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Toxicity of Selected Indigenous Plant Extracts against *Aedes albopictus* (Diptera: Culicidae): A Potential Dengue Vector in Dengue Positive Areas

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Abstract.- The present study aims at assessing the larvicidal potential of essential oils from some indigenous plants against larvae of *Aedes albopictus* (Diptera: Culicidae), a dengue vector in dengue positive areas. Results revealed that after 24, 48 and 72 h of exposure to essential oils, musambi (*Citrus sinensis* L. var. musambi) caused highest mortality (64.32%, 76.05% and 89.25, respectively) followed by eucalyptus (*Eucalyptus camaldulensis* L.) (60.93%, 72.78% and 85.49, respectively). *Ae. albopictus* larvae showed highest susceptibility, both in terms of LC_{50} and LT_{50}, to musambi (*Citrus sinensis* L. var. musambi) essential oil after 24 h, but were highly and equally susceptible to essential oils from darchini (*Cinnamomum cassia*), musumbi (*Citrus sinensis* L. var. musambi), bakhra (*Tribulus terrestris* L.), eucalyptus (*Eucalyptus camaldulensis* L.) and succari (*Citrus sinensis* L. var. succari) after 48 and 72 h. Results are discussed with possible mode of action of plant extracts in mosquitoes.

Key words: Plant extracts, larvicide, dengue vector, *Aedes albopictus*.

INTRODUCTION

Although some vector borne diseases such as Japanese encephalitis and yellow fever have been reasonably brought under control by vaccination, however, no effective vaccine is available yet for dengue (Pitasawat *et al.*, 2007). Therefore, the only effective approach to minimize the incidence of these diseases is to eradicate and control mosquito vectors mainly by applying insecticides to larval habitats, destroying unwanted containers and enhancing public awareness (Corbel *et al.*, 2004). Organophosphorus compounds such as temephos, fenthion and chlorpyrifos, are commonly used larvicides against mosquitoes and other aquatic insects. However, because of the known hazardous effects of chemical insecticides (Din *et al.*, 2011), major consideration has been shifted steadily on the use of plant based products as larvicides or adulticides which can provide alternatives to synthetic chemicals (Junwei *et al.*, 2006). Plants serve as one of the most important sources of several compounds which possess potential insecticidal or repellent properties and are free from harmful effects (Isman, 1995). Many plant products have been explored against mosquitoes either as larvicides and/or adulticides or as adult repellents (Sukumar *et al.*, 1991). In the present study we explored the larvicidal potential of some indigenous plants against *Aedes albopictus*.

MATERIALS AND METHODS

Insects

Ae. albopictus larvae and pupae were collected round the year from all artificial containers and natural habitats of Faisalabad and reared in steel trays of 3 inch depth and were fed on fish food (Tetramin®) till adult emergence (Akram *et al.*, 2010). Adults were maintained in transparent plastic cages (70×35×35 cm), where the males were provided with cotton wicks soaked in 10% sucrose solution while females were fed with blood of white rats every alternate day (Shaalan *et al.*, 2006). A glass beaker with strips of moistened filter paper was provided for gravid females to lay their eggs. The population was maintained at set conditions of 27±2°C, 65% RH and L14:D10 photoperiod.

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Table I.- Plants and their parts used for oil extraction.

English name	Binomial name	Part
Darchini	*Cinnamomum cassia*	Bark
Musambi	*Citrus sinensis* L. var. musambi	Leaf
Bakhra	*Tribulus terrestris* L.	Seed
Eucalyptus	*Eucalyptus camaldulensis* L.	Seed
Black Pepper	*Piper nigera* L.	Seed
Castor	*Ricinus communis* L.	Seed
Garlic	*Allium sativum* L.	Rhizome
Linseed	*Linum usitatissimum* L.	Seed
Succari	*Citrus sinensis* L. var. succari	Peel

Extraction of oil and preparation of solution

The plants with their parts selected for oil extraction are listed in Table I. These plants were collected from Faisalabad (31°30'N, 73°05'E) and got identified from the Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

The seeds of citrus cultivars were washed with tap water to remove the pulp and then dried in electric oven for 48 hours at 60°C and later were grounded in an electric grinder (Anex Germany). While parts of other plants like seeds, leaves and rhizomes were also dried. The grounded material was put in Soxhlet apparatus for the extraction of oil using di-ethyl ether as a solvent (Vogel, 1978). 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 99 ml of acetone that was considered as 1% stock solution from which series of concentration (mg/L) were prepared (Murugan *et al.,* 2007).

Bioassay

The extracted oils were used in six different concentrations including controls. Each concentration was replicated thrice having 200 ml of oil solution placed in 250ml glass beakers. Thirty late third instar larvae of the *Ae. albopictus* (F_1 Lab. generation) were exposed in each beaker containing oil solution (Mohtar *et al.*. 1999). Larvae in control were only treated with acetone. The bioassays were performed under lab conditions at 27±2°C and 65±5% RH. The percentage mortality was calculated from the average of three replicates using the following formula given by Sumroiphon *et al*. (2006).

Percentage mortality = (Number of dead larvae / Number of larvae tested) ×100

Mortality data ware recorded after 24, 48 and 72 hours of exposure to plant oils.

Data analysis

Abbott's formula (Abbott, 1925) was used for corrected mortality and the data so obtained was analyzed by probit analysis (Finney, 1971) using Minitab-15 statistical software (2009) for dose and time mortality regression lines. Due to the inherent variability of bioassays, pair wise comparisons of LC_{50} (mg/L) values were made, and if 95% fiducial limits of two treatments did not overlap at 1% level of significance, they were considered significant (Litchfield and Wilcoxon, 1949). The data on percent mortalities were analyzed with Statistix 8.1 (Analytical Software, 2005) and means were compared using Tukey's Honestly Significant Difference (HSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Plant extracts exhibited strong larvicidal activities against *Ae. albopictus*. With respect to percent mortalities, musambi caused highest mortality (64.32%) after 24 h of exposure followed by eucalyptus (60.93%), darchini (56.48%) and succari (49.14%) (F=507; df=8,18; P<0.001). After 48 and 72 h of exposure, musambi caused highest percent mortality (76.05% for 48 h; 89.25% for 72 h) followed by eucalyptus (72.78% for 48 h; 85.49% for 72 h), darchini (68.03% for 48 h; 79.94 % for 72 h) and succari (57.78% for 48 h; 71.61% for 72 h), later both were statistically at par at both time intervals (F=460; df=8,18; P<0.001 for 48 h; F=317; df=8,18; P<0.001 for 72 h) (Fig. 1).

In terms of lethal concentration to kill 50 % population (LC_{50}) of the subjected mosquito larvae, musambi proved to be the most effective larvicide particularly after 24 h of exposure by having least LC_{50} value (40.0 mg/L) while black pepper, castor, garlic and linseed proved least effective (70.3 mg/L, 71.5 mg/L, 66.5 mg/L and 75.5 mg/L, respectively) and were statistically at par (overlapping of 95% FL_s). Darchini, musumbi, bakhra, eucalyptus and succari had lowest LC_{50} values (35.8 mg/L, 32.2, 39.0, 37.3 and 43.6 mg/L, respectively) after 48h

Table II.- Toxicity of plant extracts against larvae of *Aedes albopictus.*

Plant extract	Observation (h later)	*n*	LC_{50} (mg/L) (95% FL)	Slope±SE	χ^{2b} (df=4)	*P*
Darchini (*Cinnamonum cassia*)	24	540	46.7 (42.5-50.9)b	1.32±0.17	2.91	0.57
Musambi (*Citrus sinensis* L. var. musambi)	24	540	40.0 (35.9-40.5)a	1.50±0.18	4.63	0.33
Bakhra (*Tribulus terrestris* L.)	24	540	52.7 (47.5-58.6)b	1.09±0.16	0.18	0.99
Eucalyptus (*Eucalyptus camaldulensis* L.)	24	540	49.8 (42.6-52.0)b	0.81±0.14	0.16	0.99
Black Pepper (*Piper nigera* L.)	24	540	70.3 (65.8-75.4)c	1.94±0.21	1.06	0.90
Castor (*Ricinus communis* L.)	24	540	71.5 (64.8-79.1)c	1.51±0.19	1.93	0.75
Garlic (*Allium sativum* L.)	24	540	66.5 (62.2-72.3)c	1.81±0.20	0.60	0.96
Linseed (*Linum usitatissimum* L.)	24	540	75.5 (69.3-86.5)c	1.55±0.20	0.37	0.98
Succari (*Citrus sinensis* L. var. succari)	24	540	54.6 (49.0-60.8)b	1.11±0.17	0.32	0.99
Darchini (*Cinnamonum cassia*)	48	540	35.8 (31.6-39.2)a	1.57±0.18	9.50	0.06
Musambi (*Citrus sinensis* L. var. musambi)	48	540	32.2 (27.9-35.5)a	1.65±0.19	8.90	0.09
Bakhra (*Tribulus terrestris* L.)	48	540	39.0 (29.2-45.5)a	0.74±0.16	0.40	0.98
Eucalyptus (*Eucalyptus camaldulensis* L.)	48	540	37.3 (29.6-42.7)a	0.98±0.18	0.55	0.97
Black Pepper (*Piper nigera* L.)	48	540	55.1 (50.3-59.5)b	1.40±0.17	0.22	0.99
Castor (*Ricinus communis* L.)	48	540	58.9 (54.2-67.6)b	1.22±0.17	0.75	0.95
Garlic (*Allium sativum* L.)	48	540	56.8 (52.9-61.4)b	1.56±0.18	0.28	0.99
Linseed (*Linum usitatissimum* L.)	48	540	60.1 (55.4-63.7)b	1.66±0.19	0.46	0.97
Succari (*Citrus sinensis* L. var. succari)	48	540	43.6 (38.9-47.8)a	1.24±0.21	2.15	0.71
Darchini (*Cinnamonum cassia*)	72	540	29.9 (23.6-33.2)a	1.76±0.21	7.26	0.12
Musambi (*Citrus sinensis* L. var. musambi)	72	540	26.8 (22.2-30.2)a	1.82±0.23	4.69	0.32
Bakhra (*Tribulus terrestris* L.)	72	540	35.8 (29.1-36.6)a	1.04±0.175	5.20	0.27
Eucalyptus (*Eucalyptus camaldulensis* L.)	72	540	32.6 (24.5-36.8)a	1.54±0.24	5.18	0.27
Black Pepper (*Piper nigera* L.)	72	540	43.6 (39.2-47.2)b	1.32±0.17	1.21	0.84
Castor (*Ricinus communis* L.)	72	540	44.7 (38.2-49.6)b	0.46±0.07	0.68	0.96
Garlic (*Allium sativum* L.)	72	540	46.5 (42.6-50.2)b	1.47±0.17	1.27	0.87
Linseed (*Linum usitatissimum* L.)	72	540	45.7 (42.2-49.5)b	1.64±0.18	1.19	0.88
Succari (*Citrus sinensis* L. var. succari)	72	540	35.5 (29.0-38.1)a	1.53±0.18	2.22	0.70

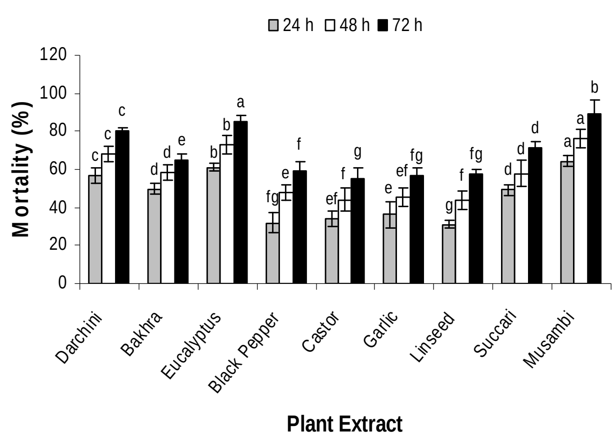

Fig. 1. Effect of plant extracts on mortality of mosquito larvae. Bars represent mean percent mortality (± S.E.) at different time intervals. Bars of specific time duration, followed by the same letters are not significantly different at 5% level of significance (Tukey's Honestly Significant Difference [HSD] test, Statistix 8.1 v)

and (29.9, 26.8, 35.8, 32.6 and 34.5 mg/L, respectively) after 72 h and were statistically at par (overlapping of 95% FL_s) (Table II).

With regards to lethal time to kill 50% population (LT_{50}) of *Aedes* larvae, musambi took least time (13.44 h) followed by darchini and bakhra (18.91 h and 25.08 h) both having non significant difference while black pepper, castor, garlic and linseed took more than 2 days (51.22 h, 57.48 h, 55.11 h and 56.50 h respectively) to cause 50% mortality and were statistically at par (overlapping of 95% FL_s) (Table III).

In view of the environmental hazards and development of insecticide resistance in insects, particularly disease vectors, the recent trend is to explore eco-friendly plant extracts with the potential to suppress pest populations. Though several plant based insecticides have been reported worldwide, there is still a wide scope to investigate more potent plant products with enhanced activity, eco-friendly

Table III.- Time mortality response of *Aedes albopictus* larvae against plant extracts.

Plant extract	LT_{50} h (95% FL)	Slope ± SE	χ^2 (df=1)	*P*
Darchini (*Cinnamonum cassia*)	18.91 (16.26-22.74)b	0.58±0.07	2.40	0.12
Musambi (*Citrus sinensis* L. var. musambi)	13.44 (09.01-15.22)a	0.58±0.07	1.24	0.27
Bakhra (*Tribulus terrestris* L.)	25.08 (16.64-31.17)b	0.35±0.06	0.15	0.70
Eucalyptus (*Eucalyptus camaldulensis* L.)	27.74 (23.30-32.74)bc	0.51±0.06	0.81	0.36
Black Pepper (*Piper nigera* L.)	51.22 (45.32-56.38)d	0.72±0.07	0.11	0.74
Castor (*Ricinus communis* L.)	57.48 (50.35-68.85)d	0.48±0.05	1.70	0.19
Garlic (*Allium sativum* L.)	55.11 (48.09-65.93)d	0.46±0.07	1.21	0.27
Linseed (*Linum usitatissimum* L.)	56.50 (51.12-64.63)d	0.62±0.08	2.46	0.13
Succari (*Citrus sinensis* L. var. succari)	27.76 (22.65-31.94)bc	0.53±0.09	3.33	0.08

$LT_{50,}$ lethal time to kill 50% population of subjected larvae

and could replace objectionable synthetic chemicals for mosquito control (Shaalan *et al*., 2006).

The ether extracts of 9 indigenous plants proved sufficiently effective against *Ae. albopictus* larvae. The varying results obtained in terms of percent mortalities LC_{50} and LT_{50} could probably be due to the differences in levels of toxicity among the insecticidal ingredients of each plant, and the effect of plant extract may vary with the time of collection and season as well (Tawatsin *et al.*, 2006; Din *et al.*, 2011). The studied plant extract caused high mortality in larval stages of *Ae. albopictus*, therefore, this intervention for public authorities (Silva *et al.*, 2003) to use plant products could provide ideal approach in public health management especially mosquito control. Plant extracts are safer for non target organisms including man (Din *et al.*, 2011), therefore, plant based formulations would be more feasible from environmental perspective than synthetic mosquitocides (Bhat and Kempraj, 2009). Essential oils against mosquitoes have been studied extensively. Senthilkumar *et al.* (2008) reported larvicidal effect of *Blumea mollis* essential oil against *Culex quinquefasciatus*, with LC_{50} of 52.2 mg/L. Similarly, Amer and Mehlhorn (2006) studied 41 plants against *Aedes*, *Anopheles* and *Culex* genera and found that *Citrus limon* was the most promising larvicide. Tiwary *et al.* (2007) applied *Zanthoxylum armatum* DC against three mosquito disease vectors and found it very effective. The mode of action of these oils on mosquito larvae is not known, however, previous studies documented that phytochemicals could interfere with the proper functioning of mitochondria particularly at the proton transferring sites (Usta *et al.,* 2002). The constituents of plant extracts primarily affect the midgut epithelial surface and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae (Rey *et al.*, 1999; David *et al.*, 2000). Moreover, the plant extracts could be more effective compared to the individual active compounds, due to natural synergism that delays the development of resistance to a maximum, particularly in disease vectors (Maurya *et al.*, 2007). However, further studies are required to confirm the exact mode of action of these essential oils.

In conclusion, the present study clearly indicates the efficacy of indigenous plant oils against *Ae. albopictus* which encourages the development of alternative active ingredients as effective mosquitocides.

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REFERENCES

ABBOTT, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.,* **18**: 265-267.

AKRAM, W., KHAN, H.A.A., HAFEEZ, F., BILAL, H., KIM, Y.K. AND LEE, J.J., 2010. Potential of citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pakistan J. Bot.*, **42**: 3343-3348.

AMER, A. AND MEHLHORN, H., 2006. Larvicidal effects of various essential oils against *Aedes*, *Anopheles* and

Culex larvae (Diptera: Culicidae). *Parasitol. Res.,* **99**: 466-472.

ANALYTICAL SOFTWARE, 2005. Statistix 8.1 for Windows. Analytical Software, Tallahassee, Florida.

BHAT, S.K. AND KEMPRAJ, K., 2009. Biocidal potential of clove oil against *Aedes albopictus* - A comparative study. *Afr. J. Biotechnol.,* **8**: 6933-6937.

CORBEL, V., DUCHON, S., ZAINM, M. AND HOUGAND, J.M., 2004. Dinotefuran: A potential neonicotinoid insecticide against resistant mosquitoes. *J. med. Ent.,* **41**: 712-717.

DAVID, J.P., REY, D., PAUTOU, M.P. AND MEYRAN, J.C., 2000. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. *J. Inverteb. Pathol.,* **75**:9–18.

DIN, S., AKRAM, W., KHAN, H.A.A., HUSSAIN, A. AND HAFEEZ, F., 2011. Citrus Waste-derived essential oils: Alternative larvicides for dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pakistan J. Zool.,* **43**: 367-372.

FINNEY, D.J., 1971. *Probit analysis: A statistical treatment of the sigmoid response curves*, 3rd edn. Cambridge University Press, London, p. 333.

ISMAN, M.B., 1995. Leads and prospects for the development of new chemistry insecticides. In: *Review in pesticide toxicology* (eds. R.M. Roe and R.J. Khur). Toxicology Communication Inc. NC. Vol. 3, pp. 1-20.

JUNWEI, Z., XIAOPENG, Z., YANMA, TING, L., KUEN, Q., YUHUA, H., SUQIN, X., TUCKER, B., SCHULTZ, G., COATS, J., ROWLEY, W. AND AIJUN, Z., 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J. Am. Mosq. Contr. Assoc.,* **3**: 515-522.

LITCHFIELD, J.T. AND WILCOXON, F., 1949. A simplified method of evaluating dose effect experiments, *J. Pharmacol. exp. Ther.,* **99**:99-103.

MAURYA, P., MOHAN, L., SHARMA, P., BATABYAL, L. AND SRIVASTAVA, C.N., 2007. Larvicidal efficacy of *Aloe barbadensis* and *Cannabis sativa* against the malaria vector *Anopheles stephensi* (Diptera:Culicidae). *Entomol. Res.,* **37**:153–156.

MINITAB *statistical software, releases 15 for Windows*, State College, PA, 2009.

MOHTAR, M., YARMO, M. AND KARDRI, A., 1999. The effects of *Warium indicum* leaf extract on *Aedes aegypti* larvae. *J. trop. Forest. Prod.,* **5**: 87-92.

MURUGAN, K., MURUGAN, P. AND NOORTHEEN, A., 2007. Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilium* Linn. against dengue vector, *Aedes aegypti*. *Bioresour. Technol.,* **98**: 198-201.

PITASAWAT, B., CHAMPAKAEW, D., CHOOCHOTE, W., JITPAKDI, A., CHAITHONG, U., KANJANAPOTHI, D., RATTANACHANPICHAI, E., TIPPAWANGKOSOL, P., RIYONG, D., TUETUN, B. AND CHAIYASIT, D., 2007. Aromatic plant-derived essential oil: Analternative larvicide for mosquito control. *Fitoterapia,* **78**: 205-210.

REY, D., PAUTOU, M.P. AND MEYRAN, J.C., 1999. Histopathological effects of tannic acid on the midgut epithelium of some aquatic dipteral larvae. *J. Inverteb. Pathol.,* **73**: 173–181.

SENTHILKUMAR, A., KANNATHASAN, K. AND VENKATESALU, V., 2008. Chemical constituents and larvicidal property of *Blumeamollis* (D.Don) *merr*. against *Culex quinquefasciatus. Parasitol. Res.,* **103**: 959–962.

SHAALAN, E.A.S., CANYON, D.V., BOWDEN, B., YOUNES, M.W.F., ABDET, W.H. AND MANSOIN, A.H., 2006. Efficacy of botanical extracts from *Callitris glaucophylla* against *Aedes aegypti* and *Cules annulirostris* mosquitoes. *Trop. Biomed.,* **2**: 180-185.

SILVA, I., ZANON, G. AND SILVA, H.H.G., 2003. Larvicidal activity of *Copaifera reticulata* Ducke Oil Resin against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Neo. Trop. Ent.,* **32**: 729-732.

SUKUMAR, K., PERICH, M.J. AND BOOBAR, L.R., 1991. Botanical derivatives in mosquito control. *J. Am. Mosq. Contr. Assoc.,* **7**: 210-237.

SUMROIPHON, S., YUWAREE, C., ARUNLERETAREE, C., KOMALAMISRA, N. AND RONGSRIYAM, Y., 2006. Bioactivity of citrus seeds for mosquito borne diseases and larval control. *Southeast Asian J. trop. Med. Publ. Hlth.,* **37**: 123-127.

TAWATSIN, A., ASAVADACHANUKORN, P. AND THAVARA, U., 2006. Repellency of essential oils extracted from plants in Thailand against four mosquito vectors and oviposition deterrent effects against *Aedes aegypti*. *Southeast Asian J. trop. Med. Publ. Hlth.,* **37**: 915–931.

TIWARY, M.S., NAIK, N., TEWARY, D.K., MITTAL, P.K. AND YADAV, S., 2007. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. *J. Vect. Borne Dis.,* **44**: 198-204.

USTA, J., KREYDIYYEH, S., BAKAJIAN, K. AND NAKKASH-CHMAISSE, H., 2002. *In vitro* effect of eugenol and cinnamaldehyde on membrane potential and respiratory complexes in isolated rat liver mitochondria. *Fd. Chem. Toxicol.,* **40**:935–940.

VOGEL, A.I., 1978. *Text book of practical organic chemistry*. The English Language Book Society and Longman, London, pp. 1368.

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