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Comparative study on the larvicidal effect of neem extract on *Culex*, *Anopheles*, and *Aedes* Species

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Abstract
The widespread use of synthetic insecticides in mosquito control has raised concerns regarding environmental impact, toxicity to non-target organisms, and the development of resistance among vector species. In light of these concerns, there is increasing interest in alternative, environmentally safe insecticides derived from plant sources. This study investigates the larvicidal potential of neem (*Azadirachta indica*) leaf extract on the fourth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensii*, and *Aedes aegypti*. Neem extract was obtained via Soxhlet extraction using different solvents to prepare concentrations ranging from 0 to 1%. Larvicidal assays were conducted over 24, 48, and 72 hours, with mortality rates recorded at each time point. Probit analysis was applied to determine each species' LC50 and LC90 values. Results revealed significant larvicidal activity of neem extract, particularly against *Aedes aegypti*, which exhibited the highest mortality rates. The findings highlight neem extract as a promising eco-friendly and cost-effective alternative to synthetic insecticides for mosquito vector control.

Keywords: Neem, larvicidal activity, *Culex quinquefasciatus*, *Anopheles stephensii*, *Aedes aegypti*, phytochemicals, eco-friendly insecticides, mosquito control

Introduction

Mosquitoes are vectors for numerous human diseases, including malaria, dengue, and Zika virus, which pose significant public health challenges worldwide. Traditional control measures primarily involve the application of synthetic insecticides such as organochlorines and organophosphates. While effective in reducing mosquito populations, these chemicals have been associated with adverse environmental effects, toxicity to non-target organisms, and the development of insecticide resistance (Brown, 1986; Russell *et al*., 2009) [3, 12]. These limitations have prompted the search for alternative, environmentally benign mosquito control agents that are biodegradable and less likely to contribute to resistance (Benelli, 2015) [2].

Among the various alternatives, plant-based insecticides have gained attention due to their eco-friendly properties and broad-spectrum efficacy against insect pests (Isman, 2015) [5]. Neem (*Azadirachta indica*), a plant native to the Indian subcontinent, has long been used in traditional medicine and agriculture due to its potent insecticidal properties (Rongnoparut, 2016) [11]. Neem contains bioactive compounds, primarily azadirachtin, demonstrating larvicidal, repellent, and growth-regulatory effects on mosquitoes (Senthil-Nathan, 2013) [14]. This study aimed to evaluate the larvicidal efficacy of neem leaf extract against *Culex quinquefasciatus*, *Anopheles stephensii*, and *Aedes aegypti*, which are important vectors for filariasis, malaria, and dengue, respectively.

Materials and Methods

Neem Leaf Extract Preparation

Fresh neem leaves (1,000 g) were thoroughly washed, air-dried in the shade for 24 hours, and ground into a fine powder. The powder was subjected to a Soxhlet extraction using hexane, dichloromethane, ethyl acetate, and methanol in ascending order of polarity. Each solvent was employed for a 72-hour extraction process to obtain various fractions. The methanol-soluble fraction was concentrated under reduced pressure using a rotary evaporator, yielding a dark yellow viscous mass further purified by column chromatography over silica gel. The extract was then dissolved in distilled water and prepared at 0%, 0.125%, 0.250%, 0.500%, 0.750%, and 1.000% for the larvicidal assays.

Larvicidal Assay

The fourth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensii*, and *Aedes aegypti* were exposed to the neem extract at varying concentrations. Each concentration was tested in triplicates, with 100 larvae per replicate. The larvae were placed in glass containers containing 500 mL of water, and the respective concentrations of neem extract were added. Mortality was observed at 24, 48, and 72 hours post-exposure. Control groups were maintained without neem extract. Larval mortality was recorded at each time point, and any morphological deformities, such as elongated larvae or malformed pupae and adults, were noted (Fig. 1-3) (Table 1-3).

Probit Analysis

To determine the efficacy of neem extract, probit analysis was performed on the observed mortality data to calculate the LC50 and LC90 values at the 24-hour exposure point. The data analysis was conducted using statistical software, and confidence intervals for each concentration were calculated to assess the precision of the estimates (Table 4).

Results

The present work aims to determine the Effect of neem extract on the 4th instar larvae of *Culex quinquefaciatus*, *Anopheles stephensii*, and *Aedes aegypti.*

The Probit analysis of the observations obtained from the larvicidal assay is as follows.

LC_{50} and LC_{90} for the efficacy of Neem extract on *Culex quinquefaciatus* is 1.89 % and 11.45 %, respectively, after 24 hours. (Table. 5). LC_{50} and LC_{90} for the efficacy of Neem extract on *Anopheles stephensii* is 0.86% and 6.27%, respectively, after 24 hours (Table. 6). LC_{50} and LC_{90} for the efficacy of Neem extract on *Aedes aegypti* is 0.25 % and 0.76% respectively after 24 hours. (Table. 7)

Table 1: Data showing Effect of neem extract on *Culex quinquefaciatus*

Effect of Neem leaf extract on Larvae of *Culex quinquefaciatus*							
Concentration (%)	**No of Larvae Exposed**	**No of Dead Larvae**			**Mortality %**		
		24 Hrs	**48 Hrs**	**72 Hrs**	**24 Hrs**	**48 Hrs**	**72 Hrs**
0	100	0	0	0	0	0	0
0.125	100	3	7	11	3	7	11
0.250	100	7	10	12	7	10	12
0.500	100	16	23	29	16	23	29
0.750	100	27	34	36	27	34	36
1.000	100	32	37	40	32	37	40

Table 2: Data showing Effect of neem extract on *Anophles stephensii*

Effect of Neem leaf extract on Larvae of *Anophles stephensii*							
Concentration	**No of Larvae Exposed**	**No of Dead Larvae**			**Mortality %**		
		24 Hrs	**48 Hrs**	**72 Hrs**	**24 Hrs**	**48 Hrs**	**72 Hrs**
0	100	0	0	0	0	0	0
0.125	100	14	26	29	14	26	29
0.250	100	19	25	36	19	25	36
0.500	100	27	39	44	27	39	44
0.750	100	48	56	63	48	56	63
1.000	100	59	71	74	59	71	74

Table 3: Data showing Effect of neem extract on *Aedes aegypti*

Effect of Neem leaf extract on Larvae of *Aedes aegypti*							
Concentration	**No of Larvae Exposed**	**No of Dead Larvae**			**Mortality %**		
		24 Hrs	**48 Hrs**	**72 Hrs**	**24 Hrs**	**48 Hrs**	**72 Hrs**
0	100	0	0	0	0	0	0
0.125	100	26	42	59	26	42	59
0.250	100	45	57	71	45	57	71
0.500	100	67	81	89	67	81	89
0.750	100	92	100	100	92	100	100
1.000	100	100	100	100	100	100	100

Table 4: Probit analysis data on effect of neem extract on *Culex, Anopheles and Aedes*

Table 4.1 Cell Counts and Residuals (*Culex quinquefaciatus*)							
	Number	**Concentration**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**	**Probability**
PROBIT	1	-.903	100	3	2.630	.370	.026
	2	-.602	100	7	7.434	-.434	.074
	3	-.301	100	16	17.102	-1.102	.171
	4	-.125	100	27	25.426	1.574	.254
	5	.000	100	32	32.415	-.415	.324

Table 4.2 Cell Counts and Residuals (*Anopheles Stephensii*)							
	Number	**Concentration**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**	**Probability**
PROBIT	1	-.903	100	14	10.470	3.530	.105
	2	-.602	100	19	21.007	-2.007	.210
	3	-.301	100	27	36.049	-9.049	.360
	4	-.125	100	48	46.237	1.763	.462
	5	.000	100	59	53.661	5.339	.537

Table 4.3 Cell Counts and Residuals (*Aedes aegypti*)							
	Number	**Concentration**	**Number of Subjects**	**Observed Responses**	**Expected Responses**	**Residual**	**Probability**
PROBIT	1	-.903	100	26	20.090	5.910	.201
	2	-.602	100	45	49.034	-4.034	.490
	3	-.301	100	67	78.524	-11.524	.785
	4	-.125	100	92	89.730	2.270	.897
	5	.000	100	100	94.567	5.433	.946

Table 5: Confidence Limits for Effect of Neem Extract on *Culex quinquefaciatus*

Probability		**95% Confidence Limits for Concentration**			**95% Confidence Limits for log (Concentration)[a]**		
		Estimate	**Lower Bound**	**Upper Bound**	**Estimate**	**Lower Bound**	**Upper Bound**
Probit	.010	.073	.028	.122	-1.140	-1.558	-.915

	.020	.106	.048	.164	-.973	-1.318	-.786
	.030	.135	.068	.198	-.868	-1.167	-.703
	.040	.163	.088	.229	-.789	-1.054	-.641
	.050	.189	.109	.257	-.724	-.963	-.589
	.060	.214	.130	.285	-.669	-.885	-.545
	.070	.239	.152	.312	-.621	-.818	-.506
	.080	.264	.175	.339	-.578	-.758	-.470
	.090	.289	.198	.366	-.539	-.704	-.437
	.100	.314	.221	.393	-.503	-.655	-.406
	.150	.443	.347	.538	-.354	-.460	-.269
	.200	.582	.477	.717	-.235	-.322	-.144
	.250	.736	.606	.950	-.133	-.218	-.022
	.300	.909	.735	1.248	-.042	-.134	.096
	.350	1.104	.870	1.625	.043	-.060	.211
	.400	1.329	1.016	2.101	.124	.007	.322
	.450	1.590	1.175	2.702	.201	.070	.432
	.500	1.896	1.353	3.470	.278	.131	.540
	.550	2.262	1.555	4.462	.354	.192	.650
	.600	2.706	1.790	5.768	.432	.253	.761
	.650	3.256	2.069	7.527	.513	.316	.877
	.700	3.958	2.408	9.970	.597	.382	.999
	.750	4.885	2.834	13.513	.689	.452	1.131
	.800	6.176	3.397	18.968	.791	.531	1.278
	.850	8.118	4.193	28.179	.909	.623	1.450
	.900	11.450	5.461	46.395	1.059	.737	1.666
	.910	12.442	5.820	52.337	1.095	.765	1.719
	.920	13.617	6.238	59.660	1.134	.795	1.776
	.930	15.037	6.731	68.901	1.177	.828	1.838
	.940	16.799	7.327	80.928	1.225	.865	1.908
	.950	19.062	8.072	97.232	1.280	.907	1.988
	.960	22.114	9.044	120.636	1.345	.956	2.081
	.970	26.542	10.400	157.276	1.424	1.017	2.197
	.980	33.832	12.522	223.783	1.529	1.098	2.350
	.990	49.593	16.774	390.225	1.695	1.225	2.591

a. Logarithm base = 10.

Table 6: Confidence Limits of Effect of Neem extract on *Anopheles Stephensii*

Probability		**95% Confidence Limits for Concentration**			**95% Confidence Limits for log (Concentration)[a]**		
		Estimate	**Lower Bound**	**Upper Bound**	**Estimate**	**Lower Bound**	**Upper Bound**
Probit	.010	.024	.008	.045	-1.621	-2.087	-1.344
	.020	.036	.014	.063	-1.438	-1.841	-1.198
	.030	.048	.021	.079	-1.322	-1.686	-1.105
	.040	.058	.027	.092	-1.235	-1.569	-1.034
	.050	.068	.034	.105	-1.164	-1.474	-.977
	.060	.079	.040	.118	-1.104	-1.393	-.928
	.070	.089	.048	.130	-1.051	-1.323	-.885
	.080	.099	.055	.142	-1.004	-1.259	-.846
	.090	.110	.063	.154	-.960	-1.202	-.811
	.100	.120	.071	.167	-.921	-1.150	-.779
	.150	.175	.117	.228	-.756	-.934	-.642
	.200	.237	.172	.295	-.626	-.765	-.530
	.250	.306	.237	.372	-.514	-.625	-.430
	.300	.386	.312	.464	-.413	-.505	-.334
	.350	.479	.396	.579	-.320	-.402	-.237
	.400	.587	.488	.728	-.231	-.311	-.138
	.450	.715	.590	.920	-.146	-.229	-.036
	.500	.868	.703	1.171	-.062	-.153	.069
	.550	1.054	.832	1.499	.023	-.080	.176
	.600	1.283	.984	1.935	.108	-.007	.287
	.650	1.573	1.167	2.528	.197	.067	.403
	.700	1.950	1.393	3.357	.290	.144	.526
	.750	2.458	1.683	4.567	.391	.226	.660
	.800	3.181	2.075	6.443	.503	.317	.809
	.850	4.298	2.645	9.636	.633	.422	.984
	.900	6.274	3.585	16.011	.798	.555	1.204
	.910	6.875	3.858	18.103	.837	.586	1.258
	.920	7.592	4.178	20.687	.880	.621	1.316

	.930	8.468	4.559	23.959	.928	.659	1.379
	.940	9.566	5.027	28.230	.981	.701	1.451
	.950	10.993	5.618	34.040	1.041	.750	1.532
	.960	12.944	6.402	42.416	1.112	.806	1.628
	.970	15.823	7.515	55.594	1.199	.876	1.745
	.980	20.665	9.300	79.672	1.315	.968	1.901
	.990	31.477	13.007	140.526	1.498	1.114	2.148

a. Logarithm base = 10.

Table 7: Confidence Limits for Effect of Neem Extract on *Aedes aegypti*

Probability		95% Confidence Limits for Concentration			95% Confidence Limits for log (Concentration)[b]		
		Estimate	**Lower Bound**	**Upper Bound**	**Estimate**	**Lower Bound**	**Upper Bound**
Probit[a]	.010	.035	.001	.089	-1.453	-3.055	-1.050
	.020	.044	.002	.104	-1.352	-2.796	-.985
	.030	.051	.002	.114	-1.288	-2.632	-.943
	.040	.057	.003	.123	-1.240	-2.509	-.911
	.050	.063	.004	.130	-1.201	-2.409	-.885
	.060	.068	.005	.137	-1.168	-2.324	-.863
	.070	.073	.006	.144	-1.139	-2.249	-.843
	.080	.077	.007	.150	-1.113	-2.183	-.825
	.090	.082	.008	.155	-1.089	-2.123	-.809
	.100	.086	.009	.161	-1.067	-2.067	-.794
	.150	.106	.014	.186	-.976	-1.839	-.730
	.200	.125	.022	.210	-.904	-1.659	-.678
	.250	.144	.031	.234	-.842	-1.507	-.631
	.300	.163	.042	.259	-.787	-1.373	-.586
	.350	.184	.056	.286	-.736	-1.251	-.543
	.400	.206	.073	.317	-.687	-1.138	-.498
	.450	.229	.093	.354	-.640	-1.033	-.452
	.500	.255	.116	.398	-.593	-.934	-.400
	.550	.284	.144	.454	-.547	-.842	-.343
	.600	.317	.175	.529	-.499	-.756	-.277
	.650	.354	.210	.633	-.451	-.677	-.199
	.700	.399	.249	.783	-.399	-.604	-.106
	.750	.453	.291	1.010	-.344	-.535	.004
	.800	.522	.339	1.373	-.282	-.470	.138
	.850	.617	.396	2.006	-.210	-.402	.302
	.900	.760	.471	3.304	-.119	-.327	.519
	.910	.799	.490	3.736	-.097	-.310	.572
	.920	.844	.511	4.273	-.074	-.292	.631
	.930	.896	.535	4.958	-.047	-.272	.695
	.940	.959	.562	5.858	-.018	-.250	.768
	.950	1.035	.594	7.093	.015	-.226	.851
	.960	1.133	.634	8.890	.054	-.198	.949
	.970	1.265	.685	11.752	.102	-.164	1.070
	.980	1.466	.758	17.065	.166	-.120	1.232
	.990	1.849	.887	30.820	.267	-.052	1.489

a. A heterogeneity factor is used. b. Logarithm base = 10.

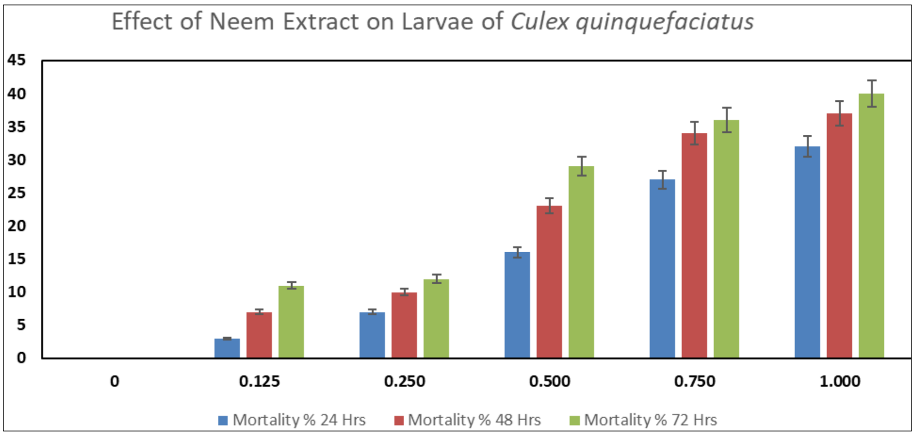

Fig 1: Effect of Neem extract on Larvae of *Culex quinquefaciatus*

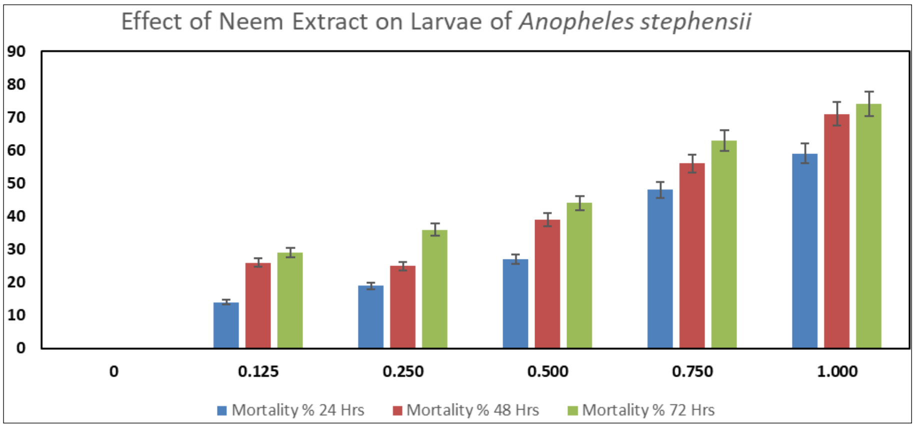

Fig 2: Effect of Neem extract on Larvae of *Anopheles stephensii.*

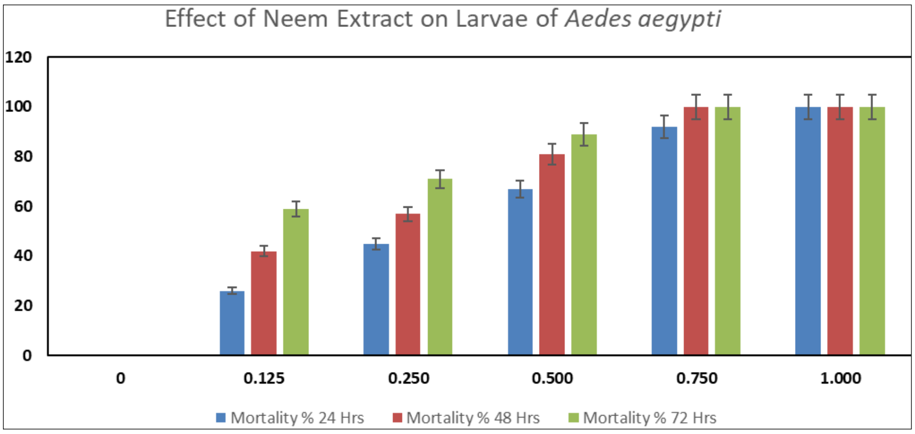

Fig 3: Effect of Neem extract on Larvae of *Aedes aegypti.*

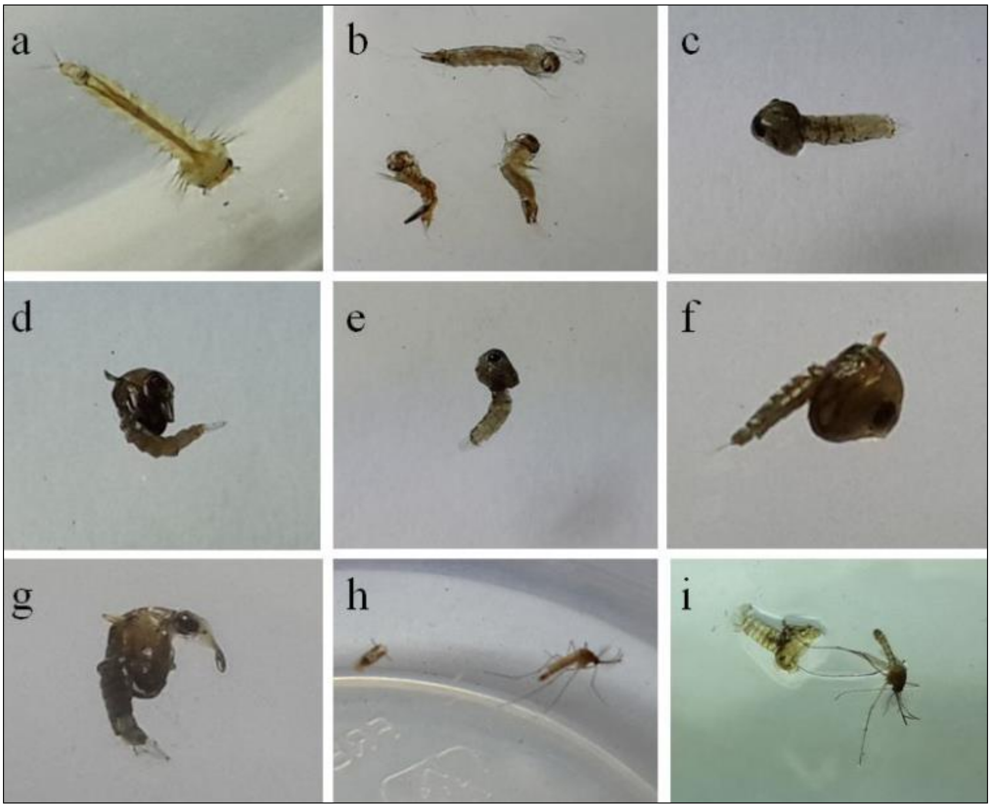

Fig 4: Photographs showing morphological deformities and growth/molting disruptive symptoms in *Cx. quinquefasciatus*, induced after the treatment with neem at different concentrations after 24 h. (a) a healthy larva from control, (b) morphologically deformed larvae, (c)Larva–pupa intermediate, (d) a healthy pupa from control, (e) and (f) morphologically deformed pupa, (g) Pupa–adult intermediate, (h) a healthy adult from control, (i) incomplete/malformed adults.

Fig 5: Photographs showing morphological deformities and growth/molting disruptive symptoms in *Ae. aegypti*, induced after the treatment with Neem extract at different concentrations after 24 h. (a) a healthy larva from control, (b) and (c) larvae with elongated guts, (d) morphologically deformed larvae, (e) Larva–pupa intermediate, (f) a healthy pupa fromcontrol, (g) morphologically deformed pupa, (h) and (i) Pupa–adult intermediate, (j) a healthy adult from control, (k) and (l) incomplete/malformed adults.

Discussion

This study demonstrates the significant larvicidal activity of neem extract against various mosquito species. Notably, *Aedes aegypti*, a primary vector for dengue and Zika viruses, showed 100% mortality even at low concentrations of 0.75%. These results agree with prior research reporting the potent larvicidal effects of neem against different mosquito species (Benelli, 2015; Senthil-Nathan, 2013) [2, 14]. Neem also exhibited effectiveness against *Anopheles stephensii*, a malaria vector, suggesting its potential for malaria control. While neem extract was less effective against *Culex quinquefasciatus*, it still showed moderate larvicidal effects.

Probit analysis revealed that *Aedes aegypti* and *Anopheles stephensii* had lower LC50 values, indicating that these species are more susceptible to neem's bioactive compounds, particularly azadirachtin, which interferes with insect growth and development (Suryawanshi, 2011) [17]. Additionally, morphological deformities in surviving larvae and pupae further supported the extract's toxic impact on mosquito development. These findings align with earlier studies that highlight neem as a promising botanical alternative to chemical insecticides, with its eco-friendly nature and low toxicity to non-target organisms, making it suitable for sustainable mosquito control (Isman, 2015) [5].

The effectiveness of plant-based products in repelling mosquitoes has been a focus of several studies. Sukumar *et al*. (1991) [16] investigated 346 plant species for their mosquito control potential, identifying six plant families—Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae, and Rutaceae—as having the highest potential to produce mosquito repellents (Raghavendra *et al*., 2013) [9]. Additionally, research by Rodrigues *et al*. (2020) [10] found that plants from the Piperaceae and Annonaceae families exhibit strong larvicidal abilities against *Stegomyia* species. A variety of plant parts, including flowers, stems, leaves, seeds, and roots, have been extracted using different solvents, showing effectiveness against mosquito larvae (Dharmshaktu *et al*., 1987; Singh *et al*., 2003; Bagavan & Rahuman, 2011) [1, 4, 15].

Many plant species produce secondary metabolites such as alkaloids, flavonoids, terpenoids, and other compounds that help defend against insects (Sathiyamoorthy *et al*., 1997; Markouk, 2000) [6, 13]. Research on India's southwest coast found that extracts from around 100 plant species were effective against *Culex quinquefasciatus*, with 17 species showing LC50 values under 100 mg/l (Nazar *et al*., 2009) [7]. Furthermore, over 400 plant species have been tested for their ability to repel mosquitoes, with 29 demonstrating excellent larvicidal activity, showing LC50 values below 10 ppm against major vectors (Pavela *et al*., 2019) [8].

In conclusion, plant extracts, including neem, represent a viable alternative to chemical mosquito control, with various species showing effective larvicidal properties.

Conclusion

The neem extract is more effective on *Aedes aegypti* and *Anopheles stephensii* than *Culex quinquefaciatus.* (Fig. 4.). Present studies reveal the highest mortality rate of 100% was found in *Aedes aegypti* at 0.75% concentration, followed by *Anopheles stephensii* with a mortality rate of 59 % at 1 % concentration, and the lowest mortality was seen in *Culex quinquefaciatus* is 32 % at 1% concentration at 24 hours. However, after emergence, the survived larvae and pupa showed morphological deformities and malformed adults (Fig. 5).

Hence, the neem leaf extract may be used as a potential larvicide as a tablet or spray in the market. However, it is very economical compared to other commercially available larvicides. In this way, the mosquito population may be controlled.

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