



Murraya paniculata leaf extracts as effective larvicides against the larvae of *Aedes vittatus*. (Insecta; Diptera; Culicidae)

Madhavi M¹, Mahesh L^{2*}

¹ Professor & HoD, Department of Zoology, Osmania University, Hyderabad, Telangana, India

² Assistant Professor, Department of Zoology, Tara Govt. College, Sangareddy, Telangana, India

Abstract

Arboviruses transmitted by arthropods are a global public health threat due to their expanding geographic range, attributed to the availability of efficient vectors. While *Aedes aegypti* and *Ae. albopictus* are well-known vectors, other *Aedes* species, such as *Ae. japonicus*, *Ae. vexans*, and *Ae. vittatus*, are becoming a concern. This article provides a comprehensive review of the global distribution, breeding habitats, and virus transmission potential of *Ae. vittatus*, a mosquito species known to transmit yellow fever, Zika, chikungunya, and dengue virus, as well as reports on the potential use of natural products as mosquito control agents.

This research article describes a study in which extracts from *Murraya paniculata* were tested for their larvicidal activity against the 4th instar larvae of *Ae. vittatus*. The extracts were prepared using different solvents and screened for the presence of secondary metabolites which exhibit insecticidal properties. The prepared extracts were tested at various concentrations using larvae in different stages. Methanolic extracts contained all tested secondary metabolites and exhibited the highest insecticidal activity, with a mortality rate of 100% at 250 ppm. Aqueous and Ethanolic extracts also showed significant insecticidal activity, while n-Hexane extracts demonstrated moderate activity. These findings suggest that *M. paniculata* leaves contain insecticidal compounds that can be utilized for mosquito control. Further research is needed to isolate and identify the active compounds responsible for the observed activity.

Keywords: mosquito-borne diseases, vector-borne diseases, natural control, mosquito larvicides, biological control, natural insecticides

Introduction

Arboviruses, or viruses transmitted by arthropods, pose a significant threat to global public health due to their expanding geographic range. This expansion is primarily attributed to the availability of efficient vectors. While *Aedes aegypti* and *Ae. albopictus* are well-known vectors, other *Aedes* species, such as *Ae. japonicus*, *Aedes vexans*, and *Aedes vittatus*, are becoming a concern due to their potential to spread arboviruses like dengue, chikungunya, and Zika viruses (Outammassine, *et al.* 2022) ^[1]. *Ae. vittatus*, a mosquito species found in Africa, tropical Asia, and southern Europe, is known to transmit yellow fever, Zika, chikungunya, and dengue virus (Alarcón-Elbal, *et al.*, 2020) ^[2].

The presence of *Ae. vittatus* has been reported in several studies conducted in different regions of India, including rural and urban areas of Jodhpur, Jaipur, and Kota districts of Rajasthan (Angel, B., & Joshi, V., 2008) ^[3], Thiruvananthapuram district in Kerala (Samuel, *et al.* 2014) ^[4], Jalna district of Maharashtra (Patil, *et al.* 2020) ^[5], and various localities of Delhi (Singh, *et al.* 2014) ^[6]. In Tiruchirappalli district of Tamilnadu State, *Ae. vittatus* and *Ae. aegypti* were the most predominant container-breeding mosquitoes, each accounting for 45% of the population (Rajesh, *et al.* 2013) ^[7].

Ae. vittatus has been recorded from almost all parts of the world, including the Ashanti region of Ghana, southern Nigeria, the Jafna peninsula in northern Sri Lanka, Galicia, Metema and Humera in Ethiopia, southwestern Iran and Khuzistan province, Jarabacoa in the Dominican Republic, and urban Islamabad area of Pakistan (Addy, *et al.* 1996;

Philip, C. B., 1962; Surendran, *et al.* 2021; Domínguez-Costas, M. & Ayres, C., 2020; Ferde, *et al.* 2018; Nasirian, *et al.* 2014; Alarcón-Elbal, *et al.*, 2020; Jabeen, *et al.*, 2022) ^[8, 9, 10, 11, 12, 13, 2, 15]. The discovery of *Ae. vittatus* on Cuba in 2019 and sequences from the Dominican Republic in 2020 suggest that the species may be present on other Caribbean islands (Pagac *et al.* 2020) ^[16].

Ae. Vittatus has gained attention in recent years due to its association with various viruses of public health importance such as Zika virus (ZIKV), yellow fever virus (YFV), dengue virus (DENV), and chikungunya virus (CHIKV). It has also been reported to be the potential vector for Japanese encephalitis (JEV), West Nile (WNV), Chandipura (CHPV), and Chittoor (CHITV) viruses (Sudeep, *et al.* 2020) ^[17]. Chikungunya outbreaks in various regions of Kenya were also found to be due to *Ae. vittatus* (Mulwa, *et al.* 2018) ^[18]. *Ae. vittatus* mosquitoes are susceptible to all four types of dengue viruses and were able to facilitate the growth of the DEN-2 virus (Mavale, *et al.* 1992) ^[19]. In addition, *Ae. vittatus* was discovered to vector *Setaria digitata* (Varma *et al.*, 1971) ^[20].

Understanding the breeding habits of *Ae. vittatus* is crucial for developing effective control measures. The mosquito is predominantly found breeding in rock pools, but it can also breed in various microhabitats such as ammonia and nitrate-rich waters of latrines and septic tanks (Nwoke, *et al.* 1993) ^[21]. *Ae. vittatus* larvae appear in granite rock pools during the long dry season. The eggs of *Ae. vittatus* survived 5 months of dryness and prolonged exposure to temperatures over 40°C. Higher humidity levels promoted better egg survival rates, with 65% of eggs hatching at 30°C even after

16 weeks of desiccation. However, at 45°C, survival was high (88%) for only 2 days, and at 50°C, no eggs survived (Roberts, 2004) [22]. *Ae. vittatus* eggs survived the dry season and hatched when the pools were flooded, which can lead to a rapid population explosion when the rains start (Irving-Bell *et al.*, 1991) [23].

Natural products have been explored as potential sources of mosquito control agents. For instance, this study aimed to isolate and evaluate the repellent properties of different fractions from *Lantana camara* flowers against *Aedes* mosquitoes (Dua, *et al.* 2003) [24]. The EO of *Kaempferia galangal* L. (Zingiberaceae), commonly known as aromatic ginger and its major chemical constituents demonstrated promising larvicidal activity against *Ae. vittatus* (Alsalthi, *et al.* 2020) [25]. n-Hexane extract of *Persea americana* seeds exhibited the highest potency against the larvae of *Ae. vittatus* with an LC50 value of 0.827 ppm, while the ethanol and ethyl acetate extracts had lower potency with LC50 values of 1.79 ppm and 2.732 ppm, respectively. The most effective fraction isolated from the n-Hexane extract contained dominant fatty acid and fatty acid methyl esters (Nzelibe and Albaba, 2015) [26]. The present study investigated the insecticidal activity of *Murraya paniculata* leaf extracts against the 4th instar larvae of *Ae. vittatus* at different concentrations (50, 100, 150, 200, and 250 ppm).

Material & methods

Test Plant

Fresh leaf samples of *M. paniculata* were obtained from Sangareddy town of Telangana State in India. The samples were air-dried for 14 days, pulverized separately in an electric blender, and 100 grams of powdered leaf were weighed and macerated in one liter of each solvent (Methanol, Ethanol, n-Hexane, and distilled water) for 48 hours. The samples were filtered using Whatman No.1 filter paper, and the filtrates were concentrated using a rotary evaporator and evaporated to dryness in a water bath kept at 50°C. The samples were stored in clear sample containers at room temperature until use.

Identification of Secondary metabolites in Test Extracts

Different tests were conducted to identify the secondary metabolites present in the prepared extracts. Alkaloids were identified using the Mayor's Test, which involved adding the Mayor's reagent and observing the formation of a cream-colored precipitate. Flavonoids were identified using the Alkaline Reagent Test, where the addition of NaOH results in yellow color. Terpenoids were identified using the Salkowski Test, where concentrated H₂SO₄ was added, resulting in a red or orange color. Saponins were identified through the Froth Test, where vigorous shaking resulted in the formation of foam. Glycosides were identified using the Keller-Killiani Test, where HCl and FeCl₃ were added, leading to a red or violet color. Finally, the NaOH Test was conducted to identify polyphenols, where the addition of NaOH resulted in yellow color.

Mosquito Culture

Larvae of *Ae. vittatus* was collected from the rock pools near the lake located at Osmania University, Hyderabad, India. They were identified using morphology-based keys. Larvae were maintained on a diet of yeast and biscuits in a 1:3 ratio. Early fourth instar stages of *Ae. vittatus* was selected for the rest of the study. Experiments were carried

out at a temperature of 29±2°C under 12:12 light and dark cycles.

Test Solutions Preparation

1 gram of the residues from the extraction was added to 985 mL of Distilled water, 5 mL of Tween 80 and 10 mL of respective solvents separately to prepare 1000 ppm stock solutions. By serial dilution, 5 different concentrations (50, 100, 150, 200 and 250 ppm) were prepared using distilled water. Control solutions were prepared using the respective solvents excluding the extracts.

Larvicidal Bioassay

For each extract, a total of three trials were conducted with five replicates in each trial. 100 mL of prepared test solutions were taken in 250 mL disposable test cups separately and 25 larvae of the early fourth instar of both species were separately transferred by means of a strainer into separate test cups. Small, unhealthy, or damaged larvae were not used. The number of dead larvae was counted after 12, 24, and 48 hours. The percentage mortality (PM) was determined using the equation:

$$\% \text{ PM} = (\text{No of dead larvae} / \text{Total larvae population}) \times 100.$$

Corrected mortalities were calculated using Abbott's (1925) formula.

$$\text{Corrected Mortality}(\%) = \frac{\%MT - \%MC}{100 - \%MC} \times 100$$

Statistical Analysis

The corrected larval mortality data were subjected to probit analysis using the SPSS 29.0 (Statistical Package of Social Sciences) software. The level of significance was set at P < 0.05.

Results & discussion

Table No. 1 displays the results of the biochemical analysis of the test extracts in the present study. It is evident from the results that Methanolic extracts of the *M. paniculata* leaves are rich in all the secondary metabolites tested for their presence. In Ethanolic and Aqueous extracts also all the secondary metabolites presence were detected, but Ethanolic extracts are rich in alkaloids, saponins and polyphenols while Aqueous extracts are rich in flavonoids, terpenoids and polyphenols. However, in n-Hexane extracts, saponins, terpenoids and polyphenols presence were identified, and the remaining secondary metabolites were not present.

Table 1: *M. paniculata* leaf extracts mean mortality rates ± SD against the 4th instar larvae of *Ae. vittatus*.

S. No.	Phytochemical	Water	Ethanol	Methanol	Hexane
1	Alkaloids	+	++	+++	-
2	Flavonoids	++	+	++	-
3	Saponins	+	++	++	+
4	Terpenoids	+	+	++	+
5	Polyphenols	++	++	+++	+
6	Glycosides	+	+	++	-

Table No. 2 and Figure 1 display the results of the present study. The results revealed that all the tested extracts exhibited insecticidal activity against *Ae. vittatus* larvae, with concentration dependant mortality rates and the highest

mortality rate being observed at 250 ppm for all extracts. The Methanolic extract showed the most potent insecticidal activity, with a mortality rate of 100% at 250 ppm. The Aqueous and Ethanolic extracts exhibited significant insecticidal activity, with mortality rates of 98.33% and 83.33% respectively at 250 ppm. The n-Hexane extracts

showed moderate insecticidal activity with an 81.66% mortality rate at 250 ppm. More than 50% mortality rates were observed at 100 ppm with Methanolic and Aqueous extracts while with n-Hexane and Ethanolic extracts more than 50% mortality rates were obtained at 150 ppm.

Table 2: Mean mortality rates ± SD of *M. paniculata* leaf extracts against the 4th instar larvae of *Ae. vittatus*.

Conc. In ppm	Aqueous	Methanolic	Ethanolic	N-Hexane
0	0	0	0	0
50	28.33 ± 0.53	33.33 ± 0.46	18.33 ± 0.46	26.66 ± 0.71
100	53.33 ± 0.53	53.33 ± 0.74	38.33 ± 0.71	43.33 ± 0.46
150	66.66 ± 0.51	73.33 ± 0.35	61.66 ± 0.75	53.33 ± 0.52
200	78.33 ± 0.35	88.33 ± 0.35	71.66 ± 0.74	61.66 ± 0.64
250	98.33 ± 1.30	100 ± 0.52	83.33 ± 0.53	81.66 ± 0.53
LC50	113.86	104.43	137.99	143.17
LC90	220.73	206.63	254.68	276.55
Regression Equation	$y = 0.3743x + 7.379$	$y = 0.3914x + 9.1248$	$y = 0.3428x + 2.6967$	$y = 0.299x + 7.0614$
R ² Value	0.9739	0.9696	0.982	0.9652

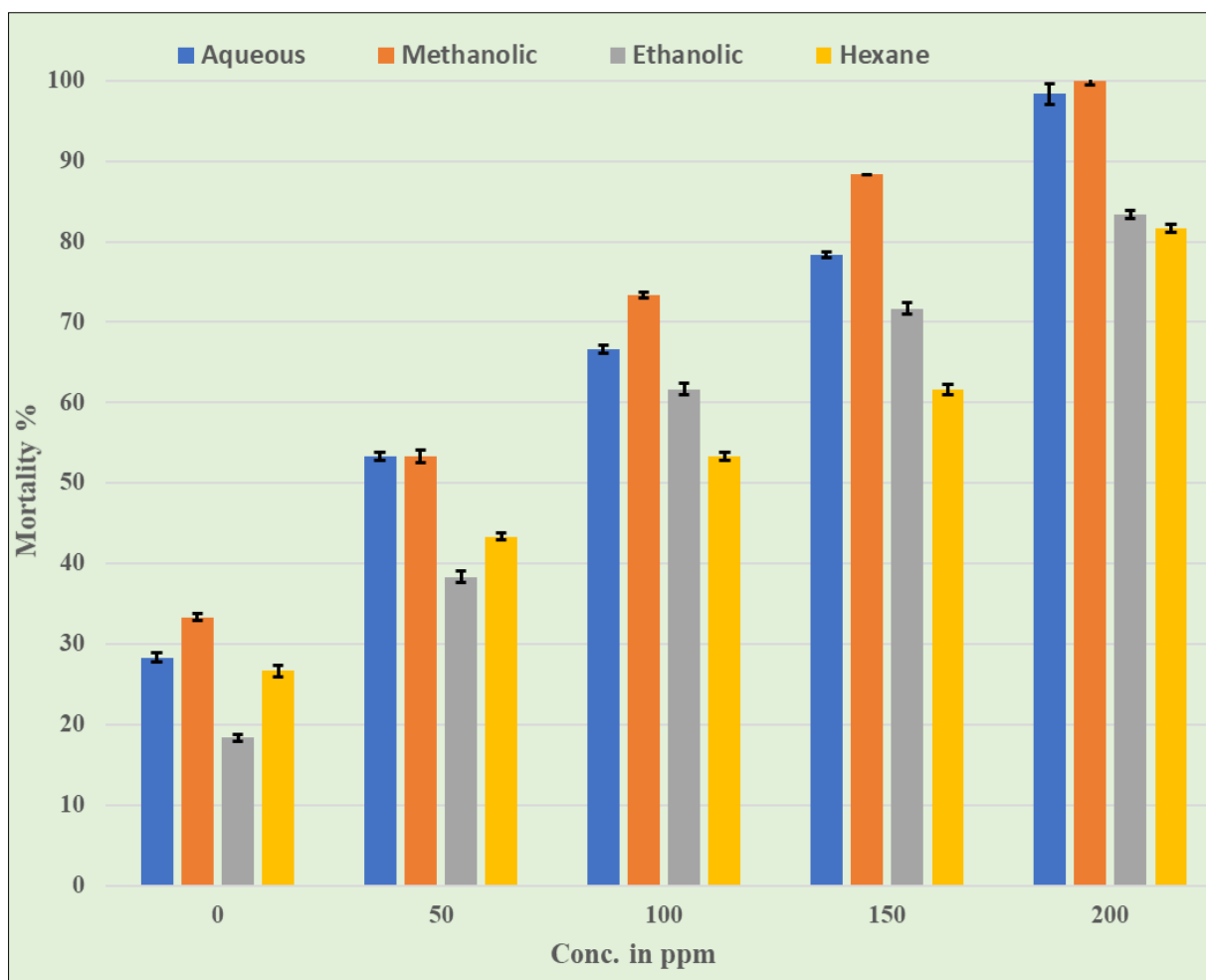


Fig 1: Larvicidal efficacy of different extracts of *M. paniculata* leaves against the 4th instar larvae of *Ae. vittatus*

Figures 2 and 3 illustrate the LC50 and LC90 values respectively for the tested extracts in the present study. The lowest LC50 and LC90 values were calculated to be 104.43 ppm and 206.63 ppm respectively for Methanolic extracts while the highest values were found to be 143.17 ppm and

276.55 ppm respectively for n-Hexane extracts. For Aqueous extracts, the LC50 and LC90 values were calculated to be 113.86 ppm and 220.73 ppm respectively while for Ethanolic extracts, they were 137.99 ppm and 254.68 ppm respectively.

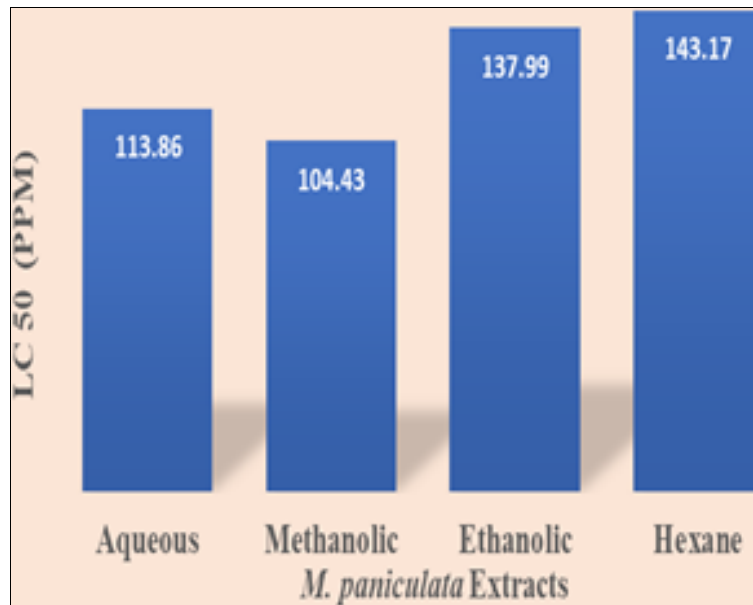


Fig 2: LC50 values of different extracts of *M. paniculata* leaves against the 4th instar larvae of *Ae. vittatus*

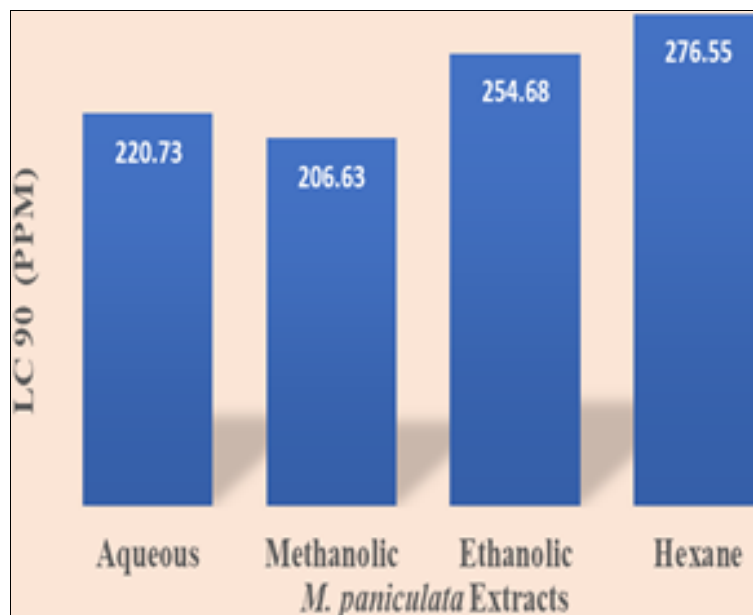


Fig 3: LC90 values of different extracts of *M. paniculata* leaves against the 4th instar larvae of *Ae. vittatus*

The findings of the present study suggest that methanol is an efficient solvent for extracting insecticidal compounds from *Murraya paniculata* leaves. The Aqueous extracts also exhibited an efficient larval mortality rate of 98.33% at 250 ppm, indicating that water is also an effective solvent for extracting active compounds from *M. paniculata* leaves. The Ethanolic extract also demonstrated significant insecticidal activity with a mortality rate of 83.33% at 250 ppm. The presence of polar compounds in the Ethanolic extract may be responsible for this activity. On the other hand, the n-Hexane extracts demonstrated moderate insecticidal activity, indicating that n-Hexane may not be a suitable solvent for extracting the active compounds from *M. paniculata* leaves.

Prevention of mosquito-borne diseases requires the control of mosquito larvae with eco-friendly plant secondary metabolites. While many plant extracts have shown mosquitocidal or repellent activities against mosquito vectors, only a few have practical utility in mosquito control. Dua, *et al.* (2003) [24] evaluated the repellent

properties of different fractions of *Lantana camara* flowers against *Aedes* mosquitoes. Very limited studies have been conducted to test the larvicidal efficacy of botanicals against the larvae of *Ae. vittatus*. In a study conducted by Alsalmi, *et al.* (2020) [25] the EO of *Kaempferia galangal* L. demonstrated promising larvicidal activity against *Ae. vittatus*.

Nzelibe and Albaba, 2015 [26] reported that the n-Hexane extract of *P. americana* seeds exhibited the highest potency against the larvae of *Ae. vittatus* with an LC50 value of 0.827 ppm, while the ethanol and ethyl acetate extracts had lower potency with LC50 values of 1.79 ppm and 2.732 ppm, respectively. However, in the present study, n-Hexane extracts failed to exhibit better results than Methanolic, Aqueous and Ethanolic extracts.

In this study, the Methanolic extract of *M. paniculata* demonstrated 100% mortality while Aqueous and Ethanolic extracts also gave significant results when compared to n-Hexane extracts. The presence of alkaloids, terpenoids, flavonoids, saponins, polyphenols and glycosides in

Methanolic, Aqueous and Ethanolic extracts may have contributed to the high mortality rate observed in this study.

Conclusion

Overall, the results of the present study suggest that *M. paniculata* leaves contain insecticidal compounds that can be utilized for controlling the population of *Ae. vittatus* larvae. The Methanolic, Aqueous and Ethanolic extracts exhibited the most potent insecticidal activity, while the n-Hexane extract showed the least activity. It is evident that plant-originated natural products are emerging as a potential source of mosquito control. Crude extracts from the plant *M. paniculata* could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. Further research is needed to identify and isolate the active compounds responsible for the observed insecticidal activity and evaluate their safety and efficacy as potential insecticides.

References

1. Outamassine A, Zouhair S, Loqman S. Global potential distribution of three underappreciated arbovirus vectors (*Aedes japonicus*, *Aedes vexans* and *Aedes vittatus*) under current and future climate conditions. *Transboundary and Emerging Diseases*,2022;69(4):e1160-e1171. <https://doi.org/10.1111/tbed.14404>
2. Alarcón Elbal PM, Rodríguez Sosa MA, Newman BC, Sutton WB. The First Record of *Aedes vittatus* (Diptera: Culicidae) in the Dominican Republic: Public Health Implications of a Potential Invasive Mosquito Species in the Americas. *Journal of Medical Entomology*, 2020. doi:10.1093/jme/tjaa128
3. Angel B, Joshi V. Distribution and seasonality of vertically transmitted dengue viruses in *Aedes* mosquitoes in arid and semi-arid areas of Rajasthan, India. *Journal of Vector Borne Diseases*,2008;45(1):56-59.
4. Samuel PP, Thenmozhi V, Nagaraj J, Dilip Kumar T, Tyagi BK. Dengue vectors prevalence and the related risk factors involved in the transmission of dengue in Thiruvananthapuram district, Kerala, south India. *J Vector Borne Dis*,2014;51:313-9.
5. Patil PB, Dasgupta SK, Reddy KVS, Char B, Zehr UB, Barwale SR. Surveillance of *Aedes* mosquito species in villages of Jalna district, Maharashtra, India. *Journal of Entomology and Zoology Studies*, 2020;8(2):669-677.
6. Singh RK, Mittal PK, Kumar G, Dhiman RC. Prevalence of *Aedes* mosquitoes in various localities of Delhi during dengue transmission season. *Entomology and Applied Science Letters*,2014;1(4):16-21.
7. Rajesh K, Dhanasekaran D, Tyagi BK. Survey of container breeding mosquito larvae (Dengue vector) in Tiruchirappalli district, Tamil Nadu, India. *Journal of Entomology and Zoology Studies*,2013;1(6):88-91.
8. Addy PA, Esena RK, Atuahene SK. Possible contributing factors to the paucity of yellow fever epidemics in the Ashanti region of Ghana, west Africa. *East African Medical Journal*,1996;73(1):3-9.
9. Philip CB. Breeding of *Aedes aegypti* and Other Mosquitoes in West African Rock Holes. *Annals of the Entomological Society of America*,1962;55(6):706-708. doi:10.1093/aesa/55.6.706
10. Surendran N, Jayadas TTP, Thiruchenthooran V, Raveendran S, Tharsan A, Santhirasegaram S, *et al.* *Aedes* larval bionomics and implications for dengue control in the paradigmatic Jafna peninsula, northern Sri Lanka. *Parasit Vectors*,2021;14(1):162. doi: 10.1186/s13071-021-04640-6.
11. Domínguez Costas M, Ayres C. New record of *Aedes vittatus* (Bigot, 1861) (Diptera: Culicidae) in Galicia. *Arquivos Entomol6xicos*,2020;22:279-280.
12. Ferede G, Tiruneh M, Abate E, Kassa WJ, Wondimeneh Y, Dامتie D, *et al.* Distribution and larval breeding habitats of *Aedes* mosquito species in residential areas of northwest Ethiopia. *Epidemiol Health*,2018;40:e2018015. doi: 10.4178/epih.e2018015.
13. Nasirian H, Sadeghi SMT, Vazirianzadeh B, MoosaKazemi SH. New record of *Aedes vittatus* and *Culiseta subochrea* (Diptera: Culicidae) and their distribution from Shadegan Wetland, South Western Iran. *Journal of Entomology and Zoology Studies*,2014;2(5):271-275.
14. Alarcón Elbal PM, RodríguezSosa MA, Newman BC, Sutton WB. The First Record of *Aedes vittatus* (Diptera: Culicidae) in the Dominican Republic: Public Health Implications of a Potential Invasive Mosquito Species in the Americas. *Journal of Medical Entomology*, 2020. doi:10.1093/jme/tjaa128
15. Jabeen A, Ansari JA, Ikram A, Khan MA. First Report of *Aedes vittatus* in Islamabad, Pakistan. *Journal of the American Mosquito Control Association*,2022;38(3): 219-220. doi: 10.2987/22-7067.
16. Pagac BB, Spring AR, Stawicki JR, Dinh TL, Lura T, Kavanaugh MD, *et al.* Incursion and establishment of the Old-World arbovirus vector *Aedes (Fredwardsius) vittatus* (Bigot, 1861) in the Americas. *Acta Tropica*, 2020, 105739. doi:10.1016/j.actatropica.2020.105739
17. Sudeep AB, Sreelekshmy Mohandas, Bhanarkar SR, Ghodke YS, Sonawane PA. Vector competence of *Aedes vittatus* (Bigot) mosquitoes from India for Japanese encephalitis, West Nile, Chandipura and Chittoor viruses. *J Vector Borne Dis*, 2020;57:234-239.
18. Mulwa F, Lutomiah J, Chepkorir E, Okello S, Eyase F, Tigoi C, *et al.* Vector competence of *Aedes bromeliae* and *Aedes vittatus* mosquito populations from Kenya for chikungunya virus. *PLoS Negl Trop Dis*,2018;12(10):e0006746. <https://doi.org/10.1371/journal.pntd.0006746>
19. Mavale MS, Ilkal MA, Dhanda V. Experimental studies on the susceptibility of *Aedes vittatus* to dengue viruses. *Acta Virologica*,1992;36(4):412-416. PMID: 1362325.
20. Varma AK, Sahai BN, Singh SP, Lakra P, Shrivastava VK. On *Setaria digitata*, its specific characters, incidence and development in *Aedes vittatus* and *Armigeres obturbans* in India with a note on its ectopic occurrence. *Zeitschrift fur Parasitenkunde*,1971;36(1): 62-72.
21. Nwoke BE, Nduka FO, Okereke OM, Ehighibe OC. Sustainable urban development and human health: septic tank as a major breeding habitat of mosquito vectors of human diseases in south-eastern Nigeria. *Applied Parasitology*,1993;34(1):1-10. PMID: 8508215.
22. Roberts D. Prolonged survival of eggs of the rock-pool mosquito, *Aedes vittatus*, in the extreme heat of the Arabian Peninsula. *Journal of Arid*

- Environments,2004:57(2):203–210. doi:10.1016/s0140-1963(03)00108-3
23. Irving Bell RJ, Inyang EN, Tamu G. Survival of *Aedes vittatus* (Diptera: Culicidae) eggs in hot, dry rockpools. Tropical Medicine and Parasitology: Official Organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), 1991;42(1):63-66. PMID: 2052860.
 24. Dua VK, Pandey AC, Singh R, Sharma VP, Subbarao SK. Isolation of repellent ingredients from *Lantana camara* (Verbenaceae) flowers and their repellency against *Aedes* mosquitoes. Journal of Applied Entomology,2003:127(9-10):509–511. doi:10.1046/j.1439-0418.2003.00789.x
 25. AlSalhi MS, Elumalai K, Devanesan S, Govindarajan M, Krishnappa K, Maggi F. The aromatic ginger *Kaempferia galanga* L. (Zingiberaceae) essential oil and its main compounds are effective larvicidal agents against *Aedes vittatus* and *Anopheles maculatus* without toxicity on the non-target aquatic fauna. Industrial Crops and Products,2020:158: 113012. doi:10.1016/j.indcrop.2020.113012
 26. Nzelibe HC, Albaba SU. Larvicidal potential of *Persea americana* seed extract against *Aedes vittatus* mosquito. British Journal of Applied Science & Technology,2015:11(2):BJAST.16392, ref.22.
 27. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology,1925:18:265-266.