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Short Communication

Bioactivity of Oils from Medicinal Plants against Immature Stages of Dengue Mosquito *Aedes aegypti* (Diptera: Culicidae)

Shabab Nasir[1*], Marriam Batool[1], Syed Makhdoom Hussain[1], Iram Nasir[2], Faisal Hafeez[3] and Mustapha Debboun[4]
[1]*Department of Zoology, Wildlife & Fisheries, Government College University, Faisalabad, Pakistan*
[2]*Department of Statistics, Government College University, Faisalabad, Pakistan*
[3]*Institute of Entomological Research, Ayub Agriculture Research Institute, Faisalabad, Pakistan*
[4]*Academy of Health Sciences, Department of Preventive Health Servicers, Fort Sam Houston, Texas*
[*]For correspondence: flourenceshabab@yahoo.com

Abstract

To evaluate the efficacy of the essential oils extracted from the branches and leaves of eucalyptus (*Eucalyptus globules* Labill.), neem (*Azadirachta indica* A. Juss), peppermint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.) and from rhizome of ginger (*Zingiber officinale* Rosc.) against the larvae and pupae of *Aedes aegypti* L. The essential oils were extracted with Soxhlet apparatus using petroleum ether as a solvent. The oils were evaluated against 1st, 2nd, 3rd, 4th instar larvae and the pupae of *Ae. aegypti* following WHO protocol. The dead individuals in all stages were counted after 8, 16, 24 and 48 hours in treatments of different concentrations (100, 200, 300 and 400 ppm). The percent mortality in each stage was determined and consequently LC_{50}s were also calculated by Probit analysis. A control treatment was also run by using petroleum ether in which mortality (<6%) of different life stages of *Aedes* mosquitoes was observed. Results showed that higher mortality was observed in early life stages than later ones. Ginger was more effective having lowest LC_{50} after 8 h (142 ppm) and 16 h (8.5 ppm) against 1st instar larvae followed by peppermint, basil, eucalyptus and neem. However, eucalyptus and peppermint were efficacious after 24 h (66 and 84 ppm) and 48 h (19.5 and 17 ppm), respectively. Ginger oil showed high efficacy in short period of the time (8 and 16 h) followed by peppermint, basil, eucalyptus and neem, whereas eucalyptus oil exhibited its lethality after 24 h, whilst peppermint has longer potency and persistence (48 h) than other plant oils. For pupal stage, peppermint had knockdown effect (8 h) followed by eucalyptus (16 h), basil (24 h) and neem (48 h). From these results, it can be concluded that the oils of *E. globules* and *M. piperita* were effective larvicide against the immature stages of *Ae. aegypti.*

Keywords: Larvae; Medicinal plants, Oils, Pupae; *Aedes aegypti*; Mortality

Introduction

Aedes aegypti L., a vector of dengue fever, has assumed an alarming position of preponderance in Pakistan. The number of death due to this fever is increasing every year. The Government of Punjab (Pakistan) has established emergence cell to combat this vector (Anonymous, 2013). In Punjab, Pakistan, there were 21,292 confirmed dengue cases with 352 deaths during 2011 (Anonymous, 2013). To date neither a vaccine nor a treatment for dengue virus is available; the only way to ward off this disease is to control the vector, *Ae. aegypti*. The immature stages of this mosquito can efficiently be controlled by source reduction and chemical application. Source reduction method has its limitations due to growth of residential areas and poor sanitation facilities. The overuse of synthetic insecticides may foster development of resistance, which alone is sufficient to cause control failure (Sarwar *et al*., 2009; Naz *et al*., 2014). Thus, new strategies for the control of immature stages of mosquito should be sorted out (Junwei *et al*., 2006). In this respect, plant extracts may be used as an alternative of the chemical insecticides, because they constitute a rich source of bioactive compounds that are easily biodegradable and limit case of resistance in mosquitoes (Gbolade *et al*., 2000; Bokhari *et al*., 2014). Many biologists have studied the effectiveness of plant oils such as *Azadirachta indica* A. Juss., *Lantana Camara* L., *Litsea elliptica* B., *Momordica charantia* L., *Syringodium isoetifolium* Asch., *Vitex agnus* L. and plants from Citrus family against the mosquitoes and found them effective against larvae and adults. These oils can be categorised as larvicide or repellent to adult stages (Chantraine *et al*., 1998; Ansari *et al*., 2000; Yang *et al*., 2002; Amer and Mehlhorn, 2006; Senthil-Nathan *et al*., 2006; Tiwary *et al*., 2007; Anees, 2008; Bakkali *et al*., 2008; Akram *et al*., 2010; Hafeez *et al*., 2011; Sadr ud Din *et al*., 2011).

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The larvicide and repellent activities of plant oils have been documented against many mosquito vector species. The main focus of the earlier researchers was on lethal concentration, lethal time and percent mortality against a single larval instar but a little work was done on total life span including pupa. The present work has been planned to find out the effects of essential oils from the branches and leaves of eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), peppermint (*Mentha piperita*), basil (*Ocimum basilicum*) and rhizome of ginger (*Zingiber officinale*) on all larval instars and pupae of *Ae. aegypti*.

Materials and Methods

Collection and Rearing of Mosquitoes

Larvae, pupae and adult mosquitoes were collected from residential areas (Faisalabad, Punjab). Larvae and pupae were collected with the help of a standard dipper, kept and stored in a plastic bottle tied up with muslin cloth replacing lid for aeration. Then the collected material was carried to the Department of Zoology, Wildlife and Fisheries, Government College University, Faisalabad for sorting and rearing. The larvae and pupae were separated. The larvae were kept in rearing trays and pupae were kept in beakers inside the cages. After emergence, the females were fed on blood of white rat for egg laying. The collected eggs were shifted in the plastic trays with fresh water for hatching inside laboratory running at 26±1°C and 75±5% RH. The larvae were fed on fish diet and 1st, 2nd, 3rd, 4th instar larvae and pupae were used for the bioassay (Kumar *et al*., 2011).

Collection and Preparation of Plant Material for Oil Extraction

Different plant materials selected for the oil extraction are presented in Table 1. These plant materials were collected from Government College University, Faisalabad (31°30'N, 73°05' E). The plant materials were washed with tap water to remove dust particles and were dried at room temperature. After that these materials were also dried for 48 hours at 60°C in an electric oven. The dried material was grinded with the help of an electrical grinder and the resultant powder was stored in the plastic bottles after sieving for oil extraction.

Extraction of Oil

Essential oils were extracted from the selected plant materials with the help of Soxhlet apparatus (Cheng *et al*., 2009). Twenty five grams powder of each plant material with 250 mL of solvent (petroleum ether) was used for 8 to 24 h to extract oil through Soxhlet apparatus. After extraction, vacuum evaporator was used to evaporate solvent to attain filtrate in dehydrated form, which was then stored in airtight jar.

Table 1: List of plants and their parts used for oil extraction

English names	Binomial names	Families	Parts
Eucalyptus	*Eucalyptus globules*	Myrtaceae	Branches and leaves
Neem	*Azadirachta indica*	Meliaceae	Branches and leaves
Mint	*Mentha piperita*	Lamiaceae	Branches and leaves
Basil	*Ocimum basilicum*	Lamiaceae	Branches and leaves
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome

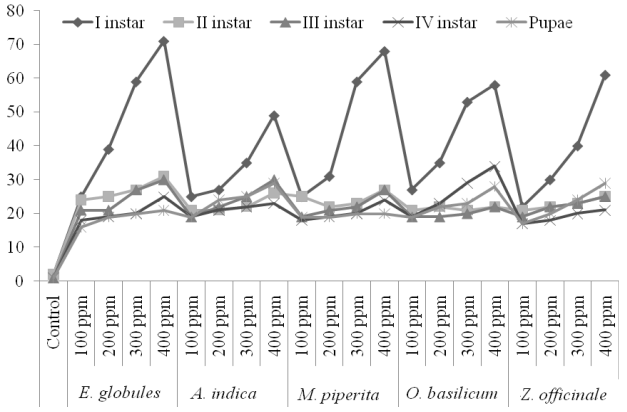

Fig. 1: Mortality (%) of various life stages of *Ae. aegypti* mosquitoes after 8 hours in the concentrations of different plant oils

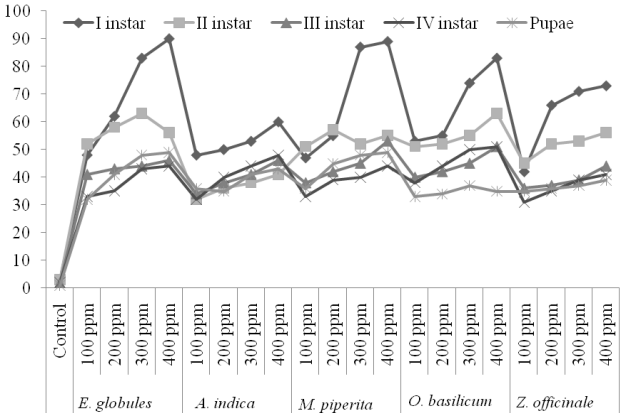

Fig. 2: Mortality (%) of various life stages of *Ae. aegypti* mosquitoes after 16 hours in the concentrations of different plant oils

Preparation of Solution

From extracted oils, 100, 200, 300 and 400 ppm concentrations were prepared by dissolving the 0.5, 1.0, 1.5 and 2.0 µL of extracted oil in 1 mL of petroleum ether, respectively and required volume was made with distilled water.

Larvicide Bioassays

Larvicide bioassays were accomplished under laboratory

Table 2: Toxicity of plant extracts against larval instars and pupae of *Ae. aegypti*

Plant extract	Life stages	After interval of 8 h				After interval of 16 h			
		LC_{50}	Slope ±S.E	χ^2	P value	LC_{50}	Slope ±S.E	χ^2	P value
Eucalyptus (*Eucalyptus globules*)	1st instar	581	1.62±0.33	2.85	0.24	189	1.95±0.19	4.92	0.00
	2nd instar	571	0.55±0.31	0.46	0.79	340	0.46±0.15	0.04	0.97
	3rd instar	591	1.01 ±0.37	0.90	0.63	496	0.25 ±0.21	0.00	0.99
	4th instar	172	1.31±0.54	0.76	0.68	810	0.81 ±0.23	1.52	0.46
	Pupae	467	0.94±0.5	0.16	0.92	519	0.92 ±0.21	0.00	1
Neem (*Azadirachta indica*)	1st instar	652	6.66±0.19	1.20	0.54	566	1.65±0.33	3.28	0.19
	2nd instar	76	0.98±0.13	0.50	0.97	357	0.93±0.49	0.90	0.63
	3rd instar	372	0.84±0.18	1.74	0.41	152	1.22±0.47	0.81	0.66
	4th instar	500	0.32±0.17	0.57	0.75	471	0.40±0.19	0.31	0.85
	Pupae	218	0.45±0.21	0.47	0.79	780	0.58±0.19	0.04	0.98
Peppermint (*Mentha piperita*)	1st instar	274	3.40 ±3.10	15.2	0.00	184	2.63±0.25	39.3	0.00
	2nd instar	463	0.7 ±0.38	0.32	0.85	395	0.43 ±0.16	0.07	0.96
	3rd instar	226	1.05 ±0.46	0.82	0.66	972	0.64 ±0.22	0.88	0.64
	4th instar	262	1.1 ±0.54	0.29	0.86	285	0.75 ±0.21	0.15	0.92
	Pupae	190	1.00±0.40	0.23	0.88	660	0.67±1.19	0.04	0.97
Basil (*Ocimum basilicum*)	1st instar	323	0.29±0.17	0.67	0.71	396	1.52±0.23	1.30	0.52
	2nd instar	53	0.83±0.13	1.47	0.47	441	0.76±0.48	0.19	0.93
	3rd instar	198	0.46±0.14	0.53	0.76	334	1.08±0.58	0.57	0.74
	4th instar	326	0.38±0.15	0.06	0.96	887	1.33±0.36	0.23	0.88
	Pupae	946	0.41±0.18	0.39	0.85	226	0.86±0.36	0.83	0.66
Ginger (*Zingiber officinale*)	1st instar	142	1.30±1.20	31.4	0.00	8.5	1.31±0.16	1.06	0.58
	2nd instar	181	0.98±0.13	0.50	0.96	74.	0.93±0.49	0.90	0.64
	3rd instar	395	0.84±0.18	1.70	0.41	372	1.20±0.45	0.81	0.66
	4th instar	589	0.33±0.17	0.57	0.75	400	0.43±0.18	0.31	0.85
	Pupae	815	0.45±0.21	0.47	0.79	578	0.57±0.19	0.04	0.97

Table 3: Toxicity of plant extracts against larvae and pupae of *Ae. aegypti*

Plants	Life stages	After interval of 24 h				After interval of 48 h			
		LC_{50}	Slope ±S.E	χ^2	P value	LC_{50}	Slope ±S.E	χ^2	P value
Eucalyptus (*Eucalyptus globules*)	1st instar	66	1.09±0.15	6.38	0.04	19.5	0.67±0.17	7.67	0.02
	2nd instar	99	0.74±0.13	0.99	0.60	86	0.69±0.13	1.24	0.53
	3rd instar	161	0.30±0.14	0.78	0.67	145	0.36±0.14	3.57	0.16
	4th instar	301	0.17±0.17	0.04	0.98	362	0.60±0.16	1.85	0.39
	Pupae	799	0.52±0.19	0.79	0.67	757	0.51±0.19	0.37	0.83
Neem (*Azadirachta indica*)	1st instar	421	0.74±0.18	9.77	0.00	181	1.05±0.15	4.70	0.09
	2nd instar	115	0.82±0.26	0.16	0.92	395	121±0.12	0.67	0.13
	3rd instar	117	0.80±0.26	0.22	0.89	597	0.23±0.17	0.20	0.90
	4th instar	524	0.35±0.18	1.14	0.56	517	0.88±0.34	0.17	0.91
	Pupae	709	0.51±0.18	1.47	0.79	229	1.80±0.51	7.92	0.19
Peppermint (*Mentha piperita*)	1st instar	84	1.2±0.17	8.4	0.01	17	0.56 ±0.14	7.47	0.02
	2nd instar	135	0.50±0.14	4.86	0.00	212	0.22 ±0.12	0.68	0.71
	3rd instar	346	0.74±0.16	1.95	0.37	342	0.24±0.14	0.15	0.93
	4th instar	549	0.36±0.19	0.80	0.67	723	0.51±0.18	0.82	0.66
	Pupae	715	0.53±0.18	0.16	0.92	323	0.29±0.17	0.67	0.75
Basil (*Ocimum basilicum*)	1st instar	206	1.37±1.07	17.0	0.00	120	0.99±0.13	0.46	0.79
	2nd instar	595	0.41±1.69	2.37	0.30	313	0.51±0.15	2.19	0.33
	3rd instar	659	0.45±0.20	0.98	0.60	523	0.33±0.15	0.33	0.84
	4th instar	589	0.81±0.20	0.23	0.89	568	0.39±0.18	0.52	0.67
	Pupae	219	0.58±0.25	0.60	0.97	946	0.44±0.22	0.40	0.81
Ginger (*Zingiber officinale*)	1st instar	466	0.64±0.08	8.77	0.00	417	1.04±0.12	3.70	0.08
	2nd instar	576	0.82±0.26	0.16	0.98	955	124±0.12	0.67	0.14
	3rd instar	152	0.80±0.26	0.22	0.89	998	0.23±0.17	0.20	0.90
	4th instar	464	0.35±0.17	1.14	0.56	422	0.88±0.33	0.17	0.91
	Pupae	777	0.52±0.18	1.47	0.79	704	1.80±0.51	7.92	0.18

conditions in accordance with WHO technique for mosquito with the transparency adjustment (WHO, 2009). In each glass beaker twenty five 1st, 2nd, 3rd and 4th instar larvae were introduced separately containing various oil solution concentrations from 100 ppm to 400 ppm (Mohtar *et al*., 1999). Control treatments had said volume of water instead of oil in petroleum ether. The treatments were repeated thrice under laboratory conditions at 27±2°C and 65±5% RH using CRD. As soon as possible the dead larvae were removed from the beaker to prevent the rapid death of further larvae, after 8, 16, 24 and 48 h. From the average of three replicates, percentage mortality was counted by using the following formula (Sumroiphon *et al*., 2006).

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

Statistical Analysis

Abbot's formula was applied to calculate the corrected mortality and then the data were analyzed by Probit analysis (Abbott, 1925; Finney, 1971), using Minitab-15 statistical software for determining LC_{50} and related parameters.

Results

The percent mortality of different life stages of *Ae. aegypti* in different concentrations of plant oils at different post-treatment time intervals are shown in Figs. 1-4. Highest mortality was seen in case of 1st instar larvae than all other immature life stages with all oils and their concentrations. After 8 h, about 70% mortality of the 1st instar larvae was seen in case of *Eucalyptus* and peppermint with 400 ppm and least mortality was seen in case of neem oil (50%). In case of all other immature life stages, the mortality percentage was almost same with all oils, i.e., close to 25%. The control treatment of this time point had shown 2% mortality (Fig. 1). After 16 h, about 90% mortality of the 1st instar larvae was seen in case of *Eucalyptus* and peppermint with 400 ppm, while basil showed about 83% mortality with 400 ppm and least mortality (~60%) was seen in case of neem oil (Fig. 2). After 24 h, more than 90% mortality of the 1st instar larvae was observed in all oils except *Eucalyptus* and mint; although with higher concentration more than 60% mortality was observed in all other life stages, while control had only 4% mortality (Fig. 3). After 48 h, 100% mortality (1st instar larvae) was seen in all oil types and their concentrations, as against 5% mortality in the control treatment (Fig. 4).

LC_{50} and related parameters of toxicity of oils for *Aedes* larvae and pupae are shown in Tables 2 and 3. Ginger showed the least value of LC_{50} (142 ppm) at 8 h to kill 1st instar larvae with p-value = 0.00 and at 16 h, LC_{50} of 1st instar larvae was 8.5 ppm with 0.58 p-value (Table 2). In case of 2nd instar larvae, the LC_{50} value of eucalyptus oil at 8 h with 0.47 p-values was very high (571 ppm) followed by peppermint (463 ppm), ginger (181 ppm), neem (76.5 ppm) and basil (53 ppm) however, eucalyptus was found to be the best after 24 and 48 h with 99 ppm and 86 ppm, respectively. In case of 3rd instar larvae, after 8 h basil was the best with LC_{50} (198 ppm) followed by peppermint (226 ppm), neem (372 ppm), ginger (395 ppm) and eucalyptus (591 ppm), however, neem was found to be the best after 24 h (117 ppm) and eucalyptus after 48 h with LC_{50} (145 ppm). Eucalyptus oil with least LC_{50} value (301 ppm) for 24 hours with 0.98 p-value was followed by ginger (464 ppm) at 24 and 48 h, respectively for 4th instar larvae. The LC_{50} value was less for 48 h with 0.833 p-value in case of pupae (Table 3).

Discussion

The oils of *E. globules* and *M. piperita* proved themselves as highly toxic to mosquito larvae (1st, 2nd, 3rd, 4th instar larvae and pupae) and this response was time and concentration dependent in all larval stages and pupae as well. These results are in agreement with earlier workers who have exhibited effectiveness and economy in using these oils where plant materials are abundantly available (Jang *et al*., 2002; Tripathi *et al*., 2002; Amer and Mehlhorn, 2006; Okumu *et al*., 2007; Aivazi and Vijayan, 2008; Kovendan *et al*., 2007; Silva *et al*., 2008; Abdel-Ghaffar *et al*., 2009). The active compounds from plant oils have been isolated and their repellent and contact activities are well reported. The limonoids from neem oil were found the effective alternative to conventional synthetic insecticides for the control of (100%) *Culex uinquefasciatus*, (90%) *Ae. aegypti* and (85%) *Ae. stephensi* within 24 h (Ansari *et al*., 2000; Senthil-Nathan *et al*., 2006). We also found 90% mortality at 400 ppm after 24 h in case of 1st instar *Aedes* larvae and more than 75% even at low concentrations (200 and 100 ppm). A total of 91% mortality was observed after 48 h. The variation of solvent for extraction of plant oils and extract almost yielded high control rate of mosquitoes. The leaf and flower

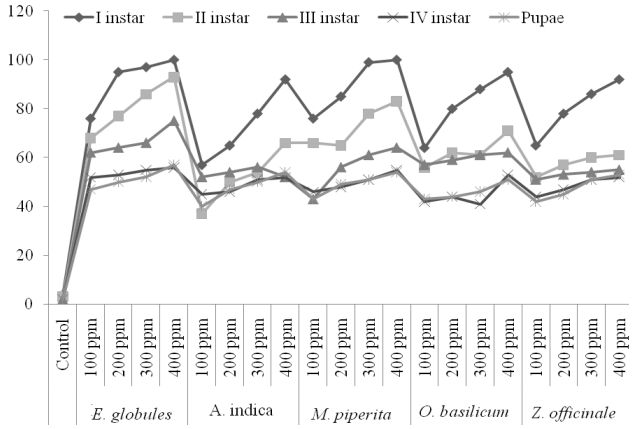

Fig. 3: Mortality (%) of various life stages of *Ae. aegypti* mosquitoes after 24 hours in the concentrations of different plant oils

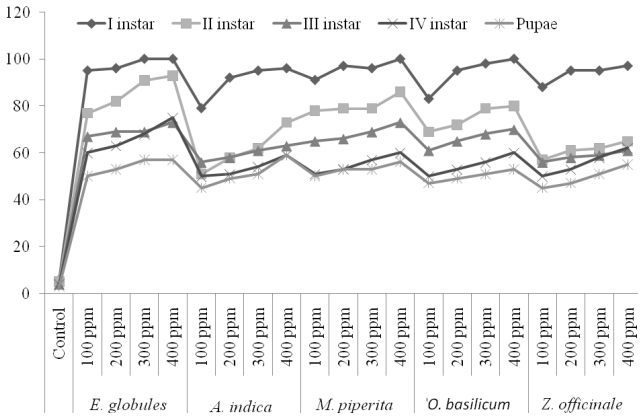

Fig. 4: Mortality (%) of different life stages of *Ae. aegypti* mosquitoes after 48 h with different concentrations of different plant oils

extracts in acetone, chloroform, ethyl acetate, hexane and methanol yielded high mortality against the 4th instar larvae of mosquito and highest mortality was observed in chloroform and hexane extract of *O. sanctum* (Anees, 2008). Our study also revealed that the solvent used for extraction also had a strong effect on the mortality of different life stages of mosquitoes. We observed up to 6% mortality after 48 h in case of 1st larvae in the control treatments and more than 3% in control treatments running along with 2nd, 3rd, 4th instars and pupae after 48 h. The essential rhizome oil from ginger was the most potent larvicide against the *An. gambiae* (Ajaiyeoba *et al*., 2008; Pushpanathan *et al*., 2008). These oils should be tested in the field with a knapsack sprayer to check their efficacy, because Prabhu *et al*. (2011) reported the efficacy of *Moringa oleifera* seed extracts against malarial vector (*Anopheles stephensi*) as more than 90% reduction in larvae after 72 h. It is concluded from this study that essential oils from *E. globules* and *M. piperita* have strong larvicide potential and could be very effective against the larvae of *Ae. aegypti*.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.,* 18: 265–267

Abdel-Ghaffar, F., M. Semmler, K. Al-Rasheid, S. Klimpel and H. Mehlhorn, 2009. Efficacy of a grapefruit extract on head lice: a clinical trial. *Parasitol. Res.,* 106: 445–449

Aivazi, A. and V.A. Vijayan, 2008. Larvicidal activity of oak *Quercusin fectoria* Oliv. (Fagaceae) gall extracts against *Anopheles stephensi* Liston. *Parasitol. Res.,* 104: 1289–1293

Ajaiyeoba, E.O., W. Sama, E.E. Essien, J.O. Olayemi, O. Ekundayo, T.M. Walker and W.N. Setzer, 2008. Larvicidal activity of turmerone rich essential oils of *Curcuma longa* leaf and rhizome from Nigeria on *Anopheles gambiae*. *Pharm. Biol.,* 46: 279–282

Akram, W., H.A.A. Khan and F. Hafeez, 2010. Potential of citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pak. J. Bot*., 42: 3343–3348

Amer, A. and H. Mehlhorn, 2006. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. *Parasitol. Res.,* 99: 478–490

Anees, A.M., 2008. Larvicidal activity of *Ocimum sanctum* Linn. *Parasitol. Res.,* 103: 1451–1453

Anonymous, 2013. *Evaluation Report on Project: Prevention and Control Program of Epidemics in Punjab*. P&D department, Civil Secretariat, Lahore–Punjab, Pakistan

Ansari, M.A., P. Vasudevan, M. Tandon and R.K. Razdan, 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. *Biores. Technol.,* 71: 267–271

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils - A Review. *Food Chem. Toxicol.,* 46: 446–475

Bokhari, N.A., I. Siddiqui, K. Perveen, I. Siddique, M.S. Alwahibi, D.W.A. Soliman and M. Al-Subeie, 2014. Potential of different parts of neem (*Azadirachta indica*) extracts in controlling *Rhizoctonia solani* infestation. *Int. J. Agric. Biol*., 16: 639–643

Chantraine, J.M., D. Laurent, C. Ballivian, G. Saavedra, R. Ibanez and L.A. Vilaseca, 1998. Insecticidal activity of essential oils on *Aedes aegypti* larvae. *Phytother. Res.,* 12: 350–354

Cheng, S.S., H.T. Chang, C.Y. Lin, P.S. Chen, C.G. Huang, W.J. Chen and S.T. Chang, 2009. Insecticidal activities of leaf and twig essential oils from *Clausena excavata* against *Aedes aegypti* and *Aedes albopictus* larvae. *Pest Manag. Sci.,* 65: 339–343

Finney, D.J., 1971. *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curves*, 3rd edition. Cambridge University Press, London

Gbolade, A.A., A.D. Dyedele, M.B. Sosan, F.B. Adewayin and O.I. Soyela, 2000. Mosquito repellent activities of essential oils from two Nigerian *Ocimum* species. *J. Trop. Med. Plants,* 1: 146–148

Hafeez, F., W. Akram, A. Suhail and M.A. Khan, 2011. Adulticidal action of ten citrus oils against *Aedes albopictus* (Diptera: Culicidae). *Pak. J. Agric. Sci.,* 47: 241–244

Jang, Y.S., M.K. Kim, Y.J. Ahn and H.S. Lee, 2002. Larvicidal activity of Brazilian plants against *Aedes aegypti* and *Culex pipiens* Pallens (Diptera: Culicidae). *Agric. Chem. Biotech.,* 44: 23–26

Junwei, Z., Z. Xiaopeng, T.L. Yanma, L. Ting, Q. Kuen, H. Yuhua, X. Suqin and T. Brad, 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J. Amer. Mosq. Cont. Assoc.,* 3: 515–522

Kovendan, K., K. Murugan and S. Vincent, 2007. Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.,* 102: 867–873

Kumar, K., R. Warikoo and N. Wahab, 2011. Relative larvicidal efficacy of three species of peppercorns against dengue fever mosquito, *Aedes aegypti* L. *J. Entomol. Res. Soc.,* 13: 27–36

Mohtar, M., M. Yarmo and A. Kardri, 1999. The effects of *Nerium indicum* leaf extract on *Aedes aegypti* larvae. *J. Trop. Forest Prod.,* 5: 87–92

Naz, S., A. Maqbool, M.U.D. Ahmad and A.A. Anjum, 2014. Toxins of *Bacillus thuringiensis* var. *israelensis* for control of malaria vector *Anopheles stephensi* under laboratory and semi field conditions. *Int. J. Agric. Biol*., 16: 966–970

Okumu, F.O., B.G. Knols and U. Fillinger, 2007. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. *Malar. J.,* 6: 63

Prabhu, K., K. Murugan, A. Nareshkumar, N. Ramasubramanian and S. Bragadeeswaran, 2011. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac. J. Trop. Biomed*., 1: 124–129

Pushpanathan, T., A. Jebanesan and M. Govindarajan, 2008. The essential oil of *Zingiber officinalis* Linn (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol. Res.,* 102: 1289–1291

Sadr ud Din, W. Akram, H.A.A. Khan, A. Hussain and F. Hafeez, 2011. Citrus waste-derived essential oils: alternative larvicides for dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pak. J. Zool.,* 43: 367–372

Sarwar, M., N. Ahmad and M. Toufiq, 2009. Host plant resistance relationships in chickpea (*Cicer arietinum* L.) against gram pod borer (*Helicoverpa armigera* Hubner). *Pak. J. Bot.,* 41: 3047–3052

Senthil-Nathan, S., S. Kalaivani and K. Sehoon, 2006. Effects of *Dysoxylum malabaricum* Bedd. (Meliaceae) extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Biores. Technol.,* 97: 2077–2083

Silva, W.J., G.A.A. Doria, R.T. Maia, R.S. Nunes, G.A. Carvalho, A.F. Blank, P.B. Alves, R.M. Marcal and S.C.H. Cavalcanti, 2008. Effects of essential oils on *Aedes aegypti* larvae: alternatives to environmentally safe insecticides. *Biores. Technol.,* 99: 3251–3255

Sumroiphon, S., C. Yuwaree, C. Arunlertaree, N. Komalamisra and Y. Rongsriyam, 2006. Bioactivity of citrus seed for mosquito-borne diseases larval control. *Southeast Asian J. Trop. Med. Public Health,* 37(Suppl. 3): 123–127

Tiwary, M.S., N. Naik, D.K. Tewary, P.K. Mittal and S. Yadav, 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

Tripathi, A.K., V. Prajapati, N. Verma, J.R. Bahl, R.P. Bansal and S.P.S. Khanuja, 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

WHO, 2009. *Report of the WHO Informal Consultation on the Evaluation on the Testing of Insecticides*, Vol. 1, p: 69. CTD/WHO PES/IC/96

Yang, Y.C., S.G. Lee, H.K. Lee, M.K. Kim, S.H. Lee and H.S. Lee, 2002. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J. Agric. Food Chem.,* 50: 3765–3767

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