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Laboratory Study on Larvicidal Activity of Different Plant Extracts against *Aedes aegypti*

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ABSTRACT

Background: Mosquitoes transmit serious human diseases causing millions of deaths every year. Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of botanical origin have been reported as useful for control of mosquitoes.

Methodology: WHO standard larvicidal bioassay method was used and 30 late 3rd and early 4th instar larvae were subjected to four different concentrations i.e. 1%, 2%, 3% and 4% against test solutions which were made by using acetone as solvent. Mortality counts were made every 24 and 48 hours in each treatment. The LC₅₀, LC₉₉, standard error, fiducial limits at 95% confidence and regression equations were calculated.

Results: The results showed neem and pine oil extract are best in terms of LC₅₀ and LC₉₉, with 100% mortality at 3% and 4% concentration after 24 hours. The trend with respect to LC₅₀ and LC₉₉ after 48 hours was Pine > Neem > Til > Kadu respectively.

Conclusion: The results suggested that plant extract oil formulations were found effective in controlling *Aedes aegypti* larvae under lab conditions. As these trees are widely distributed in Pakistan, their formulation might prove to be an effective and eco-friendly larvicide, which could be used as an alternative for dengue control.

INTRODUCTION

Dengue is one of the most important viral diseases transmitted by *Aedes aegypti* because it afflicts humans worldwide whose symptoms ranging from mild fever to severe and potentially life threatening hemorrhagic disease. *Aedes aegypti* is of supreme concern because of its wide distribution and close association with humans [1]. *Aedes aegypti* is present in heavy polluted areas like Asia, America and some Pacific Islands are infested about 2/3 of the world's population [2].

One recent estimate indicates 390 million dengue infections per year and prevalence of dengue, estimates that 3900 million people, in 128 countries, are at risk of infection with dengue viruses globally during year 2015 [3].

In Asia, the first outbreak of DHF began in the 1950s in the Philippines and Thailand. However, in the next 20 years, the disease spread throughout South East Asia and by the mid-1970s. Dengue fever epidemics were common in Asia and Pacific throughout the twentieth century (4). In Pakistan 40987 cases of dengue reported with 490 deaths during year 2006-11 [4,5]. In august 2013 dengue outbreak occurred in KPK province affecting more than 7000 people with 26 deaths [6].

Since there is no particular treatment and vaccination available so emphasis should be on control of dengue fever vector. Different mosquito control methods are being used including chemical method by targeting the adult mosquito through spraying chemical insecticides or by killing the mosquito larvae by using synthetic larvicides [7]. Insecticides though work good in terms of

vector control but imposes threats not only to human health but also to the ecosystem [8]. Other than the detrimental effect on human health, the significant increase in insecticide-based vector control in the past decade has resulted in increasing resistance among vectors. Resistance to pyrethroids had been identified in 64 countries [9]. Resistance to temephos has been recorded in *Aedes aegypti* in Asia including Cambodia [10], Thailand [11,12], and Malaysia [13]. Emergence of resistance among vector mosquitoes is recent problem. Safe and ecofriendly agents from biological origin are need of the hour [14]. The attempt of this study was to screen and identify those plants, getting their extracts and evaluating their efficacy against larvae to control dengue vector *Aedes aegypti*.

MATERIAL AND METHODS

Preparation of stock solution

Crude plants oils of *Cucurbita moschata* (Kadu), *Sesamum indicum* (Til), *Azadirachta indica* (Neem) and *Pinus roxburghii* (Chir pine) were used. These oils were collected in small vials and the quantity was measured. Stock solutions were prepared by adding 1 ml of oil from each plant in 99 ml of acetone and considered as 1% stock solution from which series of 4 concentrations (ppm) were prepared [15].

Mosquito Rearing

Adult susceptible colonies *Aedes aegypti* were maintained in insectary of MEDVC department of Health Services Academy Islamabad on 10% sugar solution and females were blood fed on live white rats. Larvae were reared in steel trays (24x36x6 cm) and fed on sterilized liver diet.

Larvicidal Bioassay

The extracted oils were used in four different concentrations (1%, 2%, 3% and 4%) and their efficacy was evaluated by standard WHO method [16]. h concentration was t; and each replicate contained 200 ml of the oil solution were placed in 500 ml glass beakers. Batches of thirty late 3rd and early 4th instar larvae were exposed in each beaker containing oil solution. A total of three replicates were conducted for each concentration [17]. And against each replicate control test was also present. The numbers of dead larvae were counted after 24 and 48 hours interval. The experiment was conducted under lab conditions at 27± 2C and 80 ± 5% relative humidity.

Data analysis

The data so obtained was subjected to probit analysis and LC₅₀ and LC₉₉ values were calculated by using MINITAB-16 software. Chi square was also calculated to check the homogeneity of tested population

Results

Larvae of *Aedes aegypti* were subjected against crude plant extracts of *Cucurbita moschata* (Kadu), *Sesamum indicum* (Til), *Azadirachta indica* (Neem) and *Pinus roxburghii* (Chir pine). Four different concentrations of crude plant extracts were tested. The results on the use of different concentration of plant extracts were recorded in terms of mortality against larvae of *Aedes aegypti* under laboratory condition. **Table 1** shows that Neem and Pine oil was considered best with LC₅₀ values 0.052 and 0.089 respectively with 100% mortality at 3% and 4% concentration after 24 hours, followed by Kadu and Til with LC₅₀ values 0.71 and 1.41 with 45% and 18.33% mortality at 4% concentration respectively after 24 hours.the mortality percentage of *Aedes aegypti* larvae at each concentration after 24 hours is shown in **Figure 1**.

Table 1. Result summary of different plant extracts against *Aedes aegypti* larvae after 24 hours

Plant Extract	Lethal concentration	LFL	UFL	Slope±SE	χ ²	P value	Regression equation
Neem	LC ₅₀	0.05	0.004	0.09	6.77±0.97	0.15	Y= -0.34+6.77x
	LC ₉₉	0.39	0.31	0.52	6.77±0.97	0.15	Y= -0.34+6.77x
Pine	LC ₅₀	0.08	0.41	0.13	6.17±0.76	0.88	Y= -0.55+6.17x
	LC ₉₉	0.46	0.39	0.58	6.17±0.76	0.88	Y= -0.55+6.17x
Kadu	LC ₅₀	0.71	0.614	0.92	2.67±0.54	4.09	Y= -1.91+2.67x
	LC ₉₉	1.58	1.25	2.34	2.67±0.54	4.09	Y= -1.91+2.67x
Til	LC ₅₀	1.41	0.91	10.85	1.1±0.51	0.58	Y= (-1.5) + (1.1)x
	LC ₉₉	3.51	2.02	32.3	1.1±0.51	0.58	Y= (-1.5) + (1.1)x

LC₅₀ =Lethal concentration 50 at which 50% of target population died.

LC₉₉ = Lethal concentration 99 at which 99% of target population died.

LFL = Lower fiducial limit UFC = Upper fiducial limit

SE = Standard error χ² = Chi-square.

p value = Level of significance p ≤ 0.05, p ≥ 0.05 non-significant

Table 2 shows that after 48 hours Neem and Pine oil presented excellent results with LC₅₀ values -0.10 and -0.18 respectively with 100% mortality at 3% and 4% concentration, followed by Til and Kadu with LC₅₀ values -0.21 and -0.27 with 98.3% and 96.6%

mortality at 4% concentration respectively. The mortality percentage of *Aedes aegypti* larvae at each concentration after 48 hours is shown in **Figure 2**.

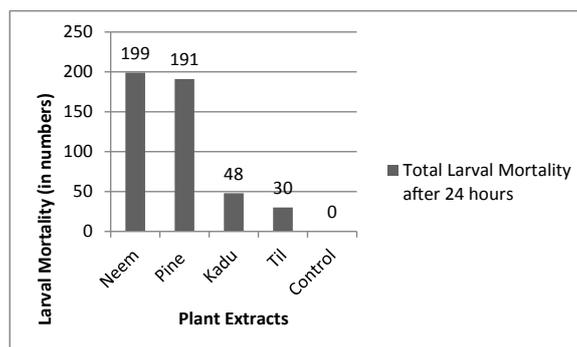


Figure 1. Total Larval mortality of *Aedes aegypti* against different plant extracts after 24 hours

Table 2. Result summary of different plant extracts against *Aedes aegypti* larvae after 48 hours

Plant Extract	Lethal Concentration	LFL	UFL	Slope±SE	χ^2	P value	Regression equation
Neem	LC ₅₀	-0.10	-0.26	-0.03	4.72±1.01	0.30	Y= 0.51+4.72x
	LC ₉₉	0.38	0.27	0.61	4.72±1.01	0.30	Y= 0.51+4.72x
Pine	LC ₅₀	-0.18	-0.49	-0.07	4.565±1.28	0.13	Y= 0.83+4.56x
	LC ₉₉	0.32	0.21	0.67	4.565±1.28	0.13	Y= 0.83+4.56x
Til	LC ₅₀	-0.21	-0.56	-0.06	2.32±0.49	0.53	Y= 0.50+2.32x
	LC ₉₉	0.78	0.60	1.19	2.32±0.49	0.53	Y= 0.50+2.32x
Kadu	LC ₅₀	-0.27	-0.75	-0.08	1.98±0.47	0.40	Y= 0.53+1.98x
	LC ₉₉	0.89	0.67	1.47	1.98±0.47	0.40	Y= 0.53+1.98x

LC₅₀ =Lethal concentration 50 at which 50% of target population died.

LC₉₉ = Lethal concentration 99 at which 99% of target population died.

LFL = Lower fiducial limit UFC = Upper fiducial limit

SE = Standard error χ^2 = Chi-square.

p value = Level of significance $p \leq 0.05$, $p \geq 0.05$ non-significant.

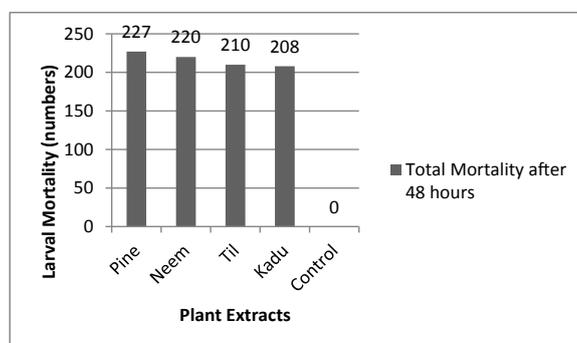


Figure 2. Total Larval mortality of *Aedes aegypti* against different plant extracts after 48 hours

DISCUSSION

Many plant based products are widely used for their insecticidal properties for the control of mosquitoes [18]. In recent years interest in plant origin products has been revived because of the development of resistance, cross resistance and toxicity hazards associated with synthetic insecticides [19]. A large number of pant products have been reported to have mosquito larvicidal activity [20].

The results of our study to evaluate the larvicidal activity of *Cucurbita moschata* (Kadu), *Sesamum indicum* (Til), *Azadirachta indica* (Neem) and *Pinus roxburghii* (Chir pine) oils were comparable with findings of other researchers like Vatandoost et al. tested 400 larvae against neem oil and the mortality rate of larvae was 15.8%. Examination of larvicidal activities of pine concluded that pine oil has varying degree of larvicidal activity with LC₅₀ value ranging between 82 and 112 ppm [16]. Larvicidal activity of five species of Cucurbitaceae plants showed extremely effective against the larvae of *Aedes aegypti* with values (LC50=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) respectively. Larvicidal activities of 100 Indian coastal plant extracts were examined against *Aedes aegypti* among which Til oil showed 34% to 100% mortality at different concentrations [14].

CONCLUSION

From these results it was concluded that Neem and Pine oil were found to have larvicidal activity under lab conditions with

best efficacy in terms of LC_{50} , LC_{99} and percentage mortality after 24 and 48 hours respectively. In search of alternative and safe methods of controlling dengue vector mosquito products from essential oils might prove to be good vector control tool which might be more safe to use and cost effective.

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