1st June, 2023

Original Article

PONGAMIA PINNATA LEAF EXTRACTS EFFICACY AGAINST THE 4TH INSTAR LARVAE OF *AEDES VITTATUS* (INSECTA: DIPTERA: CULICIDAE)

M. Madhavi* **and L. Mahesh**

Department of Zoology, Osmania University,
Hyderabad - 500007 (Telangana State), India

Date of submission : 12th April, 2023

Date of revision : 30th April, 2023

Date of acceptance : 24th May, 2023

ABSTRACT

Objective: To explore the larvicidal activity of *Pongamia pinnata* leaf extracts against the *Aedes vittatus*, an emerging threat to public health.

Methods: Secondary metabolites from the leaves of *P. pinnata* were extracted using Water, Methanol, Ethanol, Hexane, and Acetone as solvents. From each extract, 5 different concentrations (50, 100, 150, 200, 250 PPMs) were prepared and tested for their larvicidal

****Corresponding Author:***

Dr M. Madhavi; Email: prsmadhavi@gmail.com

Cite this article as:

Madhavi M, Mahesh L. *Pongamia pinnata* leaf extracts efficacy against the 4th instar larvae of *Aedes vittatus* (Insecta: Diptera: Culicidae). *J Med Arthropodol & Public Health*. 2023; 3(1): 23-34.

efficacy against the 4th instars of *Aedes vittatus* under laboratory conditions.

Results: All the extracts collected by using different solvents showed good larvicidal effects after 24 hours of exposure. However, the highest larval mortality was found in the methanolic extract of *P. pinnata* leaves followed by aqueous, ethanolic, hexane, and acetone extracts in the same order. Dose-dependent responses were observed. Mortality percentages of 100%, 98.33%, 83.33, 81.66%, and 76.66% were obtained by methanolic, Aqueous, ethanolic, hexane, and acetone extracts, respectively.

Conclusions: The Methanolic and aqueous extracts of *P. pinnata* leaves were proven to possess efficacy as ideal larvicides against the larvae of *Ae. vittatus*. Hence, the leaf extract of *P. pinnata* may be used to control this emerging threat to human health.

Keywords: Larvicides, Insecticides, Biopesticides, Vector control, Vector-borne diseases

INTRODUCTION

More than 219 million cases and 400,000 deaths occur yearly due to Malaria while 3.9 billion people in over 129 countries are at risk of Dengue, with an estimated 40,000 deaths yearly. Mosquitos also transmit many viral diseases such as chikungunya fever, Zika virus fever, yellow fever, West Nile fever, and Japanese encephalitis[1]. According to a report by WHO[2]**,** there were an estimated 241 million malaria cases and 627 000 malaria deaths worldwide in 2020. From time immemorial, man has been using plant products such as neem leaves and flowers of *Chrysanthemum cinerariaefolium* against many arthropod pests[3]. Pesticides are a major tool in controlling insect pests of agricultural and health importance[4]. Indiscriminative usage of pesticides has negative effects such as environmental pollution, loss of biodiversity, and human health issues ranging from nerve damage to cancers[5].

Aedes vittatus, an important vector and pest mosquito, has a wide distribution in many tropical and subtropical countries worldwide, such as India, the Ashanti region of Ghana, southern Nigeria, the Jaffna 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[6-13]. In recent years, *Ae. vittatus* has been linked to several viruses with public health implications, including the Zika virus (ZIKV), yellow fever virus (YFV), dengue virus (DENV), and chikungunya virus (CHIKV). The species has also been considered a potential vector for Japanese encephalitis virus (JEV), West Nile virus (WNV), Chandipura virus (CHPV), and Chittoor virus (CHITV)[14]. Studies have also identified *Ae. vittatus* as the vector for chikungunya virus in various regions of Kenya[15] and the species has been found to facilitate the growth of the DEN-2 virus[16]. Additionally, research has indicated that, *Ae. vittatus* is a carrier of *Setaria digitata*[17].

Formulations of natural origin with pesticidal action are called biopesticides[18]. Many plant products are in usage as pesticides for the past 4000 years ago[19]. Several studies have explored the potential of natural products as mosquito control agents. One such study examined different fractions from *Lantana camara* flowers and their repellent properties against Aedes mosquitoes[20]. Another study found that the essential oil of *Kaempferia galan*gal L. and its major chemical constituents exhibited larvicidal activity against *Ae. vittatus*[21]. Additionally, the n-Hexane extract of *Persea americana* seeds demonstrated high potency against *Ae. vittatus* larvae, with the most effective fraction containing dominant fatty acid and fatty acid methyl esters[22].

The current study aimed to investigate the insecticidal activity of *Pongamia pinnata* leaf extracts at various concentrations (50, 100, 150, 200, and 250 ppm) against the 4th instar larvae of *Ae. vittatus*.

MATERIALS & METHODS

(1) Test insect culture: Early-stage larvae of the *Aedes vittatus* were collected from the lake and rockpools located in the Osmania University campus, Hyderabad, Telangana State, India. They were reared in glass troughs by providing yeast and dog biscuits in a 3:1 ratio. Fourth instar larvae were used in larvicidal bioassay.

(2) *Pongamia pinnata* Leaf extracts preparation: Good-quality leaves of the *P. pinnata* were collected from the Osmania University campus, were washed first with running tap water and then with distilled water and shade dried for 15 days. Later the dried leaves were powdered by an electrical blender. Aqueous, methanolic, Ethanolic, Hexane, and Acetone extracts were collected from the powdered samples using the Soxhlet apparatus and a Rotary evaporator. Aqueous extracts were collected by boiling the powder in distilled water followed by filtration. Five different concentrations (50, 100, 150, 200, and 250 ppm) of extracts by dilution with distilled water and Tween 80.

(3) **Phytochemical Analysis:** Various tests were performed to detect the presence of secondary metabolites in the prepared extracts. Alkaloids were identified using the Mayor's Test, in which the Mayor's reagent was added and the formation of a cream-colored precipitate was observed. The Alkaline Reagent Test was used to identify flavonoids, which produced a yellow color upon adding NaOH. Terpenoids were detected using the Salkowski Test, where the addition of concentrated H_2SO_4 resulted in a red or orange color. The Froth Test was utilized to identify saponins, where the formation of foam occurred with vigorous shaking. The Keller-Killiani Test was conducted to identify glycosides, in which the addition of HCl and $FeCl_3$ produced a red or violet colour. Finally, the NaOH Test was performed to identify polyphenols, and the addition of NaOH resulted in yellow colour.

(4) **Test Solutions Preparation:** 1 g 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.

(5) **Larvicidal Bioassay:** For the larvicidal bioassay test, WHO guidelines[23] were followed. Larvae were taken in three batches of 20 in 100 mL of prepared test solutions separately in 250 mL test cups. The number of dead larvae was counted after 6, 12 and 24 h of exposure, and the percentage mortality was reported from the average of five replicates using the following equation.

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

Corrected mortalities were calculated using Abbotts's[24] formula.

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

(6) Statistical analysis: Microsoft Excel software was used to subject the results to one-way analysis of variance (ANOVA). The level of significance was set at $p<0.05$. Probit analysis was conducted to calculate LC_{50} & LC_{90} concentrations.

RESULTS & DISCUSSION

(1) Phytochemical Analysis: The results of the phytochemical analysis (Table No.1) show that *P. pinnata* leaf extracts contain a wide range of phytochemicals. The methanol extract showed the presence of the highest number of phytochemicals, including alkaloids, flavonoids, saponins, terpenoids, polyphenols, and glycosides. The ethanol extract also showed the presence of most of these phytochemicals, although in a slightly lower abundance. The water extract showed the presence of alkaloids, flavonoids, saponins, terpenoids, polyphenols, and glycosides, albeit in a lower abundance than the methanol extracts. The hexane and acetone extracts showed the presence of only a few phytochemicals, with the acetone extract containing only flavonoids and terpenoids. The methanol, ethanol and aqueous extracts showed the highest abundance of these phytochemicals, making them potentially useful in the control of pests.

Table 1. Identified secondary metabolites in the *Pongamia pinnata* leaf extracts. Absent: -; Slightly Present: +; Moderately present: ++; Heavily present: +++.

Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Polyphenols	Glycosides
Water	+	++	+	+	++	+
Ethanol	++	+	++	+	++	+
Methanol	+++	++	++	++	+++	++
Hexane	-	-	+	+	+	_
Acetone	-	+	-	+	-	-

(2) Larvicidal Bioassay: The results of the larvicidal bioassay (Table 2 and Fig. 1) revealed that the aqueous, methanolic, and ethanolic extracts of *P. pinnata* leaves possessed significant larvicidal activity against *Ae. vittatus* larvae. Among the different solvent extracts, the methanolic extract demonstrated the highest larvicidal activity, with a mortality percentage of 28.33 ± 0.53 at 50 ppm, 53.33 ± 0.74 at 100 ppm, 73.33 ± 0.35 at 150 ppm, 88.33 ± 0.35 at 200 ppm, and 100 ± 0.52 at 250 ppm. Notably, the 250 ppm concentration of the methanolic extract resulted in the complete mortality of *Ae. vittatus* larvae.

Table 2. *Pongamia pinnata* leaf extracts larval mortality percentages ± Standard Deviations against the 4_{th} Instar larvae of *Aedes vittatus*

Conc. In ppm	Aqueous	Methanolic	Ethanolic	Hexane	Acetone
0	0	0	0	0	0
50	28.33 ± 0.53	33.33 ± 0.46	18.33 ± 0.46	26.66 ± 0.71	16.66 ± 0.64
100	53.33 ± 0.53	53.33 ± 0.74	38.33 ± 0.71	43.33 ± 0.46	33.33 ± 0.46
150	66.66 ± 0.51	73.33 ± 0.35	61.66 ± 0.75	53.33 ± 0.52	41.66 ± 0.46
200	78.33 ± 0.35	88.33 ± 0. 35	71.66 ± 0.74	61.66 ± 0.64	63.33 ± 0.71
250	98.33 ± 1.30	100 ± 0. 52	83.33 ± 0.53	81.66 ± 0.53	76.66 ± 0.53

The ethanolic extract also exhibited significant larvicidal activity against *Ae. vittatus* larvae, with a mortality percentage of 18.33 ± 0.46 at 50 ppm, 38.33 ± 0.71 at 100 ppm, 61.66 ± 0.75 at 150 ppm, 71.66 ± 0.74 at 200 ppm, and 83.33 ± 0.53 at 250 ppm. Similarly, the aqueous extract demonstrated a mortality percentage of 28.33 ± 0.53 at 50 ppm, 53.33 ± 0.74 at 100 ppm, 66.66 ± 0.51 at 150 ppm, 78.33 ± 0.35 at 200 ppm, and 98.33 ± 1.30 at 250 ppm.

On the other hand, the hexane and acetone extracts of *P. pinnata* leaves exhibited relatively low larvicidal activity against *Ae. vittatus* larvae. The hexane extract showed a mortality percentage of 26.66 ± 0.71 at 50 ppm, 43.33 ± 0.46 at 100 ppm, 53.33 ± 0.52 at 150 ppm, 61.66 ± 0.64 at 200 ppm, and 81.66 ± 0.53 at 250 ppm. The acetone extract showed a mortality percentage of 16.66 ± 0.64 at 50 ppm, 33.33 ± 0.46 at 100 ppm, 41.66 ± 0.46 at 150 ppm, 63.33 ± 0.71 at 200 ppm, and 76.66 ± 0.53 at 250 ppm.

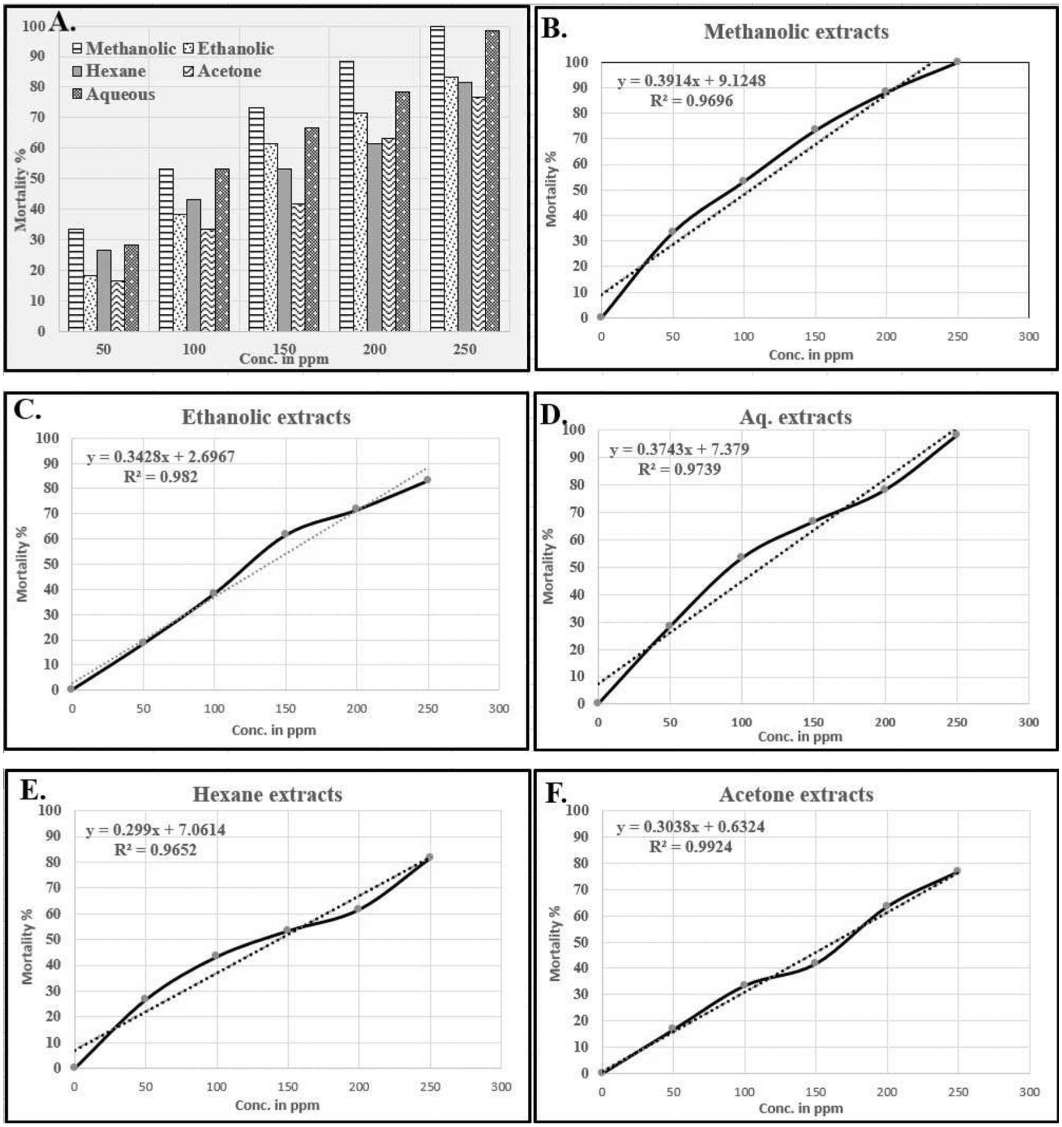

Fig 1. *Pongamia pinnata* leaf extracts larvicidal efficacy against the 4th Instar larvae of *Aedes vittatus.* (A) Bar graph of mortality percentages of different extracts; (B) Line fit plot of Methanolic extracts; (C) Line fit plot of Ethanolic extracts; (D) Line fit plot of Aqueous extracts; (E) Line fit plot of Hexane extracts; and (F) Line fit plot of Acetone extracts

Table 3 and Fig. 2 show the LC50 and LC_{90} values of the different extracts of *P. pinnata* against the 4th instar larvae of *Ae. vittatus* in the present study. Among the extracts tested, the methanolic extract displayed the lowest LC_{50} value of 104.43 ppm, with 95% confidence limits of 104.13 and 104.92, and an LC_{90} value of 206.63 ppm, with 95% confidence limits of 206.33 and 207.12. The aqueous extract showed an LC50 value of 113.86 ppm, with 95% confidence limits of 113.57 and 114.32, and an LC_{90} value of 220.73 ppm, with 95% confidence limits of 220.44 and 221.19. The ethanolic extract exhibited moderate LC_{50} and LC_{90} values with an LC50 value of 137.99 ppm, with 95% confidence limits of 137.77 and 138.42, and an LC_{90} value of 254.68 ppm, with 95% confidence limits of 254.46 and 255.11. The hexane and acetone extracts displayed the highest LC_{50} and LC_{90} values in the present study. The LC_{50} values of the hexane and acetone extracts were found to be 143.17 ppm and 162.5 ppm, respectively, and LC_{90} values were found to be 276.55 ppm and 294.16 ppm, respectively.

Table 3. LC_{50} and LC_{90} values (ppm) of *Pongamia pinnata* leaf extracts against the 4th Instar larvae of *Aedes vittatus*

Extract	**LC_{50}**	**LC_{50} 95% Confidence limits**		**LC_{90}**	**LC_{90} 95% Confidence limits**	
		LCL	**UCL**		**LCL**	**UCL**
Aqueous	113.86	113.57	114.32	220.73	220.44	221.19
Methanolic	104.43	104.13	104.92	206.63	206.33	207.12
Ethanolic	137.99	137.77	138.42	254.68	254.46	255.11
Hexane	143.17	142.98	143.50	276.55	276.36	276.88
Acetone	162.5	162.26	162.86	294.16	293.92	294.52

In summary, the methanolic extract of *P. pinnata* leaves demonstrated the highest larvicidal activity, followed by Aqueous and ethanolic extracts against *Ae. vittatus* larvae, whereas the Hexane and Acetone extracts exhibited the lowest activity.

Similar results were obtained in a previous study[25]. In that study, the methanolic extracts of *P. pinnata* showed better larvicidal efficacy than hydroalcoholic extracts against the larvae of *Culex quinquefasciatus* and *Ae. aegyptii* with LC_{50} values of 84.8 ppm and 118.2 ppm respectively and with LC_{90}

values of 184.7 ppm and 227.0 ppm, respectively. These findings indicate that the methanolic extract may have the potential to serve as a natural larvicide for controlling Aedes vittatus populations. Future studies should focus on isolating and identifying the bioactive compounds responsible for the observed larvicidal activity.

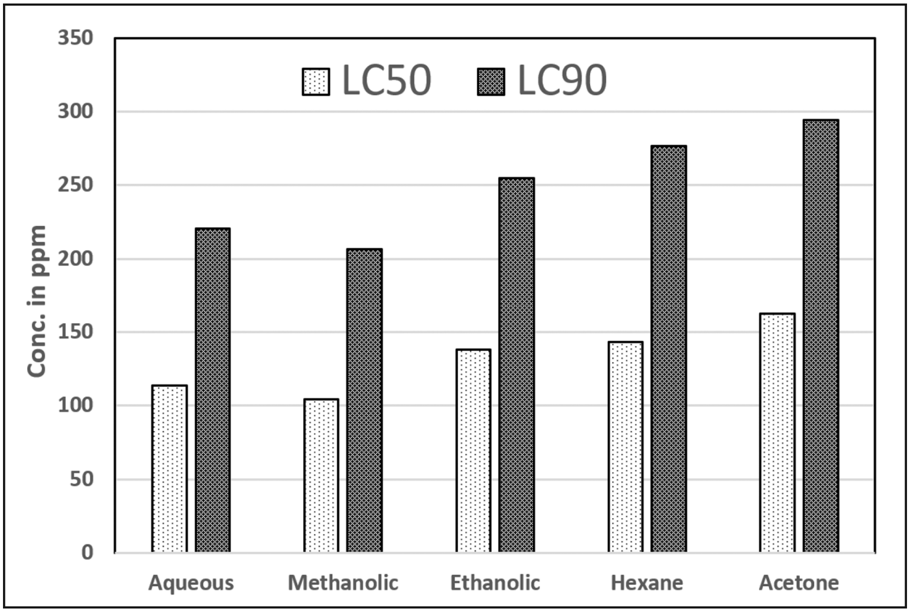

Figure 2. LC_{50} and LC_{90} concentrations of the tested *Pongamia pinnata* leaf extracts against the 4th Instar larvae of *Aedes vittatus.*

CONCLUSION

In conclusion, the phytochemical analysis of *P. pinnata* leaves revealed the presence of a diverse range of phytochemicals, with the methanol extract showing the highest abundance of phytochemicals. The larvicidal bioassay demonstrated significant larvicidal activity of the aqueous, methanolic, and ethanolic extracts of *P. pinnata* leaves against *Ae. vittatus* larvae, with the methanolic extract exhibiting the highest activity. The hexane and acetone extracts showed relatively low activity against the larvae. The methanolic extract displayed the lowest LC_{50} and LC_{90} values, while the hexane and acetone extracts had the highest values. These findings suggest that the methanolic extract of *P. pinnata* leaves has the potential as a natural pesticide against *Ae. vittatus* larvae. The results of this study are consistent with those of previous studies on the larvicidal activity of *P. pinnata.*

***Conflict of Interest*:**

The authors don't have any sort of financial or non-financial conflicts with any person or firm that can affect the results of this research.

***Author Contributions*:**

MM conceptualized, investigated, curated and analysed data, drafted manuscript and edited.

LM visualized, investigated and wrote the original draft.

REFERENCES

1. World Health Organization. Fact-sheets: Vector-Borne Diseases. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases [accessed 12th November 2022].
2. World Health Organization. World malaria report-2021. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021 [accessed 12th November 2022].
3. Pavela R, Benelli G. Essential oils as eco-friendly Biopesticides? Challenges and constraints. *Trends in Plant Sciences*. 2016; 21(2): 1000-1007. DOI: 10.1016.j.plants.2016.10.005.
4. Valbuena D, Cely-Santos M, Diana Obreg´on. Agrochemical pesticide production, trade, and hazard: Narrowing the information gap in Colombia. *Journal of Environmental Management*. 2021; 286: 112141. https://doi.org/10.1016/j.jenvman.2021.112141.
5. World atlas. Top Pesticide Using Countries. https://www.worldatlas.com/articles/top-pesticide-consuming-countries-of-the-world.html [accessed 12th November 2022].
6. 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.
7. Philip C. B. 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.

8. Surendran N, Jayadas TTP, Thiruchenthooran V, *et al.* Aedes larval bionomics and implications for dengue control in the paradigmatic Jafna peninsula, northern Sri Lanka. *Parasit Vectors*. 2021; 14(1): 162. DOIi: 10.1186/s13071-021-04640-6.
9. Domínguez-Costas M, Ayres C. New record of Aedes vittatus (Bigot, 1861) (Diptera: Culicidae) in Galicia. *Arquivos Entomolóxicos*. 2020; 22: 279-280.
10. Ferede G, Tiruneh M, Abate E, *et al.* Distribution and larval breeding habitats of Aedes mosquito species in residential areas of northwest Ethiopia. *Epidemiol Health*. 2018; 23(40): e2018015. DOI: 10.4178/epih.e2018015.
11. Nasirian H, Sadeghi SMT, Vazirianzadeh B, Moosa-Kazemi 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.
12. 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.
13. 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.
14. Sudeep AB, Mohandas S, 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.
15. Mulwa F, Lutomiah J, Chepkorir E, *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.
16. Mavale MS, Ilkal MA, Dhanda V. Experimental studies on the susceptibility of *Aedes vittatus* to dengue viruses. *Acta Virologica*. 1992; 36(4): 412-416.
17. 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.
18. Glare T. Types of biopesticides. In: Leo Nollet LML, Hamir SR. (eds.) *Biopesticides handbook*. CRC Press; 2015. P. 7-25.
19. Thakore Y. The biopesticide market for global agricultural use. *Ind. Biotechnol*. 2006; 2: 194-208.
20. 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.

21. Al Salhi 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.

22. 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.

23. World Health Organization. Guidelines For Laboratory and Field Testing of Mosquito Larvicides. https://apps.who.int/iris/bitstream/handle/10665/69101/WHO_CDS?sequence=1 [accessed 12th November 2022].

24. Abbott WS. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*. 1925; (18): 265-266.

25. Kolli GR, Balakrishnan V, Sundararajan R. Evaluation of the larvicidal activity of *Pongamia pinnata* extracts against three mosquito vectors. *Asian Pacific Journal of Tropical Biomedicine*. 2013; 3(11): 853–858. DOI:10.1016/s2221-1691(13)60168-9.

★ ★ ★ ★ ★