Evaluation of larvicidal efficacy of different solvent extracts of *Musa acuminata* leaf against the malaria vector *Anopheles stephensi*

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Abstract:

Present investigation was carried out to explore the larvicidal efficacy of the leaf extract of *Musa acuminata* (banana leave powder) against 3rdinstar larvae of malaria vector, *Anopheles stephensi*. The larval mortality of 3rd instar larvae of *Anopheles stephensi* after 24h, 48h, and 72h after testing were analyzed separately in solution of various concentrations in control 20, 40, 60, 80, 100 mg/L of the leaf extracts in (hexane, diethyl ether, ethyl acetate, dichloromethane and aqueous solvent) of *Musa acuminata*. Most effective LC_{50} and LC_{90} value observed on dichloromethane extract (48.01, 39.40 and 35.23 and 114.54, 104..83 and 94.56 mg/L) at the 24, 48 and 72 hrs respectively and least effective LC_{50} and LC_{90} value showed on aqueous extract (207.09, 171.72 and 141.84 and 1062.53, 980.27 and 1068.08 mg/L).The treatment of extracts is time- dependent and dose dependent. Further study is needed to evaluate the changes in morphogenesis of larvae after plant extract treatment and *M. acuminata* has the potential to be used as an eco-friendly alternative for managing mosquito of population *An. stephensi* being an effective larvicide.

Keywords: *Anopheles stephensi, Musa acuminata leaf extract, larvicidal efficacy, malaria vector*
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Introduction:

The Asian malaria mosquito*, Anopheles stephensi* is the most important urban malaria vector in the Middle East and Indian sub-continent. It synthetic insecticides/compounds are used to control mosquito larvae but their repeated usages lead to pesticide resistance at the same time they are costly and not beneficial for human health. Currently, the world has moved towards to utilization of natural plant-based products as compared to synthetic products. *Musa acuminata* Linn. Often referred to as plantain or the cooking banana plant is a native of India and the southern part of Asia. It belongs to Musaceae family *and* has therapeutic properties supported by numerous ethno pharmacological and ethnobotanical studies. The genus *Anopheles* includes dozens of mosquito species that are important vectors of malaria and are annually responsible for more than 200 million cases of disease and more than 600,000 deaths worldwide (WHO 2013) [1]. Mosquito control contributed significantly to the recent decrease in malaria incidence and mortality [4]. However, insecticide resistance is widely reported in mosquito populations in malaria-endemic areas of Africa and India [4]. Synthetic insecticides/compounds are

used to control mosquito larvae [3] but their repeated usages lead to pesticide resistance. They are, costly also and harmful for human health [4]. Some of these undesirable side effects initiate better control measures by alternative natural methods. Therefore, the search for new eco-friendly and sustainable strategies for reducing the mosquito population, particularly by plant-derived natural products is required. Several factors are responsible to take further the use of plant-based biological pesticides as safe alternative. They are biodegradable, economical, non-toxic to non-target pests, and have high selective specific activities towards the target pests. Plant extracts and essential oils are biodegradable and cost-effective and they have shown good larvicidal activity against the mosquito [5].

Musa acuminata is an herbaceous flower-producing plant. It is a native of southern Asia, and spreads across the Indian subcontinent and the southern part of Asia. It belongs to Musaceae family comprising of three genera, *Musa, Ensete*, and *Musella*. Musa is the largest group among the three genera, with about 35 species that include *Musa acumiata, Musa balbisiana, Musa sapietum*, etc. Recently, there had been increasing importance of plants as a substantial source of first-hand medicines. In developing countries like Nigeria, a large number of people utilize various parts of the plant for the treatment of various human ailments. Currently, the world has moved towards the utilization of natural plant-based products instead of synthetic products [6]. The *Musa acuminata* has therapeutic properties as native people utilize them for medicinal use that leads to exploring fascinating properties of plant parts supported by numerous ethno pharmacological studies and ethno pharmacological studies [7, 8]. Utilization of biowaste produced of (Banana peels) *Musa acuminata* L. for the synthesis of green silver nanoparticles (AgNPs) and biological activity was estimated for management of the dengue vector, *Aedes aegypti* [9]. The present investigation was carried out to explore the larvicidal efficacy of the leaf of *Musa acuminata* against the 3rdinstar larval stage of *Anopheles stephensi*.

MATERIAL AND METHODS:

Plant material:

Leaves of the plant *Musa acuminata* were collected from the botanical garden of the Department of Botany, University of Lucknow U.P. India.

Extraction:

Preparation of solution of leaves extract of *Musa acuminata*. The dried plant leaves (500g) were powdered mechanically using a commercial electrical stainless steel blender and extracted with different solvents of varying polarities, such viz. hexane, di-ethyl ether, ethyl acetate, dichloromethane, and aqueous(water) in a Soxhlet apparatus (Borosil).The temperature of the heating mantle was maintained at(69.0, 34.6,77.1, 39.6, and 100)°C. The process of extraction was carried out for 6–8 h. The extracts were filtered through a Buchner funnel with Whatman No.1 filter paper. The solvents were removed using a rotary evaporator. The residues obtained were stored at 4 °C till further analysis. 1% stock solution with acetone was prepared for extract solution of different concentrations viz. (20, 42, 60, 80 and 100 mg/L) separately were prepared by adding each from stock solution different concentration ranges were prepared with dechlorinated water of appropriate quantity as per requirement

Mosquitoes rearing:

Anopheles stephensi larvae were collected from the campus of University of Lucknow, Lucknow and identified by using pictorial keys [10, 11]. To start the colony larvae were kept in enamel trays with dechlorinated water and temperature was maintained at room temperature (27 ± 2 °C). Larvae were fed on the pond scum and yeast powder in a 3:1 ratio. Larvae were maintained and reared in laboratory as per the method of Kamaraj *et al* [12].

Larvicidal Bioassay:

In the larvicidal screening, 3rdinstar larvae of *Anopheles stephensi* were exposed to different concentrations of *Musa acuminata* extracts (as mentioned above). The larvicidal activity was assessed by the standard WHO procedure [13] with slight modification according to Rahuman *et al* [14].

Five different concentrations of *Musa acuminata* leaf extracts were prepared in dechlorinated tap water i.e. 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L.200 ml of each such concentration was taken in plastic beakers (250ml).Twenty-five healthy 3rd instar larvae of *Anopheles stephensi*, were then introduced in these beakers. Four replicates of each concentration were run simultaneously along with control at room temperature. The number of dead larvae was recorded at an interval of 24, 48, and 72 hrs of exposure. The dead larvae were removed soon after the mortality to avoid their decomposition in the experimental solution that may enhance the mortality of the remaining larvae. A total of three such trials were carried out over the span of two consecutive weeks.

Statistical analysis:

The percentages of larval mortality were calculated for each concentration and 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 21. Result with $p<0.05$ were considered to be statistically significant.

Result:

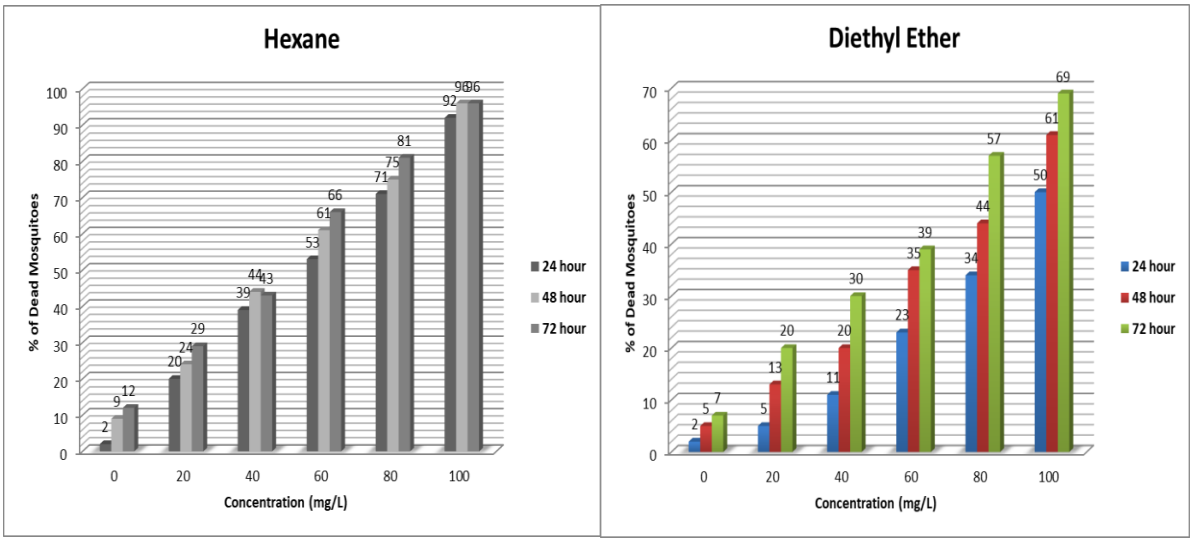
It has been found that dichloromethane extract of *M. acuminata* induces significantly maximum mortality of 3rdinstar larvae of *An. Stephensi* followed by hexane extract of *M. acuminata* with LC50 and LC90 value (48.01, 39.40 and 35.23 and 114.54, 104.83 and 94.56 mg/L its 40 mg/L) 46.63, 41.00 and 37.69 and 134.11, 116.16 and 107.78 at 24, 48 and 72 hrs respectively. Minimum effective result showed on aqueous extract (207.09, 171.72 and 141.84 and 1062.53, 980.27 and 1068.08 mg/L) at 24, 42 and 72 hrs respectively. Ethylacetate was more toxic (83.90, 66.32 and 49.08 mg/L and 288.72, 187.68 and 184.98 mg/L) than diethylaether extract (111.31, 87.16 and 66.59 and 364.37, 374.64 and 315.35 mg/L) at 24, 42 and 72 hrs respectively. It was also observed from that treatment of aqueous extract of *M. acuminata* is least effective and time and dose dependent as showed in (table-1). The mortality percentage showed after 24hr, 48hr and 72hr (hexane, diethyl ether, ethyl acetate, dichloromethane, and aqueous) extracts of *Musa acuminate* in (graph-1) and dichloromethane noticed maximum efficiency (99%)and aqueous showed minimum effect (42%) and hexane extract showed (96 %) ethylacetate (83%) and diethyl ether (69%) at 72 hrs. Mortality observed all the value are statistically significant in selected solvents at the 24, 48 and 72 hours respectively in Anova sub-table A, B, C, D and E as shown in table-2 below.

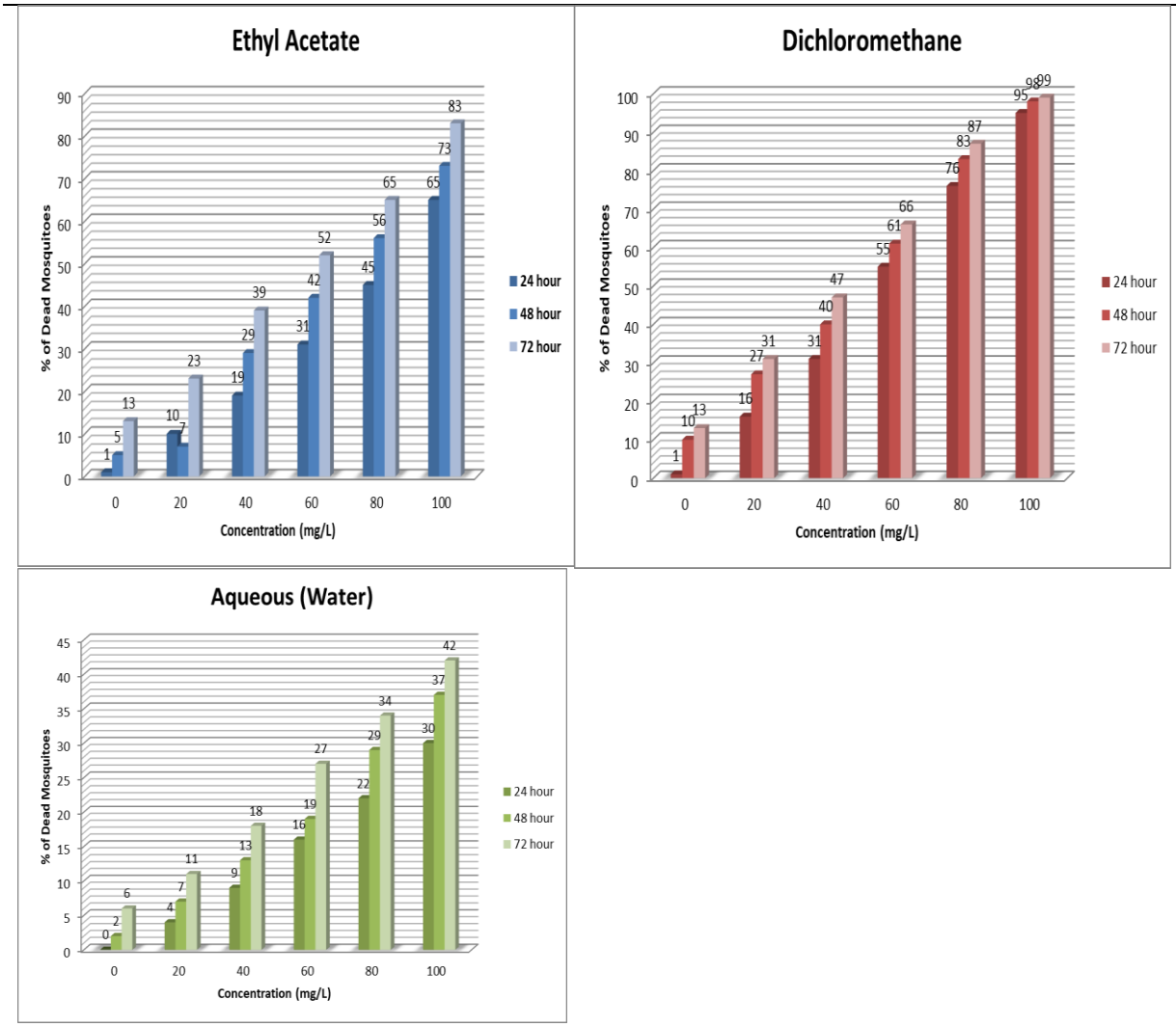
Table-1 Larvicidal activity of different solvent extract of *Musa acuminata* leaf against 3rd instar larvae of *Anopheles stephensi*.

	Time	**LC50**			**LC90**			**Regression Equation**	**χ^2 (df=3)**
		Estimate	LCL	UCL	Estimate	LCL	UCL		
Hexane	**24hrs**	46.63	27.90	67.37	134.11	85.33	711.76	y = -4.661 + 2.793x	12.84*
	48hrs	41.00	21.54	58.87	116.16	75.24	589.43	y = -4.570 + 2.834x	14.02*
	72hrs	37.69	18.23	54.04	107.78	70.32	521.95	y = -4.428 + 2.809x	14.08*
Diethyl Ether	**24hrs**	111.31	95.10	141.23	364.37	250.53	681.96	y = -5.093 + 2.489x	2.89
	48hrs	87.16	74.99	107.58	374.64	249.87	738.54	y = -3.927 + 2.024x	4.45
	72hrs	66.59	48.48	110.59	315.35	158.96	3548.06	y = -3.460 + 1.897x	5.59

Ethyl Acetate	**24hrs**	83.90	63.79	148.24	288.72	158.29	2041.02	y = -4.593 + 2.388x	6.22
	48hrs	66.32	60.16	73.87	187.68	151.50	256.43	y = -5.168 + 2.837x	1.70
	72hrs	49.08	34.32	66.53	184.98	113.80	750.79	y = -3.760 + 2.224x	6.19
Dichloromethane	**24hrs**	48.01	29.38	69.14	114.54	76.79	478.89	y = -5.708 + 3.395x	16.70*
	48hrs	39.46	13.08	62.53	104.83	65.19	1722.29	y = -4.820 + 3.020x	22.38*
	72hrs	35.23	11.56	53.39	94.56	60.50	827.09	y = -4.623 + 2.989x	19.82*
Aqueous (Water)	**24hrs**	207.09	145.25	431.55	1062.53	487.79	5711.22	y = -4.180 + 1.805x	0.40
	48hrs	171.72	125.98	313.49	980.27	468.57	4483.99	y = -3.786 + 1.694x	1.22
	72hrs	147.84	109.94	262.66	1068.08	485.89	5597.52	y = -3.238 + 1.492x	0.62

LC50 and LC90 (LCL: Lower confidence limit , UCL: Upper confidence limit), X^2 – Chi-square values are significant at $p<0.05$ level, df: degree of freedom





Graph-1 Percentage mortality of larvicidal activity of various solvent extract of *Musa acuminata* leaf against *Anopheles stephensi.*

Table no.2 one ways Anova of mortality of 3rd instar larvae of *Anopheles stephensi* in hexane, diethyl ether, ethylacetate, dichloromethane and aqueous extract of plant *Musa acuminata* where concentration and hours considered as variables.

(A) Hexane		Sumof Squares	df	Mean Square	F	Sig.
Hours24	Between Groups	1257.500	5	251.500	122.351	.000
	Within Groups	37.000	18	2.056		
	Total	1294.500	23			
Hours48	Between Groups	1289.333	5	257.867	134.539	.000

	Within Groups	34.500	18	1.917		
	Total	1323.833	23			
Hours72	Between Groups	1243.208	5	248.642	150.439	.000
	Within Groups	29.750	18	1.653		
	Total	1272.958	23			

(B) Diethylether		Sum of Squares	df	Mean Square	F	Sig.
Hours24	Between Groups	385.208	5	77.042	82.791	.000
	Within Groups	16.750	18	.931		
	Total	401.958	23			
Hours48	Between Groups	492.375	5	98.475	116.233	.000
	Within Groups	15.250	18	.847		
	Total	507.625	23			
Hours72	Between Groups	529.208	5	105.842	101.608	.000
	Within Groups	18.750	18	1.042		
	Total	547.958	23			

(C) Ethylacetate		Sum of Squares	df	Mean Square	F	Sig.
Hours24	Between Groups	660.833	5	132.167	61.000	.000
	Within Groups	39.000	18	2.167		
	Total	699.833	23			
Hours48	Between Groups	745.375	5	149.075	58.018	.000
	Within Groups	46.250	18	2.569		
	Total	791.625	23			
Hours72	Between Groups	904.833	5	180.967	132.955	.000
	Within Groups	24.500	18	1.361		
	Total	929.333	23			

(D) Dichloromethane		Sum of Squares	df	Mean Square	F	Sig.
Hours24	Between Groups	1506.333	5	301.267	361.520	.000
	Within Groups	15.000	18	.833		
	Total	1521.333	23			
Hours48	Between Groups	1421.875	5	284.375	525.000	.000
	Within Groups	9.750	18	.542		
	Total	1431.625	23			
Hours72	Between Groups	1325.333	5	265.067	329.048	.000
	Within Groups	14.500	18	.806		
	Total	1339.833	23			

(E) Aqueous		Sum of Squares	df	Mean Square	F	Sig.
Hours24	Between Groups	124.708	5	24.942	17.780	.000
	Within Groups	25.250	18	1.403		
	Total	149.958	23			
Hours48	Between Groups	158.833	5	31.767	27.229	.000
	Within Groups	21.000	18	1.167		
	Total	179.833	23			
Hours72	Between Groups	185.875	5	37.175	56.949	.000
	Within Groups	11.750	18	.653		
	Total	197.625	23			

Disscussion:

Several studies suggest that the plant extract exhibited larvicidal properties against *Anopheles* larvae. However, the susceptibility of mosquito strain and the larval stage has taken may also affect the results. Furthermore, the effect of larval mortality was depended on the concentration of leaf extract. Plant extract have a complex mixture of bioactive compounds mainly phenolics, terpenoids, flavonoids, and alkaloids which delayed the growth of larvae and induce mortality of larvae; this can be seen in the result of the present study. Some of the studies reported that acetone extract of the plant contains the maximum amount of phenols and flavonols, while methanol extracts have majorities of flavones, terpenoids, tannins, and polyphenols that show larvicidal activity [19]. Bio-insecticides derived from plants might provide a more suitable and sustainable solution against mosquito-borne diseases as effective alternative to chemical pesticides. Based on the same perspective, in a study malaria vector *An. stephensi* larvae showed susceptibility to *P. juliflora* plant extract. In other studies, have been reported that petroleum ether extracts of flowers of *P. juliflora* were also quite effective against *Cx. quinquefasciatus* followed by *An. stephensi* and *Ae. Aegypti* Sakthi vadivel and Daniel (2008) [15]. In present study hexane extract of *Musa acuminata* plant leaf was showed more effective result with LC_{50} and LC_{90} value (46.36, 41.00 and 37.69 and 134.11, 116.16 and 107.78 mg/L) than ethylacetate extract. Evaluation the

larvicidal efficacy of 10% leaf extract of *P. juliflora* and found it to be quite effective (LC_{50} 9.3 mg/L) against larvae of *An. Stephensi* by Senthil kumar *et al*., (2009) [16] But in the present study dichloromethane extract with LC50 and LC9 value (48.01, 39.40 and 35.23 and 114.54, 104.83 and 94.56 mg/L its 40 mg/L) displayed maximum effeiciency against 3rd instar *Anopheles stephensi* larvae. The methanol extract of *P. juliflora* was also reported to have an LC50 value of 128 ppm against *Ae. aegypti* larvae [17]. The plant extracts possesses various types of insecticidal and larvicidal phytochemical compounds (flavonoids, sesquiterpines, thiophene derivatives) notably, the larvicidal activities of *P. juliflora* extract are varying in different studies [18].The present study evaluates the larvicidal property of Leaf extracts of *M. acuminata* but several studies reveal the effective medicinal properties of the Banana leaves (ashes) are used in eczema [20], as cool dressings for blister and burns [21]. The decoction of the leaves of *M. acuminata* added to *Ocimum americanum* and *Ocimum gratissimum* is used to treat malarial in Comores, Ngazidja. Young leaves are placed as poultices on burns and other skin afflictions.

Our mortality percentage result showed that after 24hr, 48hr, and 72hr (hexane, diethyl ether, ethyl acetate, dichloromethane, and aqueous) extracts of *Musa acuminata* in (graph-1) dichloromethane exhibited maximum efficiency (99%) and aqueous showed minimum effect (42%) and hexane extract showed (96 %) ethylacetate (83%) and diethyl ether (69%) at 72 hrs.The roots of *Musa acuminata* are administered in digestive disorders, dysentery, and other ailments. *Musa acuminata* also has anthelmintic property [22]. Result evaluated minimum effective result showed on aqueous extract (207.09, 171.72 and 141.84 and 1062.53, 980.27 and 1068.08 mg/L) at 24, 42 and 72 hrs respectively as also found in various other studies. Effect of extract of *M. acuminata* is also time-dependent and shows higher mortality at 72h.The treatment of di-chloromethane extract showed maximum effect and also time dependent. Further study is may be conducted to evaluate the changes in morphogenesis surviving larvae after plant extract treatment.

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