Original Article

Adulticidal Activity of *Olea vera*, *Linum usitatissimum* and *Piper nigera* 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 nigera* 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 LC_{50} 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 LC_{90} 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 LT_{50} 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 LC_{50}, LC_{90}, LT_{50} 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 (Rozendaal 1997). Globally in 2009 there were 243 million cases and approximately 863000 deaths due to malaria (WHO 2009a) and 50 million cases due to dengue and DHF (WHO 2009b) were reported. While in Pakistan there were an estimated 4.5 million suspected malaria cases and 59284 confirmed cases of malaria reported in 2008 (WHO 2009a). Totally, 5164 cases of dengue and DHF in 2006 (Weekly morbidity and mortality report 2006) and 2062 confirmed cases of dengue fever including 15 deaths (WHO 2009b) 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 ever, 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 malaria and dengue are pyrethroids and organophosphates. Successive changes in the

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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 nigera*) 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 Soxhelt apparatus with extractor ID 38 mm, extractor volume 85 ml and flask volume 250 ml (Vogel 1978) for the extraction of oil by steam distillation method using Diethylether 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 400 ml 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 (Shaalan 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

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of larvae in petri dishes were shifted to rearing trays.

Aduticidal 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 LC_{50} and LC_{90} 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 LC_{50} (2.26 and 2.95) respectively, after 24 h of exposure and regarded more toxic to *Ae. aegypti*, followed by linseed (12.90), After 48 h of exposure black pepper and olive had the lowest LC_{50} value (1.56 and 1.77) respectively, followed by linseed (20.25).

Oils from black pepper and linseed had the lowest LC_{90} 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 LC_{90} 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 LC_{50} (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 LC_{50} value (5.11 and 7.16) respectively, followed by olive (11).

Oils from black pepper and linseed had the lowest LC_{90} 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 LC_{90} 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 mortality (44.75% and 38.5%) respectively, followed by linseed (35%).

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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
		Ae. aegypti			***An. stephensi***		
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

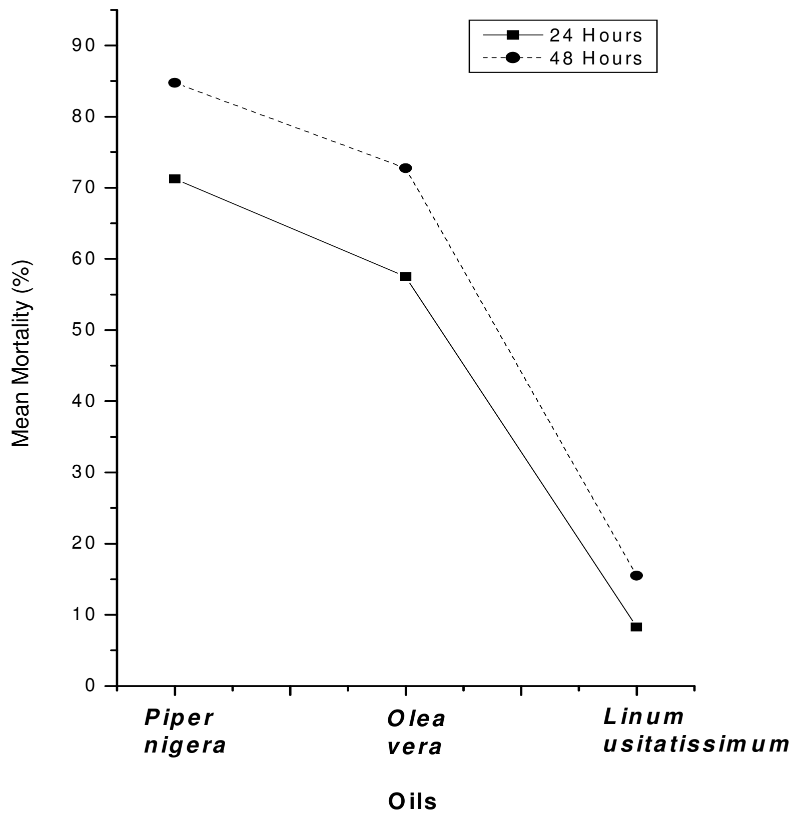

Fig. 1. Mortality of plant extracts against adult of *Ae. aegypti*

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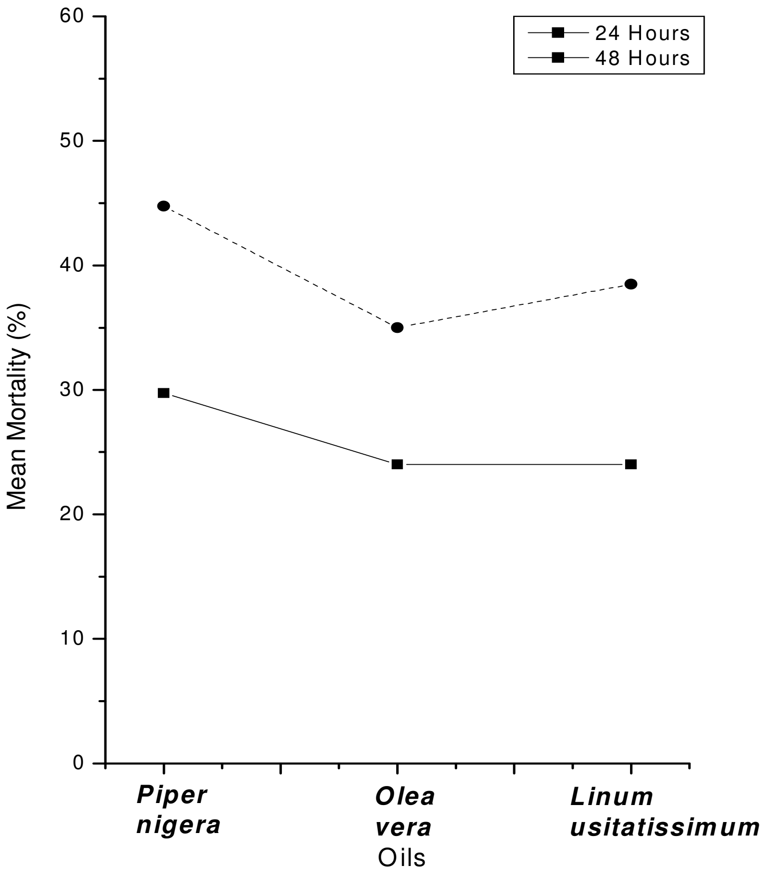

Fig. 2. Mortality of plant extracts against adult of *An. stephensi*

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
	Ae. aegypti		*An. stephensi*	
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 LC_{50}, LC_{90}, LT_{50} 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 LD_{50} and LD_{95} 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

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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. fluvialitis* 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/cm^2 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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