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Original Article

Adulticidal Efficacy of *Azadirachta Indica* (Neem Tree), *Sesamum Indicum* (Til) and *Pinus Sabinaena* (Pine Tree) Extracts Against *Aedes Aegypti* under Laboratory Conditions

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Abstract

Background: Emergence of resistance to insecticides against mosquitoes and other disease vectors has prompted the need for alternate sources of mosquito control which are more target-specific, easily bio-degradable and effective against mosquitoes.
Materials and Methods: The adulticidal efficacy of crude Neem oil extract, Pine oil extract and Til oil extract with five different concentrations i.e. 2%, 4%, 6%, and 8% was tested against five to six days old sugar fed female *Aedes aegypti* using standard WHO Bioassay test kit. The percentage mortality was observed after 24 and 48 hours under laboratory condition.
Results: The LC_{50} (Lethal Concentration)values of Neem oil extract against adults of *Aedes aegypti* were 1.17 ml and 0.09 ml after 24 and 48 hours of exposure respectively, while the LC_{99} were 4.27 ml and 1.17 ml after 24 and 48 hours of exposure respectively. The LC_{50} values of Pine oil extract after 24 and 48 hours were 0.25 and, 0.06 ml respectively, while LC_{99} values at 24 and 48 hour were and 0.89 ml and 0.64 ml respectively. The LC_{50} values of Til oil extract after 24 and 48 hours of exposure were 1.17 ml and 0.05 ml respectively while LC99 values were 4.18 ml and 1.28 ml after 24 and 48 hours of exposure respectively. The LC_{50} and LC_{99} values were indicative that the plant extracts were highly toxic after 48 hours of exposure. Among the tested plant oil extracts the maximum adulticidal efficacy was observed in the pine oil extract. No mortality was observed in controls.
Conclusion: Crude extract of Pine oil was more effective against *Aedes aegypti* as compared to Neem and Til oil.
Key words: Adulticidal activity, plant oil extracts, *Aedes aegypti*, dengue vector.

Introduction

In Pakistan, there are about 30 known species of *Aedes* out of which only two species *Aedes aegypti* and *Aedes albopictus* are considered as the primary and secondary vectors of dengue respectively[1]. Dengue is a viral infection transmitted by female *Aedes* mosquito. About 50–100 million new infections occur annually in more than 100 endemic countries[2]. According to an estimate, dengue is responsible for almost 2.5% deaths each year with a large proportion of children[3].

In Pakistan the first outbreak of dengue fever was reported from Karachi in 1994[4] and since then its prevalence has increased rapidly. In 2010, more than 9000 cases were reported across Pakistan. Approximately 5000 cases and 35 deaths were reported from Sindh; 16 deaths were from Karachi. More than 4000 cases were reported from Punjab, with three deaths[5]. In 2011, Lahore experienced a major dengue outbreak with 21,685 confirmed cases and 350 deaths due to DENV-2 [6]. In 2013 dengue fever outbreak was reported from areas not previously endemic for dengue. A huge outbreak occurred in district Swat of Khyber Pakhtunkhwa Province, where from 7th August to 25th September, 6,376 dengue fever cases and 23 deaths were reported[7].

Since there is no effective treatment and vaccine available for dengue therefore, vector control remains the sole method for its control[8].

Indoor Residual Spraying (IRS) with insecticides is a good method of mosquito control but its resistance is a big challenge. For Example adult *Aedes aegypti* from Lahore have been reported to be resistant to deltamethrin and alphacypermethrin[9,10]. The current situation demands

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exploration of alternate sources of mosquito control. Plant extracts being rich in bioactive chemicals can be a potential source of insecticidal agents against mosquitoes[11-14].

The present study was conducted to evaluate the adulticidal activity of three plant extracts i.e. *Azadirachtaindica* (Neem tree), *Sesamumindicum* (Til) *and Pinussabinaena* (Pine tree) against the adult *Aedes aegypti*.

Materials and Methods

The Plant oil extracts, glass jars (100ml) and Wattman filter paper sheets were purchased from market. Adulticidal activity of three plant oil extracts was evaluated against five concentrations (2%, 4%, 6%, 8%) prepared from the stock solutions. Briefly, filter paper (140x120mm) was dipped in known amount of water to check its water retention capacity. Different concentrations of plant extracts were then impregnated on filter papers by dipping them in the solution for 5 minutes. Filter paper impregnated with acetone (solvent) was used as a control. The impregnated papers were left to dry at room temperature for two hours.

Aedes aegypti mosquitoes were reared in the insectary of the Department of Medical Entomology and Disease Vector Control at Health Services Academy, Islamabad. The larvae were fed on liver food. Adult *Aedes aegypti* were provided with 10% sugar solution and females were fed on albino mice for oviposition. They were maintained at 27 ± 2^0C temperature and 80±10% relative humidity under a photoperiod of 12:12 hrs (light/dark).

The adulticidal activity of each plant extract was performed using standard WHO procedure[15].

The bioassay was conducted using two plastic tubes. One tube was used to expose the mosquitoes to the plant oil extract and another to hold the mosquitoes during pre and post exposure periods. The papers impregnated with various concentrations of plant extracts were rolled and placed in the exposure tube. Fifteen 5-6 days starved female mosquitoes were used in each of the three replicate. At the end of 3 hr exposure period[30] the mosquitoes were transferred to holding tubes which were kept at 27 ±2°C and 80% ± 10% relative humidity for 24 and 48 hours respectively. A cotton pad soaked in 10% sugar solution was placed in the tube during the holding period. Mortality of mosquitoes was recorded at the end of 24 and 48 hours. The adulticidal activity of plant extracts was interperated as low (1-49% mortality), moderate (50-69% mortality) and high (70-100% mortality) [12].

The data was subjected to probit analysis for the calculation of lethal concentration 50 (LC_{50}) and lethal concentration 99 (LC_{99}) using computer software Mini tab. Chi-square was also calculated to check the homogeneity of tested population.

Ethical clearance was obtained from the Internal Review Board of the Health Services Academy, Islamabad.

Results

Percentage mortality of adult female *Aedes aegypti* to various concentrations of three plant extracts (Neem, Pine and Til) were recorded under laboratory condition. Table shows adulticidal activity of the three oil extracts against *Aedes aegypti* and Figure shows mortality after 24 and 48 hours to plant oil extracts. The LC_{50} and LC_{99} values of Neem oil extract after 24 hours of exposure were 1.17 ml and 4.27 ml with mortality ranging from 26.7% to 48.9% respectively. Maximum mortality (48.9%) was observed at the concentration of 8%. The LC_{50} and LC_{99} values after 48 hours exposure were 0.9 ml and 1.17 ml with mortality ranging from 71-100%. Maximum mortality (100%) was observed at the concentration of 8%. The adulticidal activity of Pine oil extract after 24 hours of exposure was observed at almost all concentrations. The LC_{50} and LC_{99} values were 0.25 ml and 0.89 ml with mortality ranging from 87-100%. Maximum mortality (100%) was observed at the concentrations of 8%. The LC_{50} and LC_{99} values after 48 hours exposure were 0.06 ml and 0.64 ml and the mortality ranged from 90-100% respectively. Maximum mortality (100%) was observed at the concentrations of 6% and 8% respectively. Til oil extract on the other hand showed significantly lower mortality ranging from 20-40% at the end of 24 hours of exposure with LC_{50} and LC_{99} values of 1.17ml and 4.18ml respectively. At the end of 48 hours exposure the mortalities observed ranged from 73-98% with LC_{50} and LC_{99} values of 0.05ml and 1.28 ml respectively. Maximum mortality (97.8%) was observed at the concentration of 8%. No mortality was observed when mosquitoes were exposed to "control papers" treated with acetone after 24 hours and 48 hours exposure period respectively.

Discussion

The results on the use of different concentration of plant extracts were recorded in terms of mortality of adult *Aedes aegypti* under laboratory condition. The results showed that after 24 hours of exposure period the LC_{50} and LC_{99} values were 1.17 ml and 4.266 ml respectively with mortality ranging from 26.6% to 48.9%, being maximum at the concentration of 8% while after 48 hours exposure period LC_{50} and LC_{99} values were 0.9 ml and 1.173 ml respectively with mortality ranging from 71% to 100% respectively. Maximum mortality (100%) was observed at the concentrations of 8%. The adulticidal activity of Pine oil extract against adult *Aedes aegypti* after 24 hours of exposure was observed at all concentrations. The LC_{50} and LC_{99} values were 0.253ml and 0.892ml with mosquito mortality ranging from

86.7% to 100% respectively. Maximum mortality (100%) was observed at the concentrations of 8% and 10% while after 48 hours exposure period LC_{50} and LC_{99} were 0.615ml and 0.643ml with percentage mortality of *Aedes aegypti* 88.9% to 100% respectively. Maximum mortality was again observed at higher concentrations i.e. 6, 8 and 10% but Til oil extract showed significantly lower mortality in the mosquitoes exposed for 24 hours (ranging between 20-40%) with LC_{50} and LC_{99} values as 1.17ml and 4.179 respectively as compared to 48 hours exposure (ranging from 73.0-97.8%) with LC_{50} and LC_{99} values as 0.0487ml, 1.285 ml respectively. Maximum mortality was observed at the concentration of 8% (i.e. 97.8%). There was no mortality among the adult Aedes aegypti exposed to control of all plant extract oil concentrations after both exposure period i.e. 24 hour and 48 hour. Comparison of extracts of Pine oil, Neem oil and Til oil against *Aedes aegypti* showed better adult killing with Pine oil as compared to Neem or Til oil.

The growing insecticide resistance in *Aedes aegypti* populations has adversely affected the efforts towards the control of dengue vector in the country. This has led to an increased interest in the alternate methods of mosquito control which are less hazardous, inexpensive and environmental friendly. Plants being rich in bioactive chemicals and having insecticidal activity may serve as a suitable alternative to synthetic insecticides, especially for the control of mosquitoes[16]. Insecticidal properties of a number of plant extracts against mosquitoes have been documented by many authors[17-21].

Table: Adulticidal activity of neem oil, pine oil and til oil extracts against *Aedes aegypti.*

		Time	*Lethal Doses*	*Lower Fiducial Limit*	*Upper Fiducial Limit*	*Slope±SE*	χ^2	*P value*	*Regression equation*
Neem oil	L_c50	24 h	1.17	0.89	6.71	0.751±0.354	3.441	0.32	Y= -0.88+ 0.75X
		48 h	0.09	-0.30	0.27	2.150 ± 0.469	6.120	0.10	Y= -0.2 + 2.20X
	L_c99	24 h	4.27	2.56	48.25	0.751±0.354	3.4410	0.32	Y= -0.88+ 0.75X
		48h	1.17	0.98	1.63	2.150 ± 0.469	6.120	0.10	Y= -0.20 + 2.15X
Pine oil	L_c50	24 h	0.25	- 1.97	0.083	2.029 ± 0.713	2.632	0.45	Y= 0.512 + 0.2.03X
		48 h	0.06	-1.41	0.16	3.30 ± 1.25	0.38	0.94	Y=0.203+ 3.30X
	L_c99	24 h	0.89	0.69	1.78	2.029 ± 0.713	2.632	0.45	Y=0.512+ 0.2.03X
		48 h	0.64	0.50	1.40	3.30 ± 1.25	0.38	0.94	Y=0.203+ 3.30X
Til oil	L_c50	24 h	1.17	1.08	6.09	–1.233± 0.378	2.079	0.55	Y=-1.233+ 0.85X
		48 h	0.05	-0.77	0.20	1.745± 0.457	0.980	0.80	Y=0.0849+ 1.75X
	L_c99	24 h	4.18	2.60	26.60	–1.233± 0.378	2.079	0.55	Y=-1.233+ 0.85X
		48 h	1.28	1.03	2.02	1.745± 0.457	0.980	0.80	Y=0.0849+ 1.75X

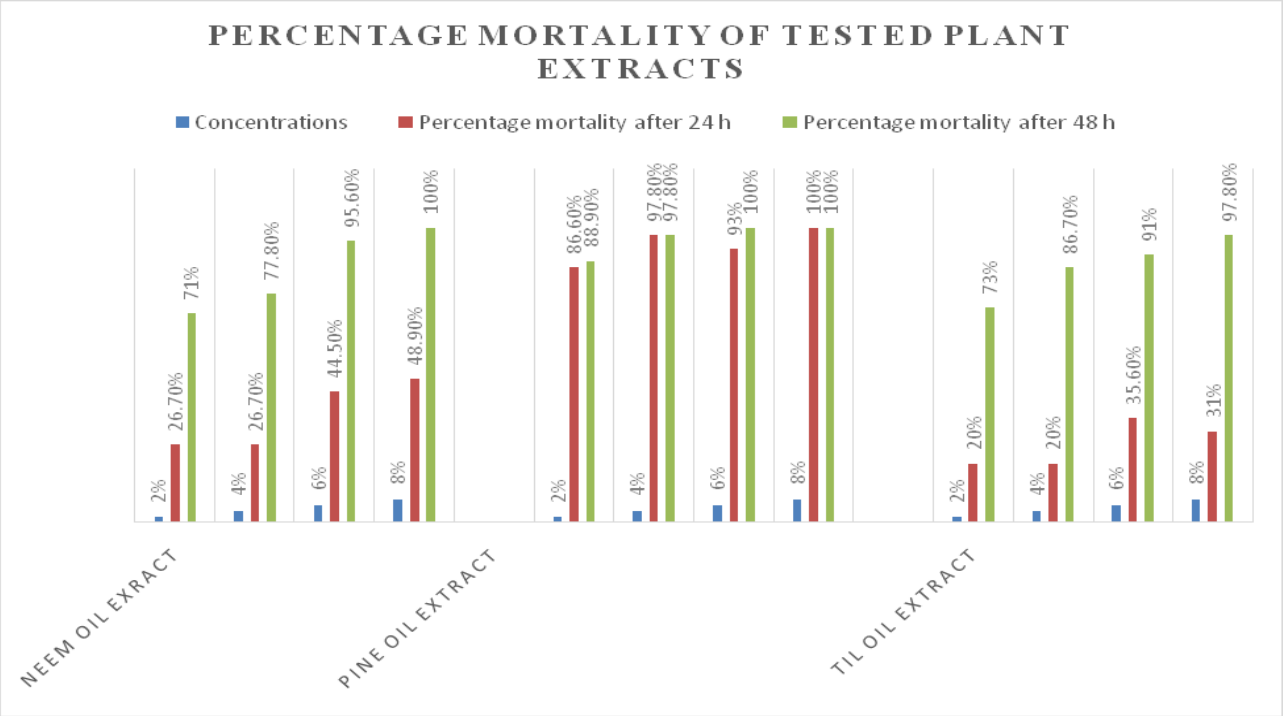

Figure: Percentage mortality of neem oil, pine oil & til oil extracts.

Excellent effects of plant derived constituents against mosquito larvae have been reported in the literature[22-26] but few studies have been carried out against adult mosquitoes.

The results of this study showed that Pine oil extract possessed marked adulticidal activity against adult *Aedes aegypti* after 24 hours of exposure with low LC_{50} value (0.253 ml) while Neem and Til Oil extracts did not exhibit effective adulticidal activity after 24 h exposure period.

Our results are comparable to studies on adulticidal and larvicidal potential of plant extracts carried out by other researchers. One study reported the bioactivity of *Cedrusdeodara*, *Eucalyptus citirodara*, *Cymbopogen flexuous*, *Pinusroxburighii*, *Syzygiumaromaticum*, *C. winteriamus* and *Tagetes minuta* against *Culex* and *Aedes* mosquitoes and reported that *S. aromaticum* and *C. winteriamus* were equally effective with LC_{50} and LC_{99} values at 0.5 and 0.9 % concentration for *Culex* and 1 and 2 % concentration for *Aedes aegypti*[27].

In the present study maximum mortality of the mosquitoes was observed at the concentration of 8% by two plant extracts i.e. Neem and Til oil while Pine oil showed mortality in all concentrations, with maximum being at 6% and 8%, respectively which, seems to be more dose dependent as compared to the other two other oils used. This observation is in line with a study carried out on the adulticidal effect of methanol extracts of seven species of *Malaysian tunicates* that showed that the mortality values of the extracts on the adult mosquitoes of *Aedes aegypti* were dose-dependent and increased with exposure period[28].

More in depth studies are needed to establish the efficacy of these oils against adult *Aede*s aegypti mosquitoes before they are generalized for commercial use.

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Authors Contribution

ABZ proposed idea for project. SAH also did collection of data regarding proposal, statistical analysis and manuscript writing. UM and SM help in arrangement of botanical extracts, formulation of solutions. MAM provided guidance regarding tabulation, manuscript writing and analysis briefing. AA helps in rearing, sorting of female mosquitoes and collection of replicates data. SAH and IAK provided guidance during statistical methodology and filter paper impregnation.

Conflict of interest: None declared.

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