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Larvicidal activity of *Musa acuminata* (Musaceae) plant leaf extracts dissolved in different chemical solvents against *Aedes albopictus* (Diptera: Culicidae)

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Abstract:
To determine the plant Musa acuminata *potential as an anti-mosquito agent. Larviciadal efficacy of hexane, diethyl ether, ethyl acetate, dichloromethane, and aqueous extracts of leaves of* Musa acuminata *were evaluated against 3rd instar larvae of* Aedes albopictus *treated with different concentrations (20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L) and a simultaneous control at 24, 48 and 72 hours. The mortality rate varies dose-dependently. The test for larvicidal activity with different solvent extracts showed significant efficiencies against* Aedes albopictus*. The outcome of the toxicity test was impressive. The study reveals that the different solvent extracts of* M. acuminata *could be an effective natural alternative to getting control over the* Aedes *mosquito vector. Further study is required to study the effective changes that occur in terms of the larval mortality pathways involved.*

Keywords: Musa acuminata, *solvent extract,* Aedes albopictus, *larvicide, mortality.*

I. Introduction:

Several diseases are transmitted by mosquito vectors, such as dengue, yellow fever, zika, chikungunya, filariasis, onchocerciasis, and mansonellosis. *Aedes albopictus is* an important mosquito vector that transmits chikungunya and dengue fever to humans. More than 100 countries, including two fifths of the world's population, are at risk of dengue, which is also responsible for the transmission of many arboviruses to laboratory animals and birds. [1, 2]. The most common means of controlling mosquito vectors is through mechanical control, which eliminates the vector and reduces mosquito-human contact by destroying its breeding sites. Another important controlling measure is biological control, which reduces the vector population by using predators or pathogens. Aside from these methods, synthetically derived chemicals are used to kill both adult and larval mosquito vectors. The use of synthetic insecticides, which are commercially available, has been widely used for controlling disease-causing mosquitoes, but a major devastating factor is the development of resistance to the synthetic insecticide after repeated use. The major threat to controlling disease-causing vectors, including *A. albopictus* [3], is the development of resistant mosquito populations. These resistant insecticides induce toxicity in humans by creating environmental pollution [4]. This evolves the requirements of natural substance development, which can obliterate the life cycle of insects at various stages such as the mosquito's egg, larva, pupa, and adult stages. Alternative strategies for vector control [5] with the help of natural agents offer less toxicity and are comparatively safer than synthetic insecticides. Several studies have confirmed that plants or specific plant parts possess larvicidal and insecticidal potential. About 479 articles have been published between 1968 and 2016, which appraise the activity of natural plant products against various mosquito vectors [6–8]. Currently, the major natural substances such as amides, quinones, and terpenoids have potential larvicidal activity against the larval stages of different vectors [6, 9], but very few studies have been carried out on *Ae. albopictus* over a long period of treatment [10]. The plant extracts contain various phytochemicals that might be acting together against the disease-causing vector and can be used long-term, are biodegradable, rarely develop resistance, and have a higher environmental safety profile than synthetic compounds [11]. As a result, in this study, we assessed the larvicidal activity of *M. acuminata* leaves, which have a variety of ethnopharmacological and ethnobotanical therapeutic properties. The *M. acuminata* plant parts, such as the leaf, fruit, and peel extracted, are known to inhibit disease-related pathways, as supported by research studies. Thus, the present investigation was carried out to evaluate the larvicidal efficacy of the leaf of *Musa acuminata against the* IIIrd instar larval stage of *Ae. albopictus.*

II. Material and Methods:

For the extraction of *M. acuminata* shade-dried leaf powder, all analytical grade solvents such as hexane, di-ethylether, ethylacetate, and dichloromethane and aqueous were used.

Plant material and extraction:

Leaves of the plant *M. acuminata* were collected from the botanical garden of the University of Lucknow, India. A commercial electric stainless steel blender was used to powder the dried leaves (about 200 g). After that, extractions were carried out with different solvents in a Soxhlet apparatus. The temperature of the heating mantle was maintained at 60–65 °C. The procedure lasted 6-8 hours. The extracts were filtered through a Buchner funnel with Whatman No. 1 filter paper. The solvents were recovered using a rotary evaporator. The thick residues obtained were stored at 4 °C for further analysis.

Mosquito vector species

Laboratory-reared, third-instar larvae of *Aedes albopictus* were collected from the field and colonised in the laboratory. The larvae were maintained at room temperature (25±2°C) and kept in dechlorinated tap water in an enamel bowl. Larvae were fed on the plant debris.

Larvicidal Activity Assay

In order to evaluate the larvicidal activity of the different extracts, 3rd instar larvae of *Aedes albopictus* were exposed to different concentrations of *M. acuminate* leaf extracts (as mentioned above). On the basis of standard WHO procedure with a slight modification, the larvicidal activity was assessed [14]. Five different concentrations of *M. acuminata* leaf extract were prepared in dechlorinated tap water, i.e., 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. 100 ml of each such concentration was taken in glass beakers (Borosil 250 ml). Twenty third-instar *Aedes albopictus* larvae were introduced into different plant extract test concentrations. In three replicates, each concentration was run concurrently with a control at room temperature. The larvae were fed plant debris on the water's surface. The number of dead larvae was counted after 24, 48, and 72 hours of exposure to various leaf extracts. In order to prevent decomposition, the dead larvae were removed soon after the mortality, which may induce death in the remaining larvae. A total of three such trials were conducted.

Statistical analysis

The percentages of larval mortality and standard deviations were calculated for each concentration of the extracts with the help of Excel. Lethal concentrations (LC_{50}) and (LC_{90}) were determined at a 95% confidence level using probit analysis. Statistical analysis was carried out using SPSS software version 25. $P< 0.05$ results were considered statistically significant.

III. Results:

The present study found that the different leaf extracts of the plant *M. acuminata* have satisfactory larvicidal activity against *Aedes albopictus* larvae. The results demonstrate that among the different extracts of leaves such as hexane, di chloromethane, ethyl acetate, di ethyl ether, and aqueous extracts (as shown in **Table 1**), the rate of mortality increased with dose, and the 100 mg/L concentration caused maximum mortality among the tested concentrations. All solvent extracts of *M. acuminata* show a moderate effect against *Aedes albopictus* larvae. When third instars larvae of *Aedes albopictus* were exposed to the non polar to polar solvent extracts of *Musa acuminate* (Banana leaf) Dichloromethane extract revealed the highest toxicity (99.16%) at 100 ppm of 72 hr of exposure followed by Ethyl acetate (93.5%) at 100 ppm of 72 hr of exposure, the Hexane with (88.3%) at 72 hr followed by Aqueous (86.8%) and Diethyl ether (86.7%) at 72 hr.

Table-1: Larvicidal activity of *Musa acuminata* (Banana plant) with different solvent extracts against IIIrd instar larvae of *Aedes albopictus*

Solvent Extracts	Doses of Concentrations	% Mortality ± S.D		
		24 hrs	48 hrs	72 hrs
Hexane	Control	0	0	0.83±0.4
	20 mg/L	13.3±0.8	25.83±0.75	32.5±0.325
	40 mg/L	20.8±0.75	32.5±1.04	41.6±0.4
	60 mg/L	40±0.63	54.16±0.98	55±0.5
	80 mg/L	65±0.89	74.16±0.4	76.6±0.8
	100 mg/L	69.16±0.75	76.6±0.8	88.3±0.8
Diethyl ether	Control	0	0.8±0.4	0.8±0.4
	20 mg/L	5.8±0.4	10.8±0.4	22.3±0.5
	40 mg/L	17.5±0.5	24.6±0.5	41.6±0.5
	60 mg/L	21.6±0.8	33.4±0.4	46.6±0.5
	80 mg/L	23.3±1.2	43.4±0.5	66.6±0.5
	100 mg/L	40.8±0.75	48.3±0.5	86.7±0.5
Ethyl Acetate	Control	0	0.8±0.4	0.83±0.4
	20 mg/L	2.5±0.5	15.8±0.4	23.3±0.5
	40 mg/L	15±0.15	28.3±0.5	36.6±0.5
	60 mg/L	22.5±0.225	40.8±0.4	46.6±0.5
	80 mg/L	41.6±0.5	47.5±0.5	58.3±0.5
	100 mg/L	48.3±1.3	72.5±0.5	93.5±0.5
Dichloromethane	Control	0	0	0.83.25±0.4
	20 mg/L	26.6±0.8	38.3±0.5	51.6±0.8
	40 mg/L	45.8±0.9	48.3±0.5	61.6±0.5
	60 mg/L	80.3±0.6	75.8±1.47	90±0.6
	80 mg/L	86.6±2.06	91.6±0.5	95±0.6
	100 mg/L	93.3±0.8	99.3±0.5	99.16±0.4
Aqueous	Control	0	0	0.8±0.4
	20 mg/L	14.16±0.75	22.5±0.8	33.3±0.5
	40 mg/L	21.6±0.5	35±0.6	42.5±0.5
	60 mg/L	28.3±0.5	43.3±0.5	71.6±0.5
	80 mg/L	41.6±0.5	60±0.8	71.6±0.8
	100 mg/L	62.5±0.5	74.16±0.98	86.67±0.86

Control- nil mortality, Mean value of 3 replicates; Significant at $p<0.05$ level.

IV. Discussion:

The larval stage in the life cycle of mosquitoes are attractive targets for pesticides, because stagnant water is their breeding sites which can be easily accessed, However, spray of chemical pesticides in water sources induces greater risks to the environment and humans [12,13]. Therefore the natural pesticides or pesticides derived from plants are a capable method for managing mosquito larvae without showing harmful effect to the environment [14–16].

Correspondingly, Rajkumar et al 2009 studied the larvicidal activity of the leaf extracts of *Cassia obtusifolia* against larval stage of *Anopheles stephensi* [17]. It has been observed from the study that extract induced high mortality at high concentrations. In another study, Chansang et al. 2005 studied the extracts of five medicinal plants *i.e. Abutilon indicum, Aegle marmelos, Euphorbia thymifolia, Jatropha gossypifolia* and *Solanum torvum* by using ethyl acetate, acetone, crude hexane, petroleum ether and methanol and were tested for their toxicity against the early fourth instar larvae of *Culex quinquefasciatus.* It was found from the study that petroleum ether extract of *Abutilon indicum* gave the highest larval mortality [18].

Another study explored the effect of ether extracts of *Emblica officinalis, Ricinus communis, Acacia coucinna, Cinnamomum tejpata, Piper nigrum, Coriandrum sativum, Olea vera, Linum usitatissimum, Syzygium aromaticum* and *Nigella sativa* against larvae of *Aedes albopictus* under laboratory conditions. The study found that, all extracts are showing moderate larvicidal activity, whereas the lowest LC_{50} was found in *Coriandrum sativum, Nigella sativa* and *Syzygium aromaticum* at a dose of 363.7 ppm, 377.5 ppm and 403.4 ppm, respectively, after 24 hrs exposure. They also found that at low concentration of extracts dose showed their larvicidal activity after 48 hrs [19]. In the present study, leaves of *M. acuminata* were used to evaluate the larvicidal activity against III instar larvae of *Ae. albopictus.* Different solvents were used to make the extracts of the leaves of *M. acuminata* such as hexane, di chloromethane, ethyl acetate, di ethyl ether, aqueous extracts. All the extracts showed remarkable larvicidal properties but di- chloromethane extract have greater toxicity towards larvae. In comparison to dose of other extracts the LC_{50} dose is lowest at 24, 48 and 72 hrs i.e 4.6, 4.2 and 3.3 mg/L respectively. It has been also observed from the study that all the extracts at 72 hrs are showing their larvicidal property at consecutively with low dose. However, the aqueous extract also feature relatively pronounced larvicidal activity at a dose of 5mg/L for 72 hrs.

V. Conclusion:

Therefore, conclusively the present study demonstrate that the plant part i.e. leaves of *M. acuminata* has notable larvicidal activity in terms of low dose and time dependant. Further study is required to study the effective changes occur in terms of larvae mortality pathways involved.

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References:

1. Gratz. Critical review of the vector status of *Aedes albopictus*. Medical and Veterinary Entomology. 2004; 18: 215–227.
2. CHEN, L.H. & WILSON, M.E. Dengue and chikungunya infections in travelers. Current Opinion in Infectious Diseases. 2010; 23(5): 438–444.
3. Gambarra WPT, Martins WFS, Lucena Filho ML, Albuquerque IMC, Apolinário OKS, Beserra EB. Spatial distribution and sterase activity in populations of *Aedes (*Stegomyia) *aegypti* (Linnaeus) (Diptera: Culicidae) resistant to temephos. *Rev Soc Bras Med Trop*. 2013; 46(2):178-84.
4. Campos J, Andrade CFS. Insecticide resistance in Simulium populations (Diptera, Simuliidae). Cad Saúde Pública. 2002; 18(3):661-71.
5. Zara ALSA, Santos SM, Fernandes-Oliveira ES, Carvalho RG, Coelho GE. Estratégias de controle do *Aedes aegypti*: uma revisão. Epidemiol Serv Saúde. 2016; 25(2):391-404.
6. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012; 135:581-98.
7. Guilhermino JF, Guerrero ATG, Almeida FS, Grillo ZCFA. Ciência no uso de produtos naturais para controle do vetor do vírus Zika (ZIKV). *Rev Fitos*. 2016; 10(1):8-12.
8. Satoto TBT, Hadianto T, Istiyana N, Badriyah S, Diptyanusa A. Mosquitocidal activities of Tagetes erecta L. compared to prallethrin against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Res J Med Plants.* 2018; 12(2):65-71.
9. Garcez WS, Garcez FR, da Silva LMGE, Sarmento UC. Naturally occurring plant compounds with larvicidal activity against *Aedes aegypti. Rev Virtual Quim.* 2013; 5(3):363-93.

10. Tankeu Nzufo Francine et al. Larvicidal activities of hydro-ethanolic extracts of three Cameroonian medicinal plants against *Aedes albopictus. Asian Pac J Trop Biomed* 2016; 6(11): 931–936
11. Maurya P, Sharma P, Mohan L, Verma MM, Srivastava CN. Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi. Asian Pac J Trop Dis*. 2012; 2(2): 110–6.
12. Subramanian J, Kovendan K, Kumar PM, Murugan K, Walton W. Mosquito larvicidal activity of *Aloe vera* (Family: liliaceae) leaf extract and Bacillus sphaericus, against Chikungunya vector, *Aedes aegypti*. *Saudi J Biol Sci*. 2012; 19:503–509. Doi: 10.1016/j.
sjbs.2012.07.003
13. Bagavan A, Rahuman AA. Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. *Asian Pac J Trop Med.* 2011; 4:29–34. doi:10.1016/S1995-7645(11) 60027-8
14. Warikoo R, Ray A, Sandhu JK, Samal R, Wahab N, Kumar S. Larvicidal and irritant activities of hexane leaf extracts of tropical biomedicine. *Asian Pac J Trop Biomed*. 2012;2(2):152–155.doi:10.1016/S2221-1691(11)60211-6
15. Mukhatar MU, Mushtag S, Arkam A, Zaki B, Hammad M, Bhatti A. Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti*. *Int J Mosquito Res.* 2015; 2(3):127–130.
16. Hemalatha P, Elumalai D, Janaki A, et al. Larvicidal activity of *Lantana camara aculeate* against three important mosquito species. *J Entomol Zool Stud.* 2015; 3(1):174–181.
17. Rajkumar, S. & Jebanesan, A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family:Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitology Research*, 2009; **104**: 337–340.
18. Chansang, U., Zahiri, N., Bansiddhi, S., Jaree, B., Thidarat, T., Ratom, M., Jiranuch, B.N. & Mulla, M.S. Mosquito larvicidal activity of aqueous extracts of long pepper (*Piper retrofractum* Vahl) from Thailand. *Journal of Vector Ecology*. 2005; **30**: 195–200.
19. Bilal H, Akram W, Din S, Khan I.A, Hassan S.A & Arshad M. Larvicidal activity of plant extracts against *Aedes albopictus. African Entomology*. 2012; **20**(1): 8–12.