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POTENTIAL OF CITRUS SEED EXTRACTS AGAINST DENGUE FEVER MOSQUITO, *AEDES ALBOPICTUS* (SKUSE) (CULICIDAE: DIPTERA)

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Abstract

Citrus seeds and peel contain certain compounds with varied level of bitterness. These compounds have been tested against insects and proved to be effective. The present study was therefore carried out to test the citrus seed extracts from 10 varieties against 4th instar larvae of dengue fever mosquito, *Aedes albopictus* (Skuse). The results indicate that the extracts from rough lemon and lemon were more effective as larvicides with lowest LC_{50} (119.993 and 137.258 ppm respectively, after 24h of exposure and 108.85 and 119.853 ppm respectively, after 48h of exposure) and LT_{50} values (2.51 and 4.91h, respectively). Seed extracts from remaining citrus varieties were less active at lower doses; however at higher doses these were biologically active against *Ae. albopictus.* Our study has suggested that citrus-seed extract are environment friendly and can be used for managing *Ae. albopictus* larvae.

Introduction

Mosquitoes are important arthropods, transmitting diseases like malaria, filariasis, Japanese encephalitis and dengue (Service, 1983) and have potential to feed on more than one individual, during a single gonotropic cycle (Mackenzie *et al*., 2004). Proper control of mosquitoes lies in personal protection and public awareness as the most economical method in eradicating breeding sites and controlling these through environment friendly larvicides (Corbel *et al*., 2004). *Aedes albopictus* which is an increasingly important disease vector (Gratz, 2004) including dengue fever (Yang *et al*., 2009), has spread with the growth of towns and poor sanitation (Anon., 1982).

The drastic effects of synthetic insecticides in the environment have received wide public apprehension (St. Lager *et al*., 1996), however its misuse in agriculture and public health programs has caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms (Sarwar *et al*., 2009). To alleviate these problems, major emphasis has been on the use of natural plant based products as larvicides which can provide an alternate to synthetic insecticides (Junwei *et al*., 2006). In general, plant essential oils are important natural alternatives to insecticides (Gbolade *et al*., 2000). Many plants have been found to contain chemicals which are helpful for the control of insects (Robert, 2001) and are useful for field applications in mosquito control programmes (Kalyanasundaram & Das, 1985). Limonoids from Rutaceae particularly citrus (Klocke & Kubo, 1982) have attracted greater apprehension due to their growth regulating activities (Champage *et al.,* 1992). Citrus which includes limonin, nomilin, obacunone, epilimonol and limonin diosphenol (Liu *et al*., 1990) can readily be extracted from citrus seeds, available in large quantities as waste products of the citrus industry (Murray *et al.,* 1995). Citrus limonoids

work both as toxicant and feeding deterrents. In structure-activity studies of limonin, it has been determined that the furan ring and epoxide groups in the citrus limonoid structure are critical for the antifeedant activity of the limonoids (Mendel *et al*., 1991). Limonoids affect the egg laying process of insects due to nutritional disruption which ultimately induce antifeedant effects (Murray *et al*., 1995).

With the growing environmental concerns and regular out break of disease since the first record of dengue from Karachi in 1994 (Chan *et al*., 1995), the present study as therefore been conducted to find best options. Citrus farming is perhaps one of the most important commercial and industrial agricultural activities of the world and Pakistan is one of the most important countries amongst citrus fruit producer. So we selected different citrus varieties and their seed extracts were tested against one of the important dengue fever vector, *Aedes albopictus*.

Materials and Methods

Collection of mosquitoes: Immature mosquitoes were collected from Faisalabad by a standard 375ml dipper (Anon., 1975) during 2007-2008. The larvae were kept at 28±2^{o}C temperature and 65±5% relative humidity in the laboratory for adult emergence. The *Ae. albopictus* were later mass reared and their larval stages were fed on Tetra-Min Tropical (Tetra TM). Adults were kept in plastic cages where they were provided 10% sucrose solution (Osghai *et al*., 2003). 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 of larvae these were shifted to rearing trays.

Collection of fruits and extraction of citrus oil: Citrus varieties viz., Brazilian sour orange (*Citrus aurantium*), chakutra (*Citrus grandis*), galgal (*Citrus pseudolimon),* grape fruit (*Citrus paradisi*), kinnow (*Citrus reticulata*), lemon (*Citrus limon*), musambi (*Citrus sinensis*), narangi (*Citrus mitis*), red blood orange (*Citrus sinensis*) and rough lemon (*Citrus jambhiri*) were collected from Punjab. The seeds were separated manually and washed with distilled water to remove the pulp and then dried for 48 hours at 60^{o}C and later ground in an electric grinder (Anex Germany). The grounded material was put in Soxhlet apparatus for the extraction of oil by steam distillation method (Vogel, 1978), using di-ethyl ether as a solvent.

Preparation of solution: Oil was collected in small vials and the quantity was measured. Stock solutions were prepared by adding 1 ml of oil from each variety in 100ml of acetone that was considered as 1% stock solution from which series of concentrations (ppm) were prepared (Murugan *et al*., 2007).

Bioassay: The extracted oils were used in six different concentrations (300, 400, 500, 600, 700, 800 ppm). For control treatments, 1 ml of acetone was mixed with 199 ml of distilled water. There were three replicates for each treatment; each replicate containing 200ml of the oil solution placed in 250ml glass beakers. Thirty early fourth instar larvae of the *Ae. albopictus* were exposed in each beaker containing oil solution (Mohtar *et al.,* 1999). The experiment was conducted using CRD under lab conditions of 28± 2^{o}C and 65± 5% relative humidity. The numbers of dead larvae were counted after 24, 48 and 72 hours of exposure.

Data analysis: Mortality data, if needed, was corrected by Abbot's formula (Abbot, 1925) and the data was analyzed by Probit analysis (Finney, 1971), using POLO-PC software (LeOra Software, 1987) for dose and time mortality regression lines. Significant differences were concluded by non-overlapping of 95% fiducial limits. Data on percent mortalities were analyzed with Statistix version 8.1 (Anon., 2005) and means were compared with least significant difference (LSD) test at 5% level of significance.

Results and Discussion

In our experiment, different citrus seed extracts were subjected to laboratory bioassay against *Ae. albopictus* larvae. These extracts provided satisfactory results as far as the susceptibility and time limit is concerned. Oils from all the citrus varieties showed strong larvicidal effects. Table 1 shows that out of the total citrus seed extracts tested, rough lemon and lemon had the lowest LC_{50} values (119.993 and 137.258 ppm, respectively), particularly after 24 hours of exposure, followed by red blood orange (295.630 ppm), chakutra (334.874 ppm), galgal (644.250 ppm), brazilian sour (905.96 ppm) and kinnow (1022.674 ppm). Narangi had the highest LC_{50} value (2069.117 ppm) after 24h of exposure followed by grape fruit (1598.15 ppm) and musambi (1389.162 ppm). After 48 hours of treatment, rough lemon and lemon had the lowest LC_{50} values (108.85 and 119.853 ppm respectively), followed by narangi (166.598 ppm), galgal (177.441 ppm), red blood orange (293.225 ppm), brazilian sour (322.36 ppm) and chakutra (342.086 ppm). Musambi had the highest LC_{50} values (587.093 ppm), followed by kinnow (532.159 ppm) and grape fruit (506.775 ppm). The χ^2 values for larval mortality tests show no heterogeneity in all the experiment.

Table 1. LC_{50} values of citrus seed extracts against 4th instar larvae of *Aedes albopictus*

Citrus extracts	Observation (h later)	LC_{50*} (ppm)	95% FL	Slope ± S.E	χ^2 (df=2)
Brazilian sour	24	905.96	701.12-2306.91 bc	2.23 ± 0.723	0.31
(*Citrus aurantium*)	48	322.36	75.95-427.689 ab	1.75 ± 0.667	0.61
Chakutra	24	334.874	169.62-429.831 ab	4.07 ± 1.169	0.86
(*Citrus grandis*)	48	342.086	200.08-418.633 ab	6.71 ± 1.943	0.71
Galgal	24	644.250	373.110-1266 b	1.58 ± 0.762	2.99
(*Citrus pseudolimon)*	48	177.441	15.497-279.418 ab	2.43 ± 0.876	0.48
Grape fruit	24	1598.15	949.700-2476 cd	1.88 ± 0.8040	0.50
(*Citrus paradisi*)	48	506.775	479.99-814.387 b	2.67 ± 0.783	2.50
Kinnow	24	1022.674	672.302-1702 bc	1.45 ± 0.731	0.16
(*Citrus reticulata*)	48	532.159	353.01-758.931 b	2.01 ± 0.729	0.07
Lemon	24	137.258	1.171-246.258 a	2.17 ± 0.878	0.56
(*Citrus limon*)	48	119.853	0.033-225.080 a	2.83 ± 1.249	1.76
Musambi	24	1389.162	904.219-2089 bc	2.13 ± 0.810	0.30
(*Citrus sinensis*)	48	587.093	441.98-1060.24 b	1.70 ± 0.661	0.11
Narangi	24	2069.117	1092.67-2290.64 d	2.08 ± 0.949	0.87
(*Citrus mitis*)	48	166.598	22.381-258.699 a	2.65 ± 0.890	3.39
Red blood	24	295.630	215.944-347.689 ab	4.52 ± 0.899	0.65
(*Citrus sinensis*)	48	293.225	239.439-326.732 ab	7.86 ± 1.651	1.75
Rough lemon	24	119.993	0.060-221.409 a	3.03 ± 1.327	4.11
(*Citrus jambhiri*)	48	108.85	65.60-153.09 a	2.53 ± 0.89	3.20

*LC_{50} i.e., lethal concentration (ppm) to kill 50% population of the subjected organism

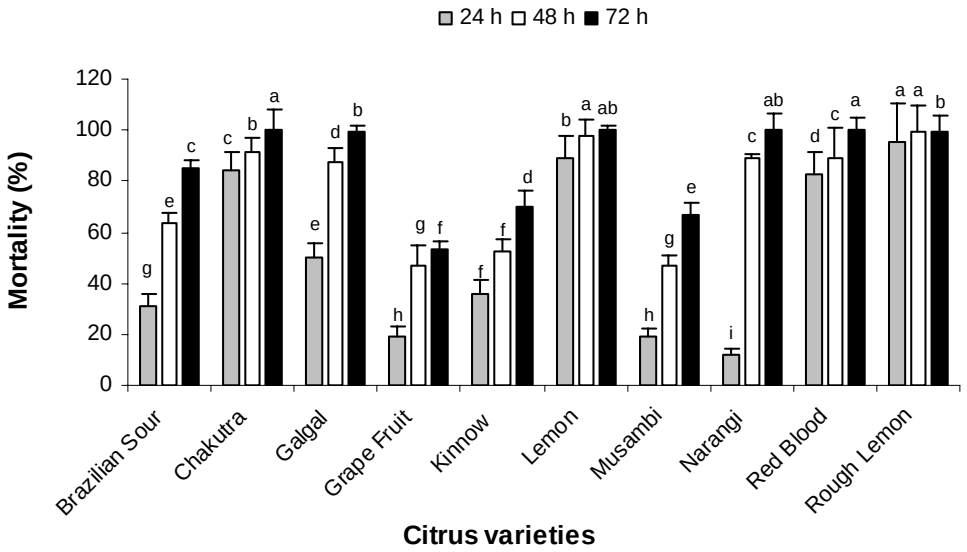

Fig. 1. Bars are mean percent mortality (± S.E.) at different time intervals. Bars sharing the same letters are not significantly different at the 5% level of significance (Least significant difference [LSD] test, Statistix 8.1).

Rough lemon and lemon showed highest percent mortalities (95.6 and 88.9% respectively), after 24 hours of exposure, followed by chakutra (84.4%) and red blood orange (82.8%) while narangi gave lowest percent mortality (11.7%). Rough lemon and lemon also showed highest percent mortalities (99.6 and 97.7%, respectively) after 48 hours of exposure followed by chakutra (91.6%) while musambi and grape fruit gave lowest percent mortalities (46.7 and 47.2%, respectively). After 72 hours of exposure, chakutra, galgal, lemon, narangi, red blood orange and rough lemon showed almost 100% mortalities (Fig. 1).

In terms of lethal time to kill 50% population of subjected organism, rough lemon and lemon took minimum time to kill 50% population i.e., 2.51 and 4.91hours, followed by red blood, chakutra and galgal (8.52, 18.90 and 26. 25 hours, respectively). Kinnow and musambi took almost two days (50.25 and 52.70 hours respectively) to kill 50% population while grape fruit took longest time (67.18 hours) to cause 50% mortality (Table 2).

The χ^2 values for larval mortality tests show no heterogeneity in all the experiment.

The results of the present study are in agreement with the scientists who worked with different plant extracts against *Ae. albopictus*. The extracts from peel of different citrus varieties (Mwaiko, 1992) also had good larvicidal potential with lemon peel oil as the best (Mwaiko & Savaeli, 1994) and are safe for human beings Murgun *et al.,* (2007) traced out the larvicidal and repellent activities of *Albizzia amara* and *Ocimum basilicum* against *Ae. albopictus* at different concentrations. The extracts of five medicinal plants i.e., *Abutilon indicum, Aegle marmelos, Euphorbia thymifolia, Jatropha gossypifolia* and *Solanum torvum* were prepared separately by using ethyl acetate, acetone, crude hexane, petroleum ether and methanol and were checked for their toxicity against the early fourth instar larvae of *Culex quinquefasciatus.* Petroleum ether extract of *Abutilon indicum* gave the highest larval mortality (Chansang *et al*., 2005). The plant derivatives are probable sources of some biologically active agents for mosquito control in the future (Mathur, 2003). Our results indicate that citrus seed extracts have good larvicidal potential against *Ae. albopictus* larvae with rough lemon and lemon being the most effective in terms of LC_{50}, LT_{50} and percent mortalities. So we suggest that citrus seed extracts as well as other plant extracts should be investigated for the control of dengue mosquitoes. This study, however, was conducted in the laboratory and the efficacy of citrus extracts against *Ae. albopictus* should now be examined in the field.

Table 2. Time mortality response of *Aedes albopictus* larvae against citrus seed extracts.

Citrus extracts	Time mortality response*			
	LT_{50}	95 % FL	Slope ± SE	χ^2
Brazilian sour (*Citrus aurantium*)	35.28	32.031-38.378c	3.21 ± 0.30	1.01
Chakutra (*Citrus grandis*)	18.90	4.693-31.382 ab	2.18 ± 0.60	0.83
Galgal (*Citrus pseudolimon)*	26.25	21.240-30.607 b	3.34 ± 0.39	3.50
Grape fruit (*Cirus paradisi*)	67.18	54.291- 83.084 d	2.40 ± 0.47	3.83
Kinnow (*Citrus reticulata*)	50.25	36.674-63.420 cd	2.17 ± 0.42	0.87
Lemon (*Citrus limon*)	4.91	0.407-10.322 a	1.6 ± 0.47	0.01
Musambi (*Citrus sinensis*)	52.70	46.092-59.745d	2.87 ± 0.37	0.04
Narangi (*Citrus mitis*)	37.15	35.140 -39.166c	6.02 ± 0.40	1.83
Red blood (*Citrus sinensis*)	8.52	3.139-14.215 a	1.72 ± 0.36	0.05
Rough lemon (*Citrus jambhiri*)	2.51	0.021-7.919 a	1.34 ± 0.40	0.94

*LT_{50} data recorded within an extract treatment denoting the same letters are not different at the 5% level of significance. FL, fiducial limit is the upper and lower limits of respective LT_{50} values.

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