



E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(6): 112-116
© 2015 JEZS
Received: 05-08-2015
Accepted: 02-10-2015

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Adulticidal efficacy of *Azadirachta indica* (neem tree), *Sesamum indicum* (til) and *Pinus sabinana* (pine tree) extracts against *Aedes aegypti* under laboratory conditions

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Abstract

Due to the development of resistance to synthetic insecticides, residue problems in the environment and toxic effects on humans and non-target organism, investigators now direct their attention towards the development of new plant based insecticides with less harmful effects. Various compounds including phenolic, terpenoids and alkaloids exist in plants and may jointly or independently contribute to the generation of larvicidal activities of mosquitoes. The results obtained from Pine oil extract used in this study were satisfactory and establish the efficacy. Mortality increases with increase in plant extract concentrations and complete mortality was observed at the concentrations of 8 percent to 10 percent. The lower concentrations were still useful as they showed restless movement for some times with abnormal wagging and then died. Keeping in views the interpretation criteria Neem oil and Til oil considered low adulticidal activity at 24 hours exposure period Pine oil showed high adulticidal activity while after 48 hours exposure period all plant oil extracts showed high adulticidal activity. From the results it can be concluded the crude extract of pine oil extract was an excellent potential for controlling dengue vector mosquito, *Aedes aegypti*.

Keywords: Adulticidal activity, Neem oil extracts, Pine oil extract and Til oil extract, *Aedes aegypti*, Dengue vector.

Introduction

Mosquitoes are important vectors of a number of human diseases. There are about 3400 species of mosquitoes belonging to 42 genera^[1]. Globally there are about 950 species have been reported under genus *Aedes*, while in Pakistan there are about 30 species of genus *Aedes*. Out of them two species are major vector for viral diseases i. e. *Aedes aegypti* and *Aedes albopictus*^[7]. *Aedes aegypti* is the primary vector of dengue while *Aedes albopictus* (Asian tiger mosquito) acts as a secondary vector^[2]. Dengue is considered as one of the most important arthropod-borne viral diseases in Southeast Asia^[3]. It is transmitted by the bite of a mosquito, infected with one of the four dengue virus serotypes (DEN1, 2, 3, and 4). It is a febrile illness that affects infants, young children and adults with symptoms appearing 3-14 days after the infective bite^[4]. Dengue fever affects 50 million people annually, and approximately two fifth population of the world is at risk of infection. More than 100 countries in South East Asia, South and Central America, Mexico and Africa are currently experiencing dengue^[5]. One recent estimate indicates 390 million dengue infections per year (95% credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically^[6]. According to World Health Organization, about 2.5% deaths occur globally every year and large proportion of which are children^[8]. The outbreak of dengue has been documented in Eastern Mediterranean Region possibly as early as 1799 in Egypt and its frequency of repeated outbreaks continue to increase^[9]. Recent outbreaks have been recorded in Pakistan, Saudi Arabia and Yemen^[10]. Dengue Surveillance Cell Sind province in 2001 reported 1,809 suspected dengue cases out of which 881 were confirmed with 5 deaths while 16 confirmed cases were reported in Islamabad. However, there was no mortality^[17]. In the year 2005, Dengue emerged as the most significant mosquito-borne viral disease affecting humans^[12].

During 2005-2011 numbers of confirmed cases and deaths dramatically increased to 55,946 and 539 respectively, affecting 105 out of 146 districts/agencies/territories [16]. From 2006-2011, 40987 cases of dengue with 490 deaths were reported from Punjab region and Karachi [13]. In addition, more than 4000 cases were reported from Punjab, with 3 deaths. About 1500 cases were reported in the last week of October, followed by 1400 cases in the first week in November, 2007 [23]. A total of 1450 confirmed cases and 20 deaths were reported from Punjab, 585 confirmed cases and 6 deaths from Karachi, 290 confirmed cases and 11 deaths from Rawalpindi and 30 confirmed cases with 4 deaths from Khyber Pakhtunkhwa [22]. During the 2008 outbreak of Lahore 17 samples were checked through real-time PCR, in which infection of DEN-4 was detected in 10 patients, DEN-2 in 5 patients and DEN-3 in 2 patients respectively [21]. The major dengue outbreak occurred in 2011 in Lahore, Punjab Province. About 20,000 cases of dengue were reported along with 300 deaths [18]. According to Medicalopedia [2012] report, 20864 cases were reported from all over the Punjab. From Lahore alone, 17256 confirmed cases were reported, resulting in 323 deaths in the Province with 279 deaths during 2011 [20]. Muhammad and Rahmat in [19] reported 70 confirmed dengue cases out of 96 suspected cases from Khwazakhela, district Swat [15]. In 2014, 5000 cases with 35 deaths were reported from Sindh out of which 16 deaths were from Karachi alone [24]. With rapid urbanization led to development of innumerable breeding places of *Aedes* mosquitoes not only in Pakistan but also in other tropical and sub-tropical areas of the world. This has resulted in significant increase in incidence of dengue cases [25]. In view of the technical and operational difficulties encountered with the use of synthetic insecticides and their implications on human and environmental health, there is a strong need to find out an alternate sources of mosquito control which are more target-specific, easily biodegradable and effective against mosquitoes. The present study was conducted to scientifically screen the adulticidal activity of three plant extracts including *Azadirachta indica* (Neem tree), *Sesamum indicum* (Til) and *Pinus sabinana* (Pine tree) against the adults of the primary dengue vector (*Aedes aegypti*). To determine efficacy of adulticidal activity of Til oil, Pine oil and Neem oil and most effective phytochemical among them that can be used against the control of adult *Aedes aegypti*.

Materials and Methods

The plant extracts were purchased from market and a Stock solution of different concentration i.e. 2%, 4%, 6%, 8% and 10% was prepared. The Adulticidal efficacy of crude Neem oil extract, Pine oil extract and Till oil extract with five different concentrations i.e. 2%, 4%, 6%, 8% and 10% of each plant oil extracts in acetone was tested against five to six days old sugar feed female of *Aedes aegypti*. The adult percentage mortality was observed after 24 hours and 48 hours under laboratory conditions. *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 while Adults were provided with 10% sucrose solution and albino rats for blood meal. Mosquitoes were reared at 28 °C and 70-85% relative humidity, with a photo period of 12-h light and 12-h dark.

Adulticidal bioassay

The adulticidal activity of each plant extract was checked by using standard World Health Organization (WHO) procedure

[47]. Five to six days old sugar-fed adult female mosquitoes were used. At least 15 female mosquitoes were used in three replicate. The different concentration of plant extracts were impregnated on filter papers by dipping them in stock solution for 5 minutes. A blank paper impregnated with only acetone (solvent) was used as control. The impregnated papers were left to dry at room temperature to evaporate off the acetone overnight. Filter paper impregnated with different concentration i.e. 2%, 4%, 6%, 8% and 10% of plant extracts from stock solution was prepared freshly prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes following the WHO method. One tube served to expose the mosquitoes to the plant extracts and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16 mesh size wire screen. Sucrose-fed five to six days old 15 mosquitoes were released into the tube. At the end of 3 h exposure periods, the mosquitoes were transferred in holding tube. Holding tube was kept in appropriate conditions (25 ± 2 °C and 80% ± 10% relative humidity) for 24 hours and 48 hours. A Cotton pad soaked in 10% sugar solution was placed in the tube during the holding period. Three levels were used to define the activity of extracts. Mortality in the range of 1%-49% indicates low activity; Mortality in the range of 50%-69% indicates moderate activity and Mortality in the range of 70%-100% indicates high activity [26]. The above procedure was carried out in three replicates for each concentration of every plant oil extract. Percentage mortality was recorded after 24 h and 48 h.

Analysis plan

The average adult mortality data were subjected to probit analysis for the calculation of lethal concentration 50 (LC₅₀) and lethal concentration 99 (LC₉₉) using computer software Mini tab. Chi-square was also calculated to check the homogeneity of tested population.

Results

The results on the use of different concentration of plant extracts were recorded in terms of mortality of adult of *Aedes aegypti* under laboratory condition. The results of the adulticidal activity of Neem oil extract against adult *Aedes aegypti* after 24 hours of exposure was observed with mosquito mortality ranging from 26.6% to 48.9% being maximum. The percentage mortality of *Aedes aegypti* after 48 hours of exposure was much higher than 24 hours exposure and ranged between 71% to 100%. 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 with mosquito mortality ranging from 86.7% to 100%. Maximum mortality (100%) was observed at the concentrations of 8% and 10%. The percentage mortality of *Aedes aegypti* after 48 hours of exposure ranged between 88.9% to 100%. Maximum mortality was again observed at higher concentrations i.e. 6, 8 and 10% but Til oil extract was shown significantly lower mortality in the mosquitoes exposed for 24 hours (ranging between 20-40%) as compared to 48 hours exposure (ranging from 73.0-97.8%). Maximum mortality was observed at the concentration of 8% (i.e. 97.8%). The results of Neem oil extract showed that after 24 hours of exposure the LC₅₀ value was 1.17 ml while it was 0.09 ml after 48 hours of exposure. The LC₉₉ value was 4.3 ml after 24 hours and 1.17ml after 48 hours of exposure but in case of Til oil extract LC₅₀ value was 1.171 ml after 24 hours

of exposure and 0.0487ml after 48 hours of exposure. The LC₉₉ value was 4.179 ml after 24 hours and 1.285ml after 48 hours of exposure. Looking at the LC₅₀ and LC₉₉ values it is very clear that the plant extract was highly toxic after 48 hours of exposure. Among the tested plant oil extracts concentration the maximum efficacy was observed in the pine oil extract. The LC₅₀ and LC₉₉ values of pine oil extract against adults of

Aedes aegypti after 24 hour and 48 hour were 0.253, 0.892 ml and 0.0615, 0.643 ml, respectively. No mortality was observed in controls. Results were considered to be statistically significant.

The results of adulticidal activity of acetone extract of Neem oil, Pine oil and Til oil are presented in Table 1.

Oil	Time	Lethal Doses	LFL	ULF	Slope±SE	χ ²	P value	Regression equation	
Neem oil	LC ₅₀	24 h	1.171	0.898	6.713	0.751±0.354	3.441	0.328	Y= -0.88+ 0.75X
		48 h	0.0917	-0.307	0.267	2.150 ± 0.469	6.120	0.106	Y= -0.2 + 2.20X
	LC ₉₉	24 h	4.266	2.559	48.250	0.751±0.354	3.4410	0.328	Y= -0.88+ 0.75X
		48h	1.173	0.976	1.632	2.150 ± 0.469	6.120	0.106	Y= -0.20 + 2.15X
Pine oil	LC ₅₀	24 h	0.253	- 1.969	0.0832	2.029 ± 0.713	2.632	0.452	Y= 0.512+ 0.2.03X
		48 h	0.0615	-1.406	0.156	3.30 ± 1.25	0.38	0.94	Y=0.203+ 3.30X
	LC ₉₉	24 h	0.892	0.689	1.785	2.029 ± 0.713	2.632	0.452	Y=0.512+ 0.2.03X
		48 h	0.643	0.497	1.393	3.30 ± 1.25	0.38	0.94	Y=0.203+ 3.30X
Til oil	LC ₅₀	24 h	1.171	1.0849	6.092	-1.233± 0.378	2.079	0.556	Y=-1.233+ 0.85X
		48 h	0.0487	-0.773	0.200	1.745± 0.457	0.980	0.806	Y=0.0849+ 1.75X
	LC ₉₉	24 h	4.179	2.575	26.594	-1.233± 0.378	2.079	0.556	Y=-1.233+ 0.85X
		48 h	1.285	1.032	2.020	1.745± 0.457	0.980	0.806	Y=0.0849+ 1.75X

Discussion

The growing resistance of *Aedes aegypti* populations to the current commercial pesticides has hampered the efforts to control dengue vector effectively. In addition, other serious problems such as high environmental and toxicity to non-target organism have been flourished by the continuous use of synthetic pesticides. Hence, there has been an increasing interest in the development of alternative methods of mosquito control which are less hazardous to humans and other living organisms. In this regard, plant derived compounds have emerged as good candidates, not only as new effective tools in vector management but also as environmentally safer agents. This study was undertaken to assess the adulticidal properties

of pine oil extract, Neem oil extract and till oil extract against the dengue vector mosquito, *Aedes aegypti*. The results from this study showed that Pine oil extract possessed marked adulticidal activity against adult *Aedes aegypti* after 24 hours of exposure with low LC₅₀ value (0.253), indicating its role as promising adulticides. The other two plant extracts from Neem and till oil exhibited comparatively lower adulticidal activity after 24 hours of exposure with LC₅₀ values of 1.1709, 1.448 respectively. When compared the LC₉₉ values of the three plant extracts after 24 hours of exposure, the LC₉₉ value of Pine Oil was much lower (0.892) as compared to that of the extracts from Neem and Til oil 4.266, 4.179 respectively.

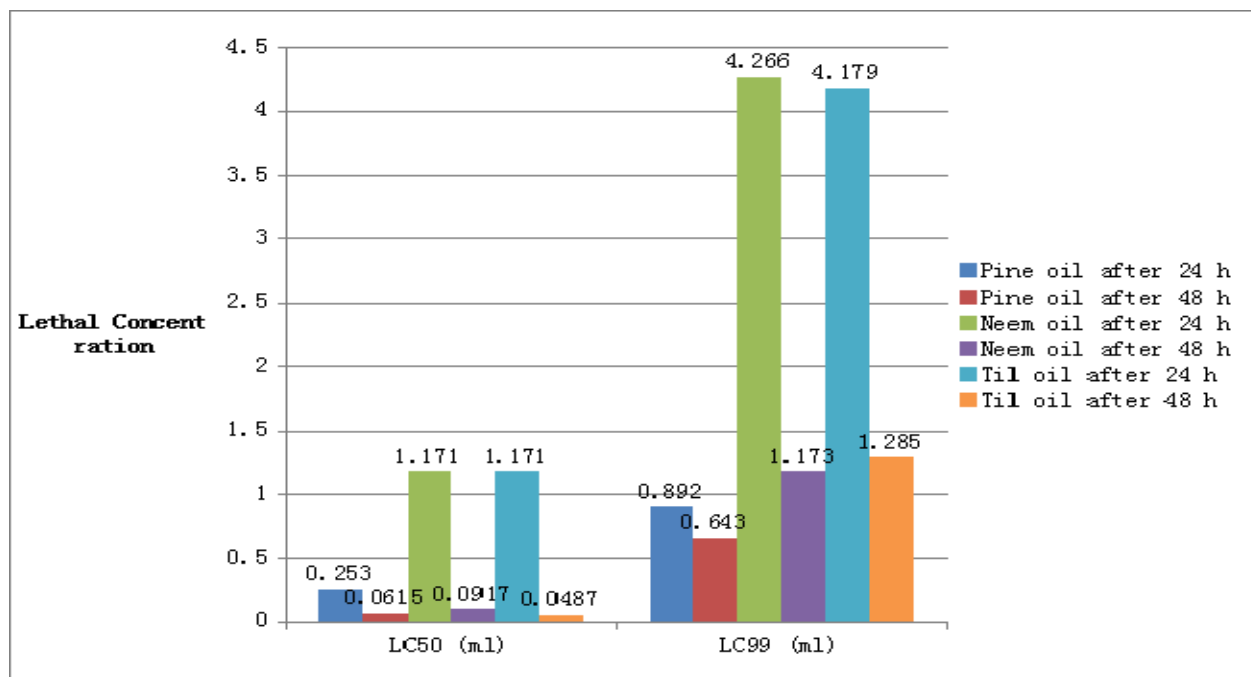


Fig 1: Adulticidal activity of acetone extract of Neem oil, Pine oil and Til oil

The percent mortality of adult *Aedes aegypti* after 24h of exposure under lab conditions showed that Pine oil had the highest percent mortalities i.e. 96 % as compared to Neem oil and Til oil (36.88% and 29.33%) respectively. At higher concentrations, the adult showed restless movement for some

times with abnormal wagging and then died. The rates of mortality were directly proportional to concentration in case of 48 h exposure for Neem and Til oil. The chi-square value are significant at $\alpha < 0.05$ level. No mortality was recorded in control after both exposure periods. In this study Neem and Til

Oil extracts did not show effective adulticidal affect after 24 h exposure period while after 48 h exposure period both plant extracts showed high *Aedes aegypti* mortality as did the Pine oil extract. The L_{c50} and L_{c99} values of Neem and Til Oil extract are presented in the tables given above. The chi-square value are significant at $\alpha < 0.05$ level. No mortality was recorded in control after both exposure periods. Our results are comparable to studies on adulticidal and larvicidal potential of plant extracts carried out by other researchers. Makhaik *et al.*, studied the bioactivity of *Cedrus deodara*, *Eucalyptus citriodora*, *Cymbopogon flexuosus*, *Pinus roxburghii*, *Syzygium aromaticum*, *C. winterianus* and *Tagetes minuta* against *Culex* and *Aedes* mosquitoes. They found that *S. aromaticum* and *C. winterianus* were equally effective with L_{c50} and L_{c99} values at 0.5 and 0.9 % concentration for *Culex* and 1 and 2 % concentration for *Aedes aegypti*. The efficacy sequence was *S. aromaticum* > *C. flexuosus* > *Eucalyptus citriodora* > *C. deodara* > *P. roxburghii* > *T. minuta* [27]. The extracts and compounds from tubers of *Neorautane niimitis* were tested against adult *Anopheles gambiae* mosquitoes and the findings suggested that traditional insecticidal application of *N. mitis* has been effective against *Anopheles gambiae* and can be extended for the control of adult mosquitoes especially the breeding sites [28]. Methanolic extract of leaves *Pinus roxburghii* were tested against the adult of *Anopheles squamosa* on *Culex quinquefasciatus*. The extract formulation produced dose dependent activity, exhibited significantly shorter knock down KD_{50} and KD_{90} values and produced significant mortality. The results suggest the potential mosquitocidal effect of this plant extract against *Anopheles squamosa* and *Culex quinquefasciatus* [29]. The methanol extracts of seven species of *Malaysian tunicates*, the mortality values of the extracts on the adult mosquitoes were dose-dependent and increased with exposure period [30]. In view of the recent increased interest in developing plant-based insecticides as an alternative to chemical insecticides. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on plant source.

Conclusion

Due to the development of resistance to synthetic insecticides, residue problems in the environment and toxic effects on humans and non-target organism, investigators now direct their attention towards the development of new plant based insecticides with less harmful effects. Various compounds including phenolic, terpenoids and alkaloids exist in plants and may jointly or independently contribute to the generation of larvicidal activities of mosquitoes. The results obtained from Pine oil extract used in this study were satisfactory and establish the efficacy. Mortality increases with increase in plant extract concentrations and complete mortality was observed at the concentrations of 8 percent to 10 percent. The lower concentrations were still useful as they showed restless movement for some times with abnormal wagging and then died. Keeping in views the interpretation criteria Neem oil and Til oil considered low adulticidal activity at 24 hours exposure period Pine oil showed high adulticidal activity while after 48 hours exposure period all plant oil extracts showed high adulticidal activity.

Recommendation

The present suggests that the pine oil has the potential of being toxic to *Aedes aegypti* therefore, this plant extract may be tested and further investigated for control of dengue vector mosquitoes under field condition.

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