector of several arboviruses, including dengue, chikungunya, and Zika viruses, making it a significant public health concern worldwide [1]. This mosquito species gets direct and indirect exposure to various insecticides due to closeness of civil area and use of household insecticide which may lead to the development of tolerance or resistance to insecticides. Control of *Aedes albopictus* populations is vital in reducing the risk of disease transmission, and a variety of chemical and biological methods have been employed for this purpose. Several reports reveal the development of resistance in mosquitoes particularly in *Aedes albopictus* against temephos, organophosphate malathion, pyrethroids deltamethrin and permethrin [2-4]. Among these methods, temephos, neem oil, and *Bacillus thuringiensis* (Bt) have gained prominence due to their potential as environmentally friendly alternatives to synthetic insecticides [5-7]. Temephos is an organophosphate larvicide commonly used to control mosquito larvae in breeding sites such as water containers [5]. Neem oil, derived from the neem tree (*Azadirachta indica*), has been shown to possess larvicidal and repellent properties, making it an attractive option for eco-friendly mosquito control [6]. *Bacillus thuringiensis* is a naturally occurring soil bacterium that produces toxins harmful to mosquito larvae, making it a biological alternative for vector control [7]. Mittal *et al*. (2004) [8] conducted a comprehensive review of the resistance status of different mosquito species, including *Anopheles dirus*, *Anopheles fluviatilis*, *Anopheles minimus*, as well as other anopheline species such as *Culex tritaeniorhynchus*, *Culex gelidus*, and *Culex vishnui*. Their findings indicated the development of resistance to chlorinated and organophosphorous insecticides in various parts of India.

Raghavendra *et al*. (2011) [9] and Singh *et al*. (2016) [10] highlighted the resistance of *Anopheles fluviatilis* to DDT and other insecticides in various regions of India.

However, there is no information on the vulnerability of *Aedes albopictus* from the Barmer and Kota areas to pesticides. Considering this, the current study assessed the susceptibility of *Aedes albopictus* strains obtained in Rajasthan's Barmer and Kota districts to temephos, neem oil, and *Bacillus thuringiensis* var. *israelensis*. We hope that the findings of this study will aid in the planning of disease control efforts in those specific locations.

2. Materials and Methods

A study was carried out in the Kota and Barmer regions of Rajasthan in September 2020. Larvae of the *Aedes albopictus* mosquito were collected for the investigation from cement tanks situated in the yards of these dwellings. Larvae were specifically gathered from 15 residences in Barmer and 21 houses in Kota. After being gathered, the mosquito larvae were moved to Career Point University's Department of Zoology in Kota, Rajasthan.

The *Aedes albopictus* mosquito larvae collected for the study were reared in a controlled laboratory environment. They were maintained at a temperature of 26±2 °C, with a relative humidity of 65±5%, and subjected to a light: dark (LD) cycle of 12:12 hours. The larvae were placed in plastic containers with a 1-liter capacity, which measured 18 cm in length and 7 cm in diameter. Each container was filled with 500 mL of dech lorinated water, and around 100 mosquito larvae were introduced into each container. To provide nourishment to the larvae, dried Brewer's yeast was added to the water at a rate of 15 mg per 500 mL daily. The water in the containers was refreshed every other day to maintain a clean and suitable rearing environment. For the susceptibility experiment conducted on both the Kota and Barmer strains, third instar larvae of *Aedes albopictus* were used.

For the larval bioassay, Temephos was provided by Hernaba Industries, GLDC, Vapi, Gujarat, India, in the form of a 95.12% technical compound. Pine and eucalyptus bioessential (BEO) were sourced from a local supplier, Barlani & Sarandhana Industries, Jhalawar. Neem oil was procured from Utkarsh Agrochem Pvt. Ltd., Surat, Gujarat, India, and *Bacillus thuringiensis Israelensis* (*Bti*) was obtained from Kota Municipal Corporation. A 1% stock solution and serial dilutions of neem oil and *Bti* were prepared in distilled water, while temephos (95.12%) was dissolved in acetone. Each bioassay involved testing 4-5 doses, ranging from 1 - 7 ppm for neem oil, 0.25 - 1.25 ppm for *Bti*, and 0.010 - 0.014 ppm for temephos. Control cups were not treated with any chemical during the larvicide evaluation. The evaluation was conducted on 3rd instar larvae of *Ae. albopictus* collected from Kota and Barmer cities. The experiment was replicated three times, with each replication consisting of three replicates.

The data collected from various larvicidal bioassays conducted in different replicates were aggregated based on the applied doses. The larvicidal effects were assessed 24 hours after treatment. Subsequently, the data from each dose-dependent larvicidal bioassay underwent probit analysis using PASW Statistics V. 18 software. Probit analysis involved plotting the transformed probit-mortality against the log10-transformed dose to estimate LC50, LC90, and LC99 values, and to generate the slope.

In addition to the LC values and slope, statistical parameters, including the chi-square value, degrees of freedom (df), and Pearson Goodness-of-Fit test (p) values, were calculated for all the larvicides tested against *Aedes albopictus* larvae from the Kota and Barmer regions. It's worth noting that the control group exhibited mortality below 5%, eliminating the need for any correction factor to adjust treatment mortality.

3. Results and Discussion

The Barmer strain of *Aedes albopictus* has shown more susceptibility against temephos, neem and *Bacillus thuringiensis* (*Bti*) in comparison to the Kota strain after 24 hours exposure.

Besides, conventional larvicides such as temephos showed approximately similar efficacy in Barmer (LC50 = 0.011 mg/l) and Kota (LC50 = 0.011 mg/l) strain (Table 1).

L C50 of Barmer strain of *Ae. albopictus* against Neem oil is 3.673 mg/l after 24 hours exposure with ranged from 3.450 – 3.880 mg/l and 4.665 mg/l for Kota strain with 4.411 – 4.912 mg/l ranges. This indicates the 1.27 fold more resistance in Kota strain of *Ae. albopictus*.

Susceptibility status of *Bacillus thuringiensis israelensis* (*Bti*) for Barmer and Kota strain was ranged from 0.435 mg/l to 0.7 mg/l. The LC50 activity of *B. thuringiensis israelensis* for Barmer strain was ranged from 0.405 to 0.464 mg/l while Kota strain had 0.637 to 0.7660 mg/l ranges with 1.6 fold more susceptibility in Barmer strain of *Ae. albopictus*.

An essential strategy in mitigating the proliferation of vector-borne diseases such as chikungunya, dengue, and zika involves the effective control of mosquito larval populations. Successfully managing disease vectors hinges on a comprehensive understanding of the mechanisms through which mosquito vectors develop resistance to different chemical agents [11]. To combat the diseases linked to *Aedes albopictus* and reduce vector transmission, the World Health Organization (WHO) has recommended a multifaceted approach. This approach includes active community involvement, health education, the administration of medications, environmental cleanliness, and targeted vector suppression measures [12]. By implementing these strategies at various levels, we can significantly diminish the impact of *Aedes albopictus* and the diseases it transmits.

Temephos is a crucial conventional synthetic insecticide that has been used to control a variety of insects, including *Simulium* [13] and *Aedes albopictus* [14-16] in a variety of habitats, including the intertidal mangrove community. The results of present study indicate that both the populations (Barmer and Kota) showed approximately equal mortality (LC50: 0.011 mg/l) against temephos. Resistance to temephos and fenthion was found in Tunisia's *Cx. pipiens*, according to Cheikh *et al*., (1998) [17]. In filarial endemic areas of Egypt, Zayed *et al*., (2006) [18] found that *Cx. pipiens* was highly resistant to temephos but still vulnerable to DDT, bendiocarb, malathion, fenitrothion, and fenthion as adulticides and larvicides, respectively. In another study, Rodriguez *et al*., (2007) [19] revealed that *Ae. aegypti* from Latin American nations were resistant to varied degrees to temephos (10-100 folds), malathion (0.5-2.2 folds), fenthion (0.9-6 folds), fentrothion (0.8-4.4 folds), chorpirifos (0.6-14.2 folds), and pirimiphos-methyl (2.5 - 50 folds). *Bacillus thuringiensis* produces three classes of insecticidal proteins during its vegetative and sporulation phases, including Cry (Crystal proteins), Cyt (Cytolytic toxin) proteins during the sporulation

phase, and Vip (vegetative insecticidal proteins) during the vegetative phase [20]. The Cyt 27 toxins present in some *B. thuringiensis parasporal* crystals were first discovered in mosquitocidal *B. thuringiensis*. subsp. *israelensis* (*Bti*) [21]. For the control of mosquitoes, microbial pesticides such as *B. sphaericus* and *B. thuringiensis* have been introduced; nevertheless, numerous cases of *B. sphaericus* resistance development have been recorded [22-24]. Mosquito tolerance to Cry toxins of *B. thuringiensis* var. israelensis is overcome or suppressed by Cyt toxins, according to research by Happi *et al*., (1997; 2005) [25]. Present study also indicates high efficacy of *B. thuringiensis* var *israelensis* against both the populations indicating absence of resistance against this insecticide in Barmer and Kota strain of *Ae. albopictus*. However, Barmer strain was found more susceptible with LC50: 0.435 mg/l than the Kota strain (LC50: 0.7 mg/l). Numerous neem formulations are suggested in Ayurveda for the treatment of various illnesses. Traditional methods include using dried neem leaves to keep stored grains and woollen clothing safe from insect pest infestations and using leaf smoke to ward off biting insects. Neem have a wide range of bioactivity against insects, 28 including reproductive fitness, oviposition, hatchability, antifeedent, repellent, metamorphosis, disruption, and death [26-30]. Neem oil's broad spectrum of activity (LC50: 3.673 - 4.665 mg/l) against the Barmer and Kota strains of *Ae. albopictus* was demonstrated in the current study. The Kota strain was found to have 1.27 times greater resistivity than the Barmer strain. In a similar manner, Kaura *et al*., (2019) [31] employed eucalyptus and neem oil to combat *Aedes* larvae and pupae. They concluded that eucalyptus oil was more effective at lower concentrations, with LC50 values of 93.3 and 144.5 ppm for larvae and pupae, respectively, compared to LC50 values of 707.9 and 741.3 ppm for neem oil.

Table 1: Probit mortality and log dose (mg/l) of Kota and Barmer strain of *Aedes albopictus* against temephos, *Bacillus thuringiensis* subsp. *Israelensis* (*Bti*) and neem oil

Temephos							
Population	**LC50**	**LC90**	**LC99**	**X^2**	**Df**	**p**	**Slope**
Barmer	0.011 (0.010 - 0.011)	0.014 (0.014 - 0.015)	0.018 (0.017 - 0.020)	28.932	43	0.950	Y = 20.492 + 10.388X
Kota	0.011 (0.011 - 0.012)	0.015 (0.015 - 0.016)	0.020 (0.018 - 0.023)	9.834	43	1.000	Y = 17.726 + 9.069X
Neem oil							
Barmer	3.673 (3.450 – 3.880)	6.315 (5.929 – 6.814)	9.824 (8.843 – 11.245)	26.779	34	0.806	Y = 5.444X-3.076
Kota	4.665 (4.411 – 4.912)	8.593 (7.974 – 9.423)	14.138 (12.474 - 16.603)	17.141	34	1.993	Y = 4.831X-3.232
***Bacillus thuringiensis* subsp. *Israelensis* (*Bti*)**							
Barmer	0.435 (0.405 - 0.464)	0.955 (0.880 - 1.053)	1.814 (1.581 - 2.151)	45.351	43	0.374	Y = 1.356 + 3.749X
Kota	0.700 (0.637 - 0.766)	1.301 (1.144 - 1.561)	2.156 (1.758 - 2.923)	139.524	43	0.0001	Y = 0.737 + 4.763X

4. Conclusion
The susceptibility experiment showed more resistance in Kota strain against temephos, *Bacillus thuringiensis* and neem oil than the Barmer strain. This indicated the Kota region needs better vector control practices than the Barmer region.

5. Conflict of interest
All the authors declare no conflict of interest.

6. Author contribution
SC -experimentation, data acquisition, analysis, designing and writing original draft
SJ - conception, designing and writing original draft

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