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Efficacy of *Phyllanthus urinaria* extracts against the larvae of filariasis vector mosquito *Culex* spp. (Diptera: Culicidae)

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Abstract
The rising demand for sustainable mosquito control strategies has increased interest in plant-derived larvicides. This study assessed the larvicidal efficacy of *Phyllanthus urinaria* extracts against *Culex* mosquito larvae. Plant materials were extracted using five solvents-acetone, 70% ethanol, chloroform, benzene, and water. Larval mortality was evaluated after 24, 48, 72, and 96 hours of exposure at concentrations of 50, 100, 150, 200, 250, and 300 ppm. Among the tested extracts, the chloroform extract exhibited the highest larvicidal activity, with LC_{50} values of 5.40, 5.19, 8.52, 4.92, and 12.97 ppm at the respective time intervals. These results emphasize the influence of solvent polarity on the extraction of bioactive compounds responsible for larvicidal action. Overall, the findings suggest that locally available weeds such as *P. urinaria* can serve as eco-friendly and effective alternatives to conventional chemical insecticides in mosquito vector control programs.

Keywords: Larvicidal efficacy, solvent polarity, weed-derived insecticides, bioinsecticide potential, natural mosquito control

Introduction
Mosquitoes are among the most significant vectors of infectious diseases worldwide, transmitting a wide range of illnesses such as malaria, dengue, chikungunya, yellow fever, tularemia, filariasis, and dirofilariasis. Among them, species of the *Culex* genus-commonly known as house mosquitoes-are of particular concern due to their preference for breeding in stagnant water and their role as vectors of diseases such as the West Nile virus [1].

Interrupting the mosquito life cycle during the aquatic stages, particularly the larval phase, remains one of the most effective strategies for reducing mosquito populations and minimizing disease transmission. Traditionally, mosquito larval control has relied on the application of synthetic insecticides such as pyrethroids, organophosphates, and carbamates [2]. However, the excessive and prolonged use of these chemical agents has resulted in environmental pollution, bioaccumulation, and the emergence of insecticide resistance in mosquito populations [3].

Mosquito-borne diseases continue to represent a major global public health challenge, especially in tropical and subtropical regions. According to the World Health Organization (WHO), these diseases are responsible for over one million deaths annually. Therefore, there is an increasing need for novel larvicidal agents that are both effective and environmentally sustainable [4].

In recent years, plant-derived products have gained significant attention as promising alternatives to conventional insecticides. Several studies have demonstrated that plant extracts, including crude extracts, essential oils, and powdered materials, exhibit potent larvicidal properties [5, 6]. The larvicidal efficacy of these botanical agents is attributed to the presence of bioactive phytochemicals such as flavonoids, alkaloids, glycosides, and terpenoids, which disrupt larval development and physiological processes through mechanisms distinct from those of synthetic insecticides [7]. Consequently, plant-based larvicides offer a safer and more sustainable approach to mosquito control while reducing the risk of resistance development [8].

Materials and Methods
1. Experimental Site: The present study was carried out in the Entomology Laboratory of

Vidyasagar College, Salt Lake Campus, Kolkata, India. All experiments were performed under controlled laboratory conditions, maintaining an ambient temperature of 30 ± 2°C and a relative humidity of 75 ± 5%.

2. Collection and Maintenance of Mosquito Larvae
Larvae of *Culex* species were collected from stagnant water bodies and drainage channels located in the vicinity of Vidyasagar College campus. The collected specimens were transported to the laboratory in clean containers filled with their native water to minimize stress. Identification of *Culex* larvae was performed based on standard morphological characteristics. The larvae were then reared in aquaria containing dechlorinated tap water and maintained under optimal environmental conditions until further use in bioassays.

3. Collection of Plant Material
Fresh leaves of *Phyllanthus urinaria* were locally sourced from the surrounding area of Vidyasagar College campus. The plant species was authenticated based on morphological features with the help of standard botanical keys. Collected leaves were washed thoroughly with tap water followed by distilled water to remove debris and dust prior to experimental use.

Preparation of plant extracts: The leaves were collected and then allowed to sun dried for 1 week. Then the leaves were finely grounded to powder using an electrical blender. After grinding the powdered leaves were transferred to the conical flasks. Then the 4 suitable solvents acetone, 70% ethanol, chloroform, benzene and water were used to homogenize the solution. These solutions were kept for 3 days in room temperature in the laboratory. The conical flasks were stirred gently for 3 time each day. After 3 days the top layer extract was isolated and kept in petri dish and then supernatant was discarded. The mixture collected in petri dish was dried in incubator for 2-3 days. Then after 2-3days the dried extract was obtained.

Larvicidal bioassay
Different concentration of the crude extracts was prepared by dissolving 0.2gms of crude extract in suitable milliliters of distilled water. The concentrations prepared were 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm. The concentrations were transferred to suitable cups. For each concentration 3 cups were prepared (labeled R1, R2 and R3). About 10 larvae were transferred using dropper, and were placed in large trays. The trays were then covered with newspaper filled with some holes for proper ventilation. The cups were observed after 24, 48, 72 and 96hrs. The number of dead larvae were counted after 24, 48, 72 and 96hrs and noted down respectively.

Statistical analysis
Statistical analysis of the experimental data was performed by using MS Excel 2020 and antilog calculator to calculate LC50 lethal concentration, regession analysis, co-efficient value, mean larval mortality, standard error etc. Probit analysis was done following Finney, 1952.

Results
During the present experiment the most common and locally available plant species leaf extracts were applied on the larvae of *C. quinquefasciatus* at different time intervals. Mosquito larvae also show different morphological changes after exposure with different concentrations of five plants at different time. The results were presented in the following tables 1, 2, 3, 4, 5, 6 and 7.

Persentage mortality of *culex quinquefasciatus* larvae when exposed to different concentration of *Phyllanthus urinaria* leaves extracts in 5 different solvents
It was revealed from Table: 1 that percentage mortality of *culex quinquefasciatus* larvae when exposed to different concentrations of *Phyllaunthus urinaria* extracts in acetone after 24hrs of treatment was 13.67, 16.67, 20, 23.33, 26.67, and 30%. After 48hrs of treatment was 33.33, 36.67, 40, 46.67, 50, and 56.67%. After 72hrs of treatment was56.67, 60, 63.33, 66.67, 73.33, 80%. After 96hrs of treatment was 80, 86.67, 90, 100, 100 and 100%. Similarly in ethanol after 24hrs of treatment was23.33, 36.67, 40, 46.67, 53.33, and 56.67%. After 48hrs of treatment was43.33, 56.67, 60, 70, 73.33 and 80%. After 72hrs of treatment was 63.33, 76.67, 80, 83.33, 90 and 96.67%. After 96hrs of treatment was 80% 90% 90% 93.33% 96.67% 100% Similarly in chloroform after 24hrs of treatment was 56.67, 70, 73.33, 83.33, 86.67 and 90%. After 48hrs of treatment was 76.67, 80, 90, 93.33, 100 and 100%. After 72hrs of treatment was 80, 83.33, 90, 100, 100 and 1000%. After 96hrs of treatment was 83.33, 100, 100, 100, 100 and 100%. Similarly in benzene after 24hrs of treatment was 33.33, 36.67, 43.33, 56.67, 63.33and 73.33%. After 48hrs of treatment was 50, 56.67, 63.33, 70, 100, and 100%. After 72hrs of treatment was 70, 80, 90, 100, 100 and 100%. After 96hrs of treatment was 80, 86.67, 100, 100, 100 and 100% Similarly in water after 24hrs of treatment was 0.33, 3.33, 6, 67, 10, 13.33and 16.67%. After 48hrs of treatment was 3.33, 13.33, 16.67, 20, 26.67, and 30%. After 72hrs of treatment was 13.33, 26.67, 30, 36.67, 43.33 and 53.33%. After 96hrs of treatment was 20, 33.33, 36.67, 66.67, 73.33 and 76.67% in the same concentrations of 50, 100, 150, 200, 250 and 300 ppm respectively (Table 1).

Table 1: Percentage mortality of *Culex* larvae in *Phyllanthus urinaria* extract in 5 different solvents-acetone, alcohol, chloroform, benzene and water

Solvent	Concentration (ppm)	No. of larvae	Mortality Rate			
			24hrs	48hrs	72hrs	96hrs
1. Acetone	50	10	13.33%	33.33%	56.67%	80%
	100	10	16.67%	36.67%	60%	86.67%
	150	10	20%	40%	63.33%	90%
	200	10	23.33%	46.67%	66.67%	100%
	250	10	26.67%	50%	73.33%	100%
	300	10	30%	56.67%	80%	100%
	control	10	0	0	0	0

2. Alcohol	50	10	16.67%	33.33%	53.33%	80%
	100	10	20%	40%	60%	83.33%
	150	10	26.67%	46.67%	66.67%	100%
	200	10	33.33%	56.67%	80%	100%
	250	10	36.67%	60%	83.33%	100%
	300	10	40%	66.67%	90%	100%
	Control	10	0	0	0	0
3. Chloroform	50	10	56.67%	76.67%	80%	83.33%
	100	10	70%	80%	83.33%	100%
	150	10	73.33%	90%	90%	100%
	200	10	83.33%	93.33%	100%	100%
	250	10	86.67%	100%	100%	100%
	300	10	90%	100%	100%	100%
	Control	10	0	0	0	0
4. Benzene	50	10	33.33%	50%	70%	80%
	100	10	36.67%	56.67%	80%	86.67%
	150	10	43.33%	63.33%	90%	100%
	200	10	56.67%	70%	100%	100%
	250	10	63.33%	100%	100%	100%
	300	10	73.33%	100%	100%	100%
	Control	10	0	0	0	0
5. Water	50	10	0.33%	3.33%	13.33%	20%
	100	10	3.3%	13.33%	16.67%	33.33%
	150	10	6.67%	16.67%	30%	36.67%
	200	10	10%	20%	36.67%	66.67%
	250	10	13.33%	26.67%	43.33%	73.33%
	300	10	16.67%	30%	53.33%	76.67%
	Control	10	0	0	0	0

Table 2: The larvicidal effect of *Phyllanthus urinaria extract* (Acetone) on *Culex* sp. After different time intervals

Treatment (ppm)	Log C	Mortality Rate 24hrs	Probit	Mortality Rate 48hrs	Probit	Mortality rate 72hrs	Probit	Mortality Rate 96 hrs	Probit
50	1.69897	13.67%	3.87	33.33%	4.56	56.67%	5.15	80%	5.84
100	2	16.67%	4.01	36.67%	4.64	60%	5.25	86.67%	6.08
150	2.176091	20%	4.16	40%	4.75	63.33%	5.33	90%	6.28
200	2.30103	23.33%	4.26	46.67%	4.9	66.67%	5.41	100%	8.95
250	2.39794	26.67%	4.36	50%	5	73.33%	5.61	100%	8.95
300	2.477121	30%	4.48	56.67%	5.15	80%	5.84	100%	8.95

Table 3: The larvicidal effect of *Phyllanthus urinaria* extract (Alcohol) on *Culex* sp. After different time intervals

Treatment (ppm)	Log C	Mortality Rate 24hrs	Probit	Mortality Rate 48hrs	Probit	Mortality rate 72hrs	Probit	Mortality Rate 96 hrs	Probit
50	1.69897	16.67%	4.01	33.33%	4.56	53.33%	5.98	80%	5.84
100	2	20%	4.16	40%	4.75	60%	5.25	83.33%	5.95
150	2.176091	26.67%	4.36	46.67%	4.9	66.67%	5.41	100%	8.95
200	2.30103	33.33%	4.56	56.67%	5.15	80%	5.84	100%	8.95
250	2.39794	36.67%	4.64	60%	5.25	83.33%	5.95	100%	8.95
300	2.477121	40%	4.75	66.67%	5.41	90%	6.28	100%	8.95

Table 4: The larvicidal effect of *Phyllanthus urinaria* extract (Chloroform) on *Culex* sp. After different time intervals

Treatment (ppm)	Log C	Mortality Rate 24hrs	Probit	Mortality Rate 48hrs	Probit	Mortality rate 72hrs	Probit	Mortality Rate 96 hrs	Probit
50	1.69897	56.67%	5.15	76.67%	5.71	80%	5.84	83.33%	5.95
100	2	70%	5.25	80%	5.84	83.33%	5.95	100%	8.95
150	2.176091	73.33%	5.61	90%	6.28	90%	6.28	100%	8.95
200	2.30103	83.33%	5.95	93.33%	6.48	100%	8.95	100%	8.95
250	2.39794	86.67%	6.08	100%	8.95	100%	8.95	100%	8.95
300	2.477121	90%	6.28	100%	8.95	100%	8.95	100%	8.95

Table 5: The larvicidal effect of *Phyllanthus urinaria* extract (Benzene) on *Culex* sp. After different time intervals

Treatment (ppm)	Log C	Mortality Rate 24hrs	Probit	Mortality Rate 48hrs	Probit	Mortality rate 72hrs	Probit	Mortality Rate 96 hrs	Probit
50	1.69897	33.33%	4.56	50%	5	70%	5.52	80%	5.84
100	2	36.67%	4.64	56.67%	5.15	80%	5.84	86.67%	6.08
150	2.176091	43.33%	4.82	63.33%	5.33	90%	6.28	100%	8.95
200	2.30103	56.67%	5.15	70%	5.52	100%	8.95	100%	8.95
250	2.39794	63.33%	5.33	100%	8.95	100%	8.95	100%	8.95
300	2.477121	73.33%	5.61	100%	8.95	100%	8.95	100%	8.95

Table 6: The larvicidal effect of *Phyllanthus urinaria* extract (water) on *Culex* sp. After different time intervals

Treatment (ppm)	Log C	Mortality Rate 24hrs	Probit	Mortality Rate 48hrs	Probit	Mortality rate 72hrs	Probit	Mortality Rate 96 hrs	Probit
50	1.69897	0.33%	2.67	3.3%	3.12	13.33%	3.87	20%	4.16
100	2	3.3%	3.12	13.33%	3.87	26.67%	4.36	33.33%	4.56
150	2.176091	6.6s7%	3.45	16.67%	4.01	30%	4.48	36.67%	4.64
200	2.30103	10%	3.72	20%	4.16	36.67%	4.64	66.67%	5.41
250	2.39794	13.33%	3.87	26.67%	4.36	43.33%	4.82	73.33%	5.61
300	2.477121	16.67%	4.01	30%%	4.48	53.33%	5.08	76.67%	5.71

Table 7: LOG Probit analysis and regression analysis of larvicidal activity of *Phyllanthus urinaria* on *culex* larvae.

Solvent	Time	Regression equation	r^2 value	antilog	LC50	Lower 95%	Upper 95%
1.Aetone	24hrs	Y=0.7683x+2.5189	0.963767	3.230729167	1701.097345	2.0657793	2.971956
	48hrs	Y=0.7368x+3.2307	0.893625	2.403940217	253.4779683	2.457578	4.003899
	72hrs	Y=0.7876x+3.7184	0.798588	1.628970775	42.55697744	2.515284	4.921524
	96hrs	Y=4.7856x-2.9	0.754432	1.650992685	44.77057637	-11.2036	5.403449
2.Alcohol	24hrs	Y=0.9829x+2.2754	0.961786	2.774949084	3.793279022	1.67947	2.871278
	48hrs	Y=1.0906x+2.6311	0.948701	2.173394495	149.0714567	1.859787	3.402466
	72hrs	Y=1.5027x+2.3663	0.878694	1.753661784	56.71027905	0.668126	4.064571
	96hrs	Y=4.8458x-2.6089	0.781021	1.570278638	37.17736778	-10.4133	5.19546
3.Chloroform	24hrs	y=1.4118x+2.6942	0.958603	1.634301914	43.08260087	1.801834	3.586492
	48hrs	Y=4.2885x-2.2867	0.667325	1.700350058	50.1591337	-11.49	6.916517
	72hrs	Y=4.84x-3.0413	0.748905	1.66142562	45.85910977	-11.5665	5.482894
	96hrs	Y=3.4501x+0.9954	0.657206	1.175362319	14.97484441	-6.63341	8.524134
4.Benzene	24hrs	Y=1.3258x+2.1345	0.849239	2.163018868	145.5522314	0.435412	3.833526
	48hrs	Y=5.1001x-4.6104	0.58522	1.884276779	76.60846825	-17.67	8.449167
	72hrs	Y=5.254x-4.0147	0.792414	0.18753308	1.540044634	-12.1949	4.165452
	96hrs	Y=4.7908x-2.4676	0.79541	1.5588726551	36.21367931	-9.85789	4.922607
4.water	24hrs	Y= 1.7529x - 0.339	0.9965	1112.02507	3.046114578	-0.657432	-0.021823
	48hrs	Y=5.1001x - 4.6104	0.5852	117.6468976	2.070580479	-37.00695	10.124062
	72hrs	Y=5.2546x-4.0147	0.7924	80.82847915	1.907564407	-24.18058	4.8052947
	96hrs	Y=4.7908x-2.4676	0.7954	44.97885417	1.653008388	-21.04303	12.966248

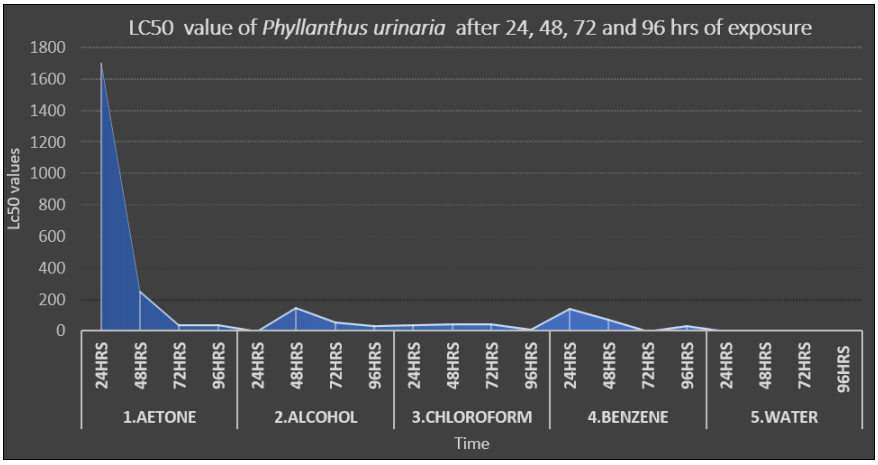

Fig 1: Graph showing LC50 values of *Phyllanthus urinaria extract* with 5 different

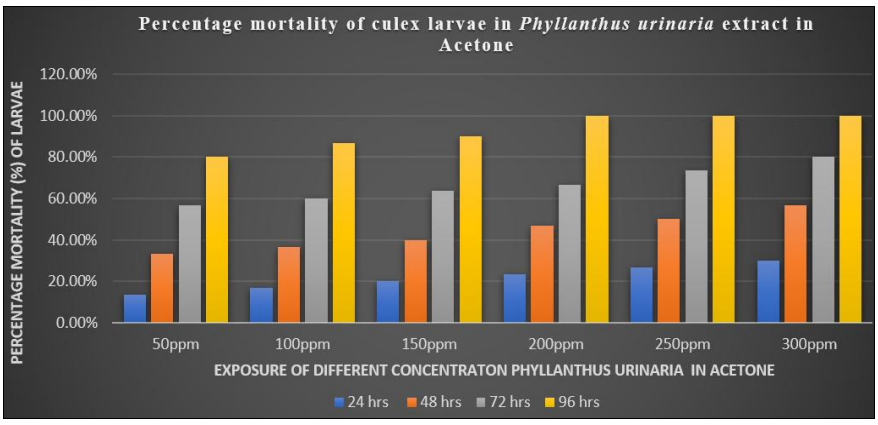

Fig 2: Percentage mortality of *culex* larvae in *Phyllanthus urinaria* extract in Acetone.

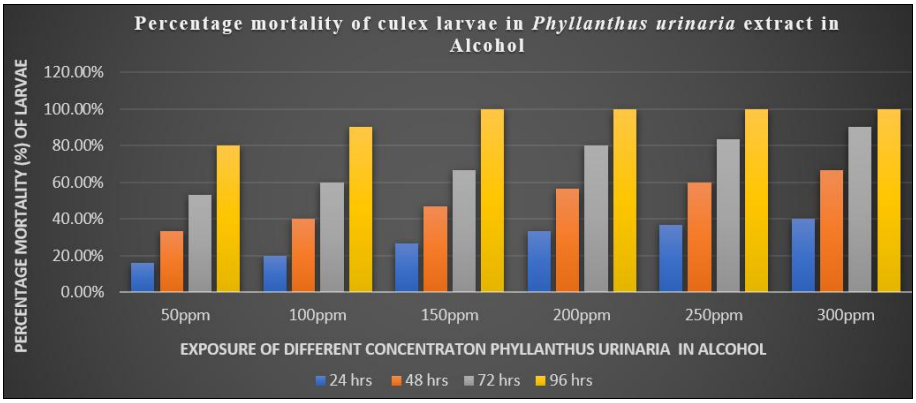

Fig 3: Percentage mortality of *culex* larvae in *Phyllanthus urinaria* extract in Alcohol

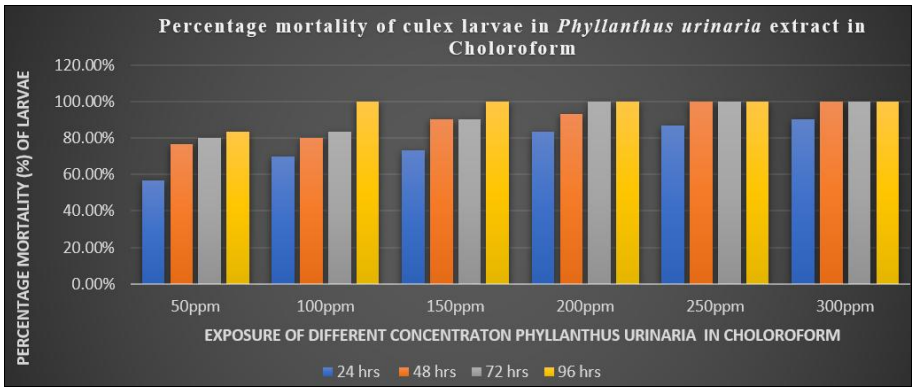

Fig 4: Percentage mortality of *culex* larvae in *Phyllanthus urinaria* extract in Chloroform

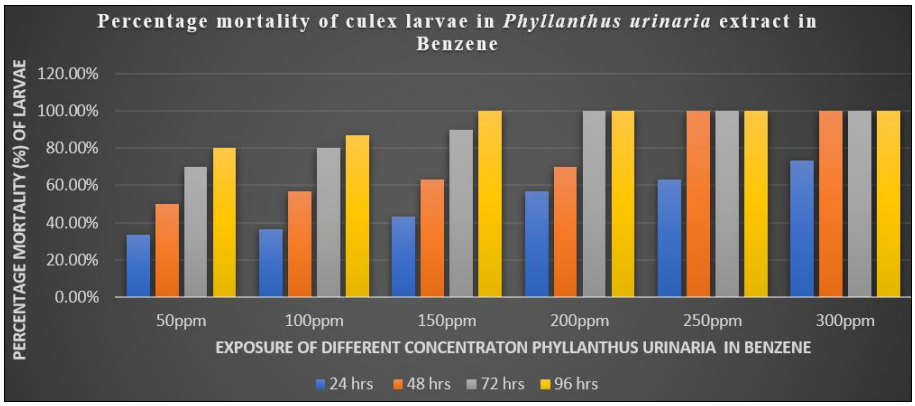

Fig 5: Percentage mortality of *culex* larvae in *Phyllanthus urinaria* extract in Benzene.

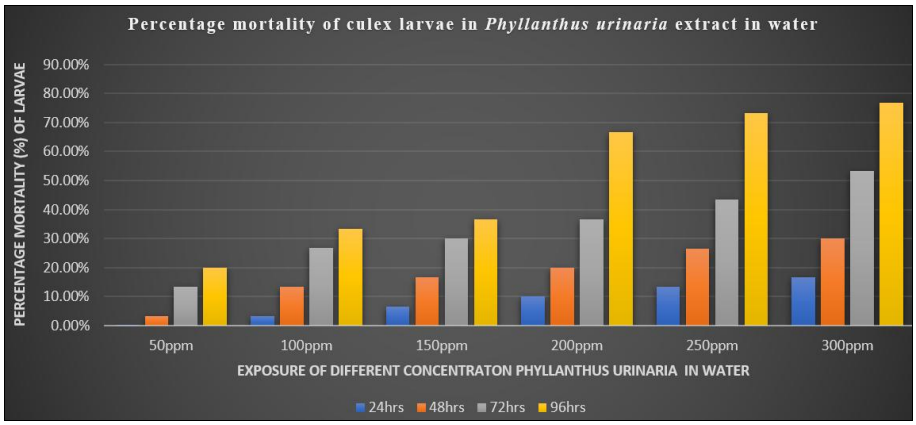

Fig 6: Percentage mortality of *culex* larvae in *Phyllanthus urinaria* extract in water.

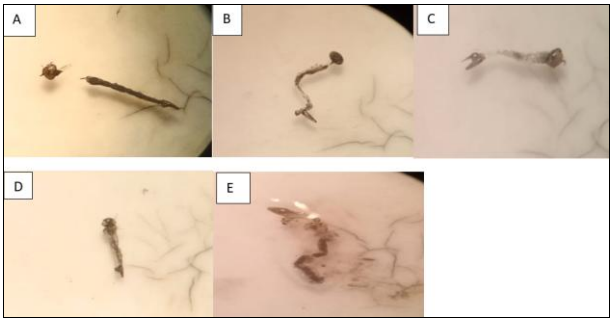

Fig 7: Larvae after treated with *Phyllanthus urinaria* A) acetone, B) alcohol, C) chloroform, D) benzene E) Water

Discussion

The present study recorded the use of *Phyllanthus urinaria extracts in:* acetone, 70% alcohol chloroform, benzene and water at 6 different concentrations of 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm. Among the tested extracts, the chloroform extract exhibited the highest larvicidal activity after 96hrs of exposure and the LC50 values of *Phyllanthus urinaria* extract after 96 hrs of exposure in acetone, 70% alcohol, chloroform, benzene and water respectively are as follows: 5.403449, 5.19546, 8.524134, 4.922607 and 12.966248 (Table 7).

In a contrasting observation, Kalimuthu *et al*. found that the ethanol leaf extract of *Cadaba indica* Lam. produced the highest larval mortality in *Aedes aegypti*, with an LC_{50} of 143.75 ppm, exceeding the effects of its hexane, chloroform, and petroleum ether fractions [9]. In a similar study, Maheswaran *et al*. reported that crude extracts of *Leucas aspera* exerted larvicidal effects on *Aedes aegypti* and *Culex quinquefasciatus*, where the hexane extract showed the strongest activity, followed in descending order by chloroform and ethanol extracts. Likewise, Warikoo & Kumar demonstrated the bio-larvicidal and pupicidal potential of *Acalypha alnifolia* against first to fourth instar larvae and pupae of *Culex quinquefasciatus*, yielding LC_{50} values of 5.388% (I), 6.233% (II), 6.884% (III), 8.594% (IV), and 10.073% for pupae [10]. Moreover, Vijayan *et al*. evaluated *Euodia ridleyi* leaf extracts against *Culex quinquefasciatus* and demonstrated notable larvicidal activity, underscoring the potential of plant-based mosquito control agents [11].

Conclusion

The present study assessed the larvicidal effects of *Phyllanthus urinaria* using extracts prepared in acetone, 70% ethanol, chloroform, benzene, and water. These extracts were tested at six different concentrations-50, 100, 150, 200, 250, and 300 ppm-against *Culex* mosquito larvae. The results indicated that the plant extracts exhibited notable larvicidal activity, with effectiveness depending on both the type of solvent and the concentration applied. In conclusion, the findings suggest that weeds like *Phyllanthus urinaria*, though often viewed as unwanted plants, possess significant potential for eco-friendly and cost-effective mosquito control, contributing to sustainable approaches in vector management.

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