

Original Article

Adulticidal Activity of *Olea vera*, *Linum usitatissimum* and *Piper nigrum* against *Anopheles stephensi* and *Aedes aegypti* under Laboratory Conditions

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Abstract

Background: There are several plant extractions which are being used for mosquito control. The aim of this study was to evaluate the efficacy of *Olea vera*, *Linum usitatissimum* and *Piper nigrum* against *Anopheles stephensi* and *Aedes aegypti* under laboratory conditions.

Methods: These tests were carried out using WHO recommended bioassay method for adult mosquitoes.

Results: The extracts from black pepper was more effective as adulticide with lowest LC₅₀ values (2.26% and 8.4%) against *Aedes aegypti* and *Anopheles stephensi* after 24 h of exposure while after 48h (1.56% and 5.11%) respectively. In terms of LC₉₀ value black pepper was best with (8.66% and 30.1%) against *Ae. aegypti* and *An. stephensi* after 24 h of exposure while after 48h (4.59% and 17.3%) respectively. In terms of LT₅₀ black pepper took 15 h to kill 50% tested population of *Ae. aegypti* while against *An. stephensi* it took more than 2 days. In terms of percentage mortality black pepper kill 84% of the population of *Ae. aegypti* and 44.75% of the *An. stephensi* population.

Conclusion: Black pepper showed best results in term of LC₅₀, LC₉₀, LT₅₀ and percentage mortality against *Ae. aegypti* and *An. stephensi*. Our study suggested that the plant extracts have potential to kill adult mosquitoes, are environment friendly and can be used for the control of mosquitoes.

Keywords: Adulticide, Plant extracts, *Anopheles stephensi*

Introduction

Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria, Japanese encephalitis, yellow fever, dengue and filariasis (WHO 1997). Globally in 2009 there were 243 million cases and approximately 863000 deaths due to malaria (World Malaria Report 2009) and 50 million cases due to dengue and DHF (Dengue and dengue haemorrhagic fever, 2009) were reported. While in Pakistan there were an estimated 4.5 million suspected malaria cases and 59284 confirmed cases of malaria reported in 2008 (World Malaria Report Pakistan 2009). Totally, 5164 cases of dengue and DHF in 2006 (Weekly mor-

bidity and mortality report, 2006) and 2062 confirmed cases of dengue fever including 15 deaths (Flood response in Pakistan 2010) were reported in 2010.

A primary element in the current global strategy for the control of vector-borne diseases is vector control, chemical control remaining a main component of integrated vector management. Major mosquito-borne diseases, including malaria, dengue, and yellow fever, are reportedly controlled with insecticides (Curtis et al. 2003, Zaim and Jambulinagam 2004). According to a WHO pesticide evaluation (Zaim and Jambulinagam 2004), the main insecticides used against

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malaria and dengue are pyrethroids and organophosphates. Successive changes in the insecticides and overuse resulted in multiple insecticides resistant in malaria vectors (Raghavendra and Subbarao 2002).

Thus, the future of vector control mainly relies on the strategies for the management of existing insecticide resistance in malarial vectors and to limit its further spread. The most important aspect of the management of resistance is to either avoid or delay the onset of resistance by effectively manipulating or influencing the factors responsible for the development of resistance. One of the possible ways of avoiding development of insecticide resistance in field is using non chemical control method, i.e., biopesticides (Amer and Mehlhorn 2006a, b). Therefore, it is the hour to launch extensive search to explore eco-friendly biological materials for control of *Anopheles stephensi* and *Aedes aegypti*.

Plant essential oils in general have been recognized as an important natural resource of insecticides (Adebayo et al. 1999, Gbolade et al. 2000). They have the potential of being acute ovicidal, fumigant, insect growth regulator and insecticidal against various insect species (Tsao et al. 1995) and concurrently being non hazardous to pesticide to eco-system (Isman 2000). Generally they are safe to humans and other mammals (Tripathi et al. 2002–2004).

For this reason, it is necessary to test those plant extracts which could be expected to contain substances to provide adequate efficacy against mosquitoes and are environment friendly (Trongtokit et al. 2008).

Therefore the present study was conducted for the evaluation of botanicals extracts against *An. stephensi* and *Ae. aegypti*.

Materials and Methods

Collection of Plants

Olive (*Olea vera*), Linseed (*Linum usitatissimum*) and Black pepper (*Piper nigrum*)

were collected from Faisalabad (31° 21' 52" N, 72° 59' 40" E) and identified from the Department of Agronomy, University of Agriculture Faisalabad.

Extraction of oil

The seeds were washed, then dried and later grounded in an electric grinder (Anex Germany). The grounded material was put in thimble and kept in extraction tube of Soxhlet apparatus with extractor ID 38mm, extractor volume 85 ml and flask volume 250 ml (Vogel 1978) for the extraction of oil by steam distillation method using Diethyl-ether as solvent (250 ml/20 g sample). The cycle time for one sample was 4–5 h. Solvent was evaporated at room temperature, leaving oil which was then collected.

Preparation of Solution

Small vials were used to collect the oil and the quantity was measured. 1% stock solution was prepared by adding 1 ml of oil from each plant in 99ml of acetone, from which series of concentration (%) were prepared (Murgan et al. 2007).

Collection of Mosquitoes

The immature mosquitoes were collected from different areas of Islamabad (33° 43' 0" N, 73° 4' 0" E) and Rawalpindi (33° 36' 0" N, 73° 2' 0" E) from 2009 and colonized in the insectary of Department of Medical Entomology and Disease Vector Control, Health Services Academy, Islamabad. The larvae were collected by dipping with a standard 400ml dipper (WHO 1975). These were mass reared at 30±2 °C temperature and 70±5% humidity. The larval population was fed on Tetra Min Tropical (Tetra TM). Adults were reared in steel cages and males were provided with 10% sucrose solution, while females were fed on blood of white rats (Shalan et al. 2006). The gravid females were allowed to lay eggs on black plastic gauze that was placed in Petri dishes. The

eggs were separated and on emergence of larvae in petri dishes were shifted to rearing trays.

Adulticidal Bioassay

The extracted oils were evaluated by standard WHO method (WHO 2006). Different concentrations (2%, 4%, 6%, and 8%) of oils were applied on the Whatman # 1 filter paper and control was treated with acetone only. 15–25, 12 hrs old female mosquitoes were introduced in WHO insecticide testing kit in holding tube and remain there for 1 h to acclimatize. After 1 h mosquitoes were transferred in exposure tube, data was collected after 1 h, 24 and 48 h.

Data analysis

Abbott's formula (Abbott 1925) was used for corrected mortality and the data so obtained was analyzed by Probit analysis (Finney 1989) dose and time mortality regression lines were calculated by using MANITAB-15 software.

Results

Colonies of *Ae. aegypti* and *An. stephensi* were maintained in our insectary, males 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 liver diet. Our studies on the bio-potentials of seed extracts have yielded results that they can be tested for application on large scale against *Ae. aegypti* and *An. stephensi*. The adults were tested against the four selected concentrations of these extracts. The data so obtained has been elaborated in results and is being explained in light of previous findings.

Table 1 indicated the LC₅₀ and LC₉₀ values of black pepper, olive and linseed against *Ae. aegypti* and *An. stephensi*. Results showed that oils from black pepper and olive was considered best with LC₅₀ (2.26 and 2.95) respectively, after 24 h of exposure and re-

garded more toxic to *Ae. aegypti*, followed by linseed (12.90), After 48 h of exposure black pepper and olive had the lowest LC₅₀ value (1.56 and 1.77) respectively, followed by linseed (20.25).

Oils from black pepper and linseed had the lowest LC₉₀ value (8.66 and 27.60) respectively, after 24 h of exposure, followed by olive (38.55), While after 48 hours of exposure black pepper and olive seed had the lowest LC₉₀ value (4.59 and 10.29) respectively, followed by linseed (128.91) against *Ae. aegypti*.

Oils from black pepper and linseed was considered best with LC₅₀ (8.14 and 13.51) respectively, after 24 h of exposure and regarded more toxic to *An. stephensi* followed by olive (21.02), After 48 h of exposure black pepper and linseed had the lowest LC₅₀ value (5.11 and 7.16) respectively, followed by olive (11).

Oils from black pepper and linseed had the lowest LC₉₀ value (30.12 and 94.81) respectively, after 24 h of exposure, followed by olive seed (347.12), While after 48 h of exposure black pepper and linseed had the lowest LC₉₀ value (17.26 and 56.61) respectively, after 48 h of exposure, followed by olive seed (237.59) against *An. stephensi*.

Percent mortality of *Ae. aegypti* larvae after 24 h of exposure under lab conditions showed that black pepper and linseed had the highest percent mortalities (71.25% and 57.50%) respectively, followed by olive (8.25%), While after 48 hours black pepper and linseed had the highest percent mortality (84.75% and 72.75%) respectively, followed by olive (15.5%) as shown in Fig. 1.

According to Fig. 2 percent mortality of *An. stephensi* larvae after 24 h of exposure under lab conditions showed that black pepper and olive had the highest percent mortalities (29.75% and 24.5%) respectively, followed by linseed (24%), While after 48 h black pepper and olive had the highest percent mor-

tality (44.75% and 38.5%) respectively, followed by linseed (35%).

In terms of lethal time (Table 2) to kill 50% population of *Ae. aegypti*, black pepper and linseed took minimum time to kill 50% of population i.e., 14.98 and 21.36 h, followed by olive (246.08 h).

In terms of lethal time (Table 2) to kill 50% population of *An. stephensi*, black pepper and olive took minimum time to kill 50% of population i.e., 53.69 and 68.16 h, followed by linseed (78.04).

Table 1. Adulticidal effect of plant extracts against *Ae. aegypti* and *An. stephensi*

Treatments	Time (h)	LC ₅₀ (%age)	LC ₉₀ (%age)	Slope±SE	LC ₅₀ (%age)	LC ₉₀ (%age)	Slope±SE
		<i>Ae. aegypti</i>			<i>An. stephensi</i>		
Black pepper	24	2.26	8.66	0.96±0.20	8.4	30.1	0.95±0.21
	48	1.56	4.59	1.18±0.25	5.11	17.3	1.05±0.20
Olive	24	2.95	38.6	0.49±0.18	21.02	347	0.45±0.20
	48	1.77	10.3	0.72±0.19	11	238	0.41±0.18
Linseed	24	12.9	27.6	1.68±0.57	13.51	94.8	0.62±0.21
	48	20.25	129	0.69±0.24	7.16	56.6	0.62±0.91

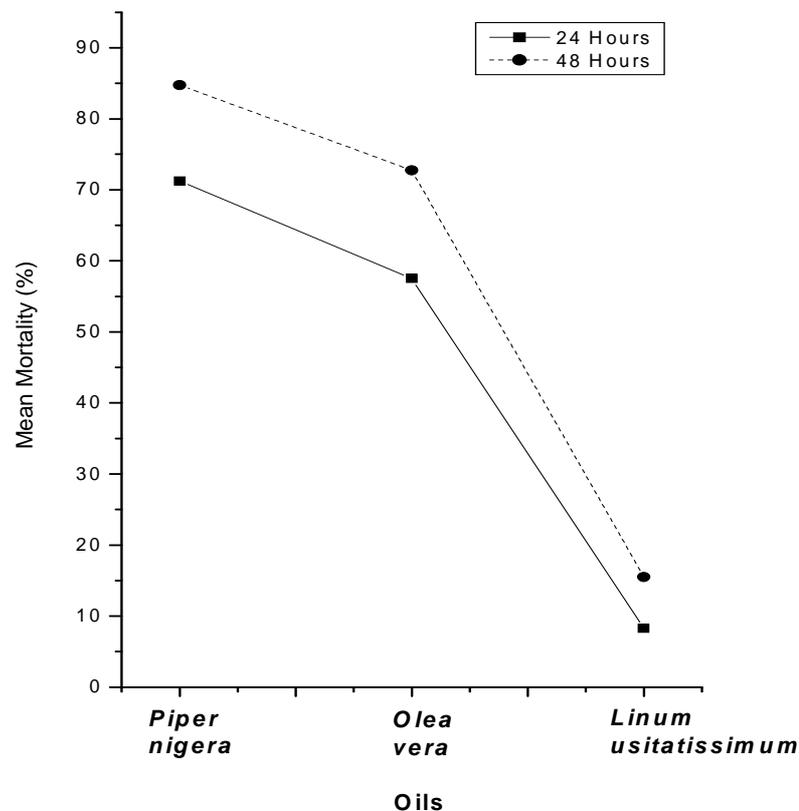


Fig. 1. Mortality of plant extracts against adult of *Ae. Aegypti*

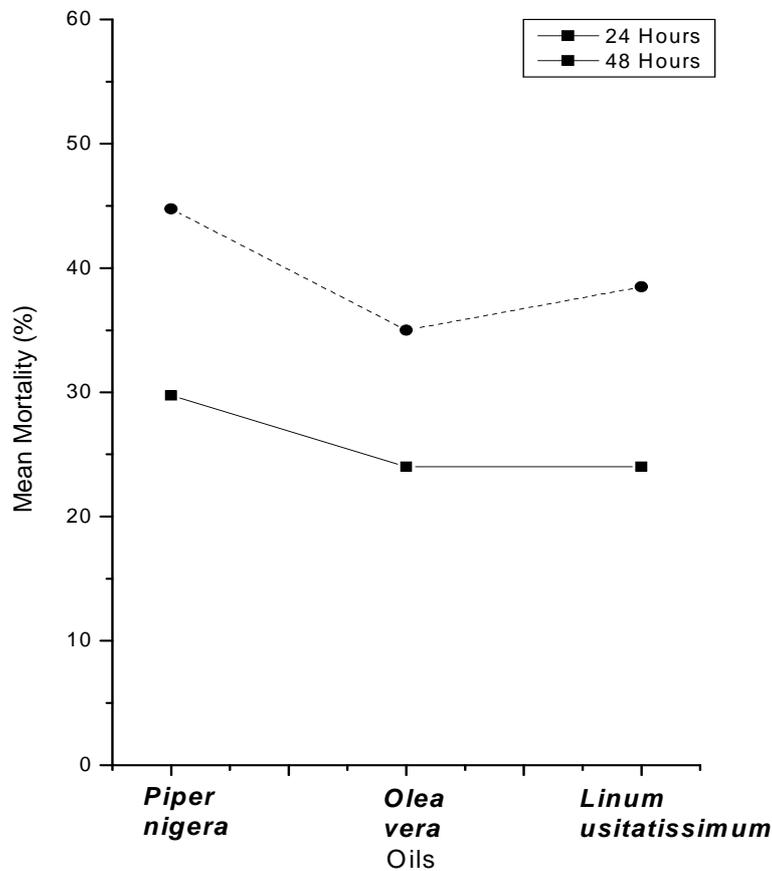


Fig. 2. Mortality of plant extracts against adult of *An. stephensi*

Table 2. Time mortality response of *Ae. aegypti* and *An. stephensi* against plant extracts

Treatments	LT ₅₀ (h)	Slope±SE	LT ₅₀ (h)	Slope± SE
	<i>Ae. aegypti</i>		<i>An. stephensi</i>	
Black pepper	15	0.99±0.11	53.7	0.72±0.12
Olive	21.4	0.88±0.10	78	0.65±0.11
Linseed	246	0.59±0.18	68.2	0.70±0.13

Discussion

Our results indicate that Black pepper seed extracts have good adulticidal potential against *Ae. aegypti* and *An. stephensi* as the most effective in terms of LC₅₀, LC₉₀, LT₅₀ and percent mortalities.

Our results that plant extracts have the potential were supported by scientists like Choochote et al. (2004) reported that crude

seed extract of celery, *Apium graveolens* extract exhibited a slightly adulticidal potency with LD₅₀ and LD₉₅ values of 6.6 and 66.4 mg/cm², respectively. The extracts and compounds from tubers of *Neorautanenia mitis* against adult *An. gambiae* mosquitoes showed good adulticidal effects and the results can be extended for the control of mosquitoes

especially at breeding sites (Joseph et al. 2004). Methanolic extract of leaves of *Annona squamosa* against the adult of *Culex quinquefasciatus* the extract showed dose dependent activity, exhibited significantly shorter knock down KD_{50} and KD_{90} values and produced significant mortality (Jaswanth 2002). 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 (Hussain et al. 2001). Investigation of the insecticidal activity of essential oil isolated from the leaves of *Lantana camara* against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis* and *An. stephensi* respectively, KDT_{50} of the oil were 20, 18, 15, 12, and 14 min and KDT_{90} values were 35, 28, 25, 18, 23 min against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis* and *An. stephensi*, respectively on 0.208 mg/cm² impregnated paper (Dua 2010). Akram et al. (2010) and Hafeez et al. (2011) investigated the seed extract of citrus cultivars against the *Ae. albopictus* larvae and found satisfactory results. Sulaiman et al. (2008) evaluated the bifenthrin and *Acorus calamus* extract against *Ae. aegypti* and *Ae. albopictus* and found bifenthrin as best.

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References

- Abbot SW (1925) A method of competing the effectiveness of an insecticide. *J Econ Entomol.* 18: 265–267.
- Adebayo TA, Gbolade AA, Olaifa JI (1999) Comparative study of toxicity of essential oils to larvae of three mosquito species. *Nigerian J Nat Prod Med.* 3: 74–76.
- Akram W, Khan HAA, Hafeez F, Bilal H, Kim YK, Lee JJ (2010) Potential of citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pak J Bot.* 42(4): 3343–3348.
- Amer A, Mehlhorn H (2006a) Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol Res.* 99: 478–490.
- Amer A, Mehlhorn H (2006b) The sensilla of *Aedes* and *Anopheles* mosquitoes and their importance in repellency. *Parasitol Res.* 99: 491–499.
- Choochote W, Benjawan T, Duangta K, Eumporn R, Udom C, Prasong C, Atchariya J, Pongsri T, Doungkrat, Benjawan (2004) Potential Of crude seed extract of celery, *Apium graveolens* L., against the Mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *J Vector Ecol.* 29: 340–346.
- Curtis CF, Jana-Kara B, Maxwell CA (2003) Insecticide treated nets: impact on vector populations and relevance of initial intensity of transmission and pyrethroid resistance. *J Vect Borne Dis.* 40: 1–9.
- Dengue and dengue haemorrhagic fever. Geneva, World Health Organization, March (2009), Fact sheet N°117.
- Dua VK, Pandey AC, Dash AP (2010) Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J Med Res.* 131: 434–439.
- Finney DJ (1989) Probit analysis, 3rd ed. Cambridge Great Britain University press, UK.
- Flood response in Pakistan. *Weekly Epidemiological Bulletin* (2010). 1(10): 6.

- Gbolade AA, Oyedele AO, Sosan MB, Adewoyin FB, Soyelu O (2000) Mosquito repellent activities of essential oils from two Nigerian *Ocimum* species. *J Trop Med Plants*.1: 146–148.
- Guidelines for testing mosquito adulticides for Indoor residual spraying and treatment of mosquito nets. Geneva, World Health Organization, (2006) (WHO/CDS/NTD/WHOPES/GCDPP).
- Hafeez F, Waseem A, Essam S (2011) Mosquito larvicidal activity of citrus limonoids against *Aedes albopictus*. *Parasit Res*. 109(1): 221–229.
- Isman MB (2000) Plant essential oils for pest and disease management. *Crop Prot*. 19: 603–608.
- Jaswanth A, Ramanathan P, Ruckmani K (2002) Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say. *Indian J Experimental Biol*. 40(3): 363–365.
- Joseph CC, Malima RC, Mohd-Hussein ZA, Jantan I, Awaludin A, Wasi AN (2001) Insecticidal activity of the methanol extracts of Some tunicate species against *Aedes aegypti* and *Anopheles maculatus*. *Pharm Biol*. 39(3): 213–216
- Murgan K, Murgan P, Noortheen A (2007) Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilium* Linn against dengue vector, *Aedes aegypti*. *Biores Technol*. 98: 198–201.
- Ndoile MM, Nkunya MHH (2004) Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. *Transac. Royal Soc Trop Med Hygiene*. 98(8): 451–455
- Raghavendra K, Subbarao SK (2002) Chemical insecticides in malaria vector control in India. *ICMR Bulletin*. 32(10): 1–7.
- Rozendaal JA (1997) Vector Control-Methods for Use by Individuals and Communities. Geneva, World Health Organization.
- Shaalan EAS, Canyon DV, Bowden B, Younes MWF, Wahab HA, Mansoin AH (2006) Efficacy of botanical extracts from *Callitris glaucophylla* against *Aedes aegypti* and *Culex annulirostris* mosquitoes. *Trop Biomed*, 2: 180–185.
- Sulaiman S, Abang Kamarudin DSF, Othman H (2008) Evaluation of Bifenthrin and *Acorus calamus* Linn. Extract against *Aedes aegypti* L. and *Aedes albopictus* (Skuse). *Iran J Arthropod-Borne Dis*. 2(2): 7–11.
- Tripathi AK, Prajapati V, Verma N, Bahl JR, Bansal RP, Khanuja SP, Kumar S (2002) Bioactivities of the leaf essential oil of *Curcuma Longa* (var. ch-66) on three species of stored-product beetles (Coleoptera). *J Econ Entomol*. 95: 183–189.
- Tripathi AK, Veena P, Ateeque A, Kishan KA, Suman PSK (2004) Piperitenone oxide as toxic, repellent and reproduction retardant toward malarial vector *Anopheles stephensi* (Diptera: Anophelinae). *J Med Entomol*. 41(4): 691–698.
- Trongtokit Y, Burivong P, Moore S, Hill N, Curtis CF (2008) Plant extracts in prevention of mosquito attacks. *Botanical medicine in clinical practice*. pp. 807–813. ISBN (978-1-84593-413-2)
- Tsao R, Lee S, Rice PJ, Jensen C, Coats JR (1995) Monoterpenoids and their synthetic derivatives as leads for new insect control agents. In: Baker, DR, Fenyves, JG, Basarab, GS (Eds), *Synthesis and Chemistry of Agrochemicals IV*. American Chemical Society, Washington, DC. pp. 312–324.
- Vogel AI (1978) *Text Book of Practical Organic Chemistry*. The English language Book Society and Longman, London.

Weekly morbidity and mortality report. Epidemiological Week No. 46 (11–17 Nov 2006). 23 Nov 06/ Vol. 52/ DEWS 2006-46.

WHO (World Health Organization) (1975) Manual on practical Entomology in Malaria, World Health Organization. Prepared by the WHO Division of Malaria and other parasites diseases. Part 1–2. Offset publication No.13. World Health Organization, Geneva.

World Malaria Report Pakistan. Geneva, World Health Organization (2009). pp 135.

World Malaria Report. Geneva, World Health Organization (2009). pp 37.

Zaim M, Jambulingam P (2004) Global insecticide use for vector borne disease control. World Health Organization Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme (WHOPES).