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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 methodology was adopted for larvicidal bioassay. Thirty late 3rd and early 4th instar larvae were subjected to four different concentrations i.e. 1%, 2%, 3% and 4% against *Cucurbita moschata* (seed)**,** *Sesamum indicum* (seed)**,** *Azadirachta indica* (Leaves) and *Pinus roxburghii* **(**Bark) oils in acetone as solvent. Mortality counts were made every 24 and 48 hours in each treatment. The $LC_{50,}$ LC_{99}, standard error, fiducial limits at 95% confidence and regression equations were calculated.
Results: The results showed neem and pine oil extracts are best in terms of LC_{50} and$LC_{99,}$ with 100% mortality at 3% and 4% concentration after 24 hours. The trend with respect to LC_{50} and LC_{99} after 48 hours was Pine > Neem> Til > Kadu respectively.
Conclusion: The results suggested these plant extract 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.

Keywords: Plant extracts, *Aedes aegypti*, Larvicidal, Dengue.

1. 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 and about2/3 of the world's population are infected [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 [5]. In August 2013, dengue outbreak occurred in Kyber Pakhtun khaw province affecting more than 7000 people with 26 deaths [6].
Since there is no particular treatment and vaccination available, emphasis should be on control of dengue 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 poses 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 a recent problem. Safe and ecofriendly agents from biological origin are need of the hour [14]. The attempt of the present study was to screen and

identify those plants, getting their extracts and evaluating their efficacy against larvae to control the dengue vector *Aedes aegypti*.

2. Material and Methods

2.1. Collection of Plants

Following plants were collected from different cultivators in Rawalpindi and Islamabad. These are:

Common Name	Botanical Name	Part used
Kadu	*Cucurbita moschata*	Seed
Til	*Sesamum indicum*	Seed
Neem	*Azadirachta indica*	Leaves
Chir pine	*Pinus roxburghii*	Stem Bark

2.2. Extraction of oil

The seeds were washed with tap water to remove the pulp and then dried in oven for 48 hours at 60 °C and later would be ground in an electric grinder (Anex Germany). While the other parts like leaves and bark were also dried. The grounded material was put in soxhelt apparatus for the extraction of oil by steam distillation method (Vogel, 1978)

2.3. Preparation of Stock Solution

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 99ml of acetone and were considered as 1% stock solution from which series of 4 concentrations (%) were prepared [15].

2.4. Mosquito Rearing

Adult susceptible colonies of *Aedes aegypti* were maintained in an insectary of Medical Entomology and Disease Vector Control 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 (24 x 36 x 6 cm) and fed on sterilized broiler chicken liver diet.

2.5. 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]. Each replicate contained 200ml of the oil solution were placed in 500ml glass beakers. Batches of thirty late 3rd and early 4th instar larvae were exposed in each beaker containing the crude oil solution. A total of three replicates were conducted for each concentration [17] and against each replicate, a control was present. The numbers of dead larvae were counted after 24 and 48 hours interval. The experiment was conducted under lab conditions at 27± 2 ºC and 80± 5% relative humidity.

2.6. Data analysis

The data obtained was subjected to probit analysis and LC_{50} & LC_{99} values were calculated using MINITAB-16 software. Chi square analysis was also calculated to check the homogeneity of the tested population.

3. Results

Larvae of *Aedes aegypti* were subjected against crude plant oils of *Cucurbita moschata* (Kadu)**,** *Sesamum indicum* (Til)**,** *Azadirachta indica* (Neem) and *Pinus roxburghii* **(**Chir pine). Four different concentrations of crude plant oils 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 LC_{50} values 0.052 and 0.089 respectively with 100% mortality at 3% and 4% concentration after 24 hours, followed by Kadu and Til with LC_{50} 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 are shown in figure 1.

Table 1: Result summary of different crude plant oils against *Aedes aegypti* larvae after 24 hours

Crude plant oil	Lethal concentration		LFL	UFL	Slope ±S.E.	χ^2	P value	Regression equation
Neem	LC_{50}	0.05	0.004	0.09	6.77±0.97	0.15	0.92	Y= -0.34+6.77x
	LC_{99}	0.39	0.31	0.52	6.77±0.97	0.15	0.92	Y= -0.34+6.77x
Pine	LC_{50}	0.08	0.41	0.13	6.17±0.76	0.88	0.64	Y= -0.55+6.17x
	LC_{99}	0.46	0.39	0.58	6.17±0.76	0.88	0.64	Y= -0.55+6.17x
Kadu	LC_{50}	0.71	0.614	0.92	2.67±0.54	4.09	0.12	Y= -1.91+2.67x
	LC_{99}	1.58	1.25	2.34	2.67±0.54	4.09	0.12	Y= -1.91+2.67x
Til	LC_{50}	1.41	0.91	10.85	1.1±0.51	0.58	0.74	Y= (-1.5) + (1.1)x
	LC_{99}	3.51	2.02	32.3	1.1±0.51	0.58	0.74	Y= (-1.5) + (1.1)x

LC_{50} =Lethal concentration 50 at which 50% of target population died.
LC_{99} = 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

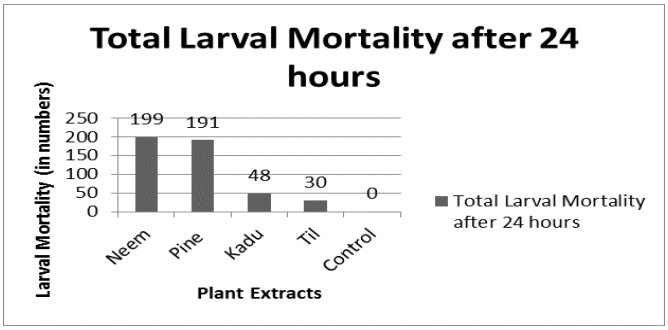

Fig 1: Total Larval mortality of *Aedes aegypti* against different crude plant oils after 24 hours

Table 2 indicates Neem and Pine oil presented excellent results with LC_{50} values 0.10 and 0.18 respectively with 100% mortality at 3% and 4% concentration, after 48 hours exposure followed by Til and Kadu with LC_{50} 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 are shown in figure 2.

Table 2: Result summary of different crude plant oils 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	0.85	Y= 0.51+4.72x
	LC_{99}	0.38	0.27	0.61	4.72±1.01	0.30	0.85	Y= 0.51+4.72x
Pine	LC_{50}	0.18	-0.49	-0.07	4.565±1.28	0.13	0.93	Y= 0.83+4.56x
	LC_{99}	0.32	0.21	0.67	4.565±1.28	0.13	0.93	Y= 0.83+4.56x
Til	LC_{50}	0.21	-0.56	-0.06	2.32±0.49	0.53	0.76	Y= 0.50+2.32x
	LC_{99}	0.78	0.60	1.19	2.32±0.49	0.53	0.76	Y= 0.50+2.32x
Kadu	LC_{50}	0.27	-0.75	-0.08	1.98±0.47	0.40	0.81	Y= 0.53+1.98x
	LC_{99}	0.89	0.67	1.47	1.98±0.47	0.40	0.81	Y= 0.53+1.98x

LC_{50} =Lethal concentration 50 at which 50% of target population died.
LC_{99} = 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.

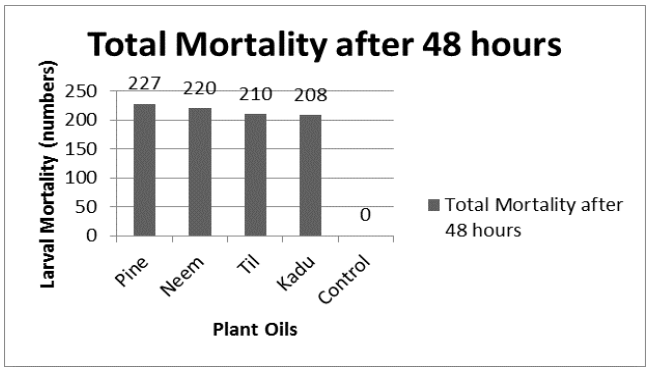

Fig 2: Total Larval mortality of *Aedes aegypti* against different crude plant oils after 48 hours

4. Discussion and conclusion

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 the present study evaluated the larvicidal activity of *Cucurbita moschata* (Kadu)**,** *Sesamum indicum* (Til)**,** *Azadirachta indica* (Neem) and *Pinus roxburghii* **(**Chir pine) oils which were comparable with findings of other researchers like Vatandoost *et al.* (2004) who 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 LC_{50} value ranging between 82 and 112 ppm (Ansari *et al.* (2005). Larvicidal activity of five species of Cucurbitaceae plants showed extremely effective against the larvae of *Aedes aegypti* with values (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) respectively (Rahuman *et al.* (2008). 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 (Nazar *et al.* (2009).

From the results of the present study 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, essential oils might prove to be a good vector control tool which might be more safe to use and cost effective.

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6. References

1. Beula JM, Ravikumar S, Ali MS. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. Asian Pacific journal of tropical biomedicine. 2011; 1(2):143-6.
2. Hahn CS, French OG, Foley P, Martin EN, Taylor RP. Bispecific monoclonal antibodies mediate binding of dengue virus to erythrocytes in a monkey model of passive viremia. The Journal of Immunology. 2001; 166(2):1057-65
3. World Health Organization Dengue Fever Fact Sheet May, 2015, 117.
4. Khan E, Kisat M, Khan N, Nasir A, Ayub S, Hasan R. Demographic and clinical features of dengue fever in Pakistan from 2003–2007: a retrospective cross-Sectional study. PloS one 2010; 5(9):12505.
5. World Health Organization (WHO) Eastern Mediterranean Regional Office (EMRO) Weekly Epidemiological Monitor, 2012, 5(23).
6. Medicalopedia. Why Pakistan Is Having The Dengue Epidemic Every Summer? "Retrieved, 19, March, 2012.
7. Tiwary M, Naik S, Tewary DK, Mittal P, Yadav S. Chemical composition and larvicidal activities of the essential oil of Zanthoxylum armatum DC (Rutaceae) against three mosquito vectors. Journal of vector borne diseases. 2007; 44(3):198.
8. Mathivanan T, Govindarajan K, Elumalai K, Ananthan A. Mosquito Larvicidal and Phytochemical Properties of Ervantaniacoronaria Stap f. (Family Apocynaceae), J Vector Borne Dis. 2000; 44:178-180
9. World Health Organization Global plan for insecticide resistance management in malaria program 2012.

http://www.who.int/malaria/publications/atoz/gpirm/en/
10. Polson KA, Curtis C, Chang M, Olson JG, Chantha N, Rawlins S. Susceptibility of two Cambodian population of Aedes aegypti mosquito larvae to temephos during 2001. Dengue Bull 2001; 25:79-83.
11. Chareonviriyahpap T, Aum-Aung B, Ratanatham S. Current insecticide resistance patterns in mosquito vectors in Thailand. The Southeast Asian journal of tropical medicine and public health. 1999; 30(1):184-94.
12. Paeporn P, Komalamisra N, Deesin V, Rongsriyam Y, Eshita Y, Thongrungkiat S. Temephos resistance in two forms of Aedes aegypti and its significance for the resistance mechanism. Southeast Asian journal of tropical medicine and public health. 2003; 34(4):786-92.
13. Chen C, Nazni W, Lee H, Sofian-Azirun M. Susceptibility of Aedes aegypti and Aedes albopictus to temephos in four study sites in Kuala Lumpur City Center and Selangor State, Malaysia. Tropical biomedicine 2005; 22(2):207-16.
14. Nazar S, Ravikumar S, Williams GP, Ali MS, Suganthi P. Screening of Indian coastal plant extracts for larvicidal activity of Culex quinquefasciatus. Indian Journal of Science and Technology. 2009; 2(3):24-7.
15. Murgan K, Murgan P, Noortheen A. Larvicidal and repellent potential of Albizziaamara Boivin and Ocimumbasilium Linn against dengue vector, *Aedes aegypti*. Bioresource Technology 2007; 98:198-201.
16. Ansari M, Mittal P, Razdan R, Sreehari U. Larvicidal and mosquito repellent activities of Pine (Pinus longifolia, Family: Pinaceae) oil. Journal of vector borne diseases. 2005; 42(3):95.
17. Sharma P, Mohan L, Srivastava C. Phytoextract-induced developmental deformities in malaria vector. Bioresource Technology 2006; 97(14):1599-604.
18. Jacobson M, Crosby DG. *Naturally occurring insecticides*. New York: Marcel Dekker Inc 1971, 585.
19. Ansari M, Mittal P, Razdan R, Sreehari U. Larvicidal and mosquito repellent activities of Pine (Pinus longifolia, Family: Pinaceae) oil. Journal of vector borne diseases. 2005; 42(3):95.
20. Ansari M, Vasudevan P, Tandon M, Razdan R. Larvicidal and mosquito repellent action of peppermint (Mentha piperita) oil. Bioresource Technology 2000; 71(3):267-71.