Bangladesh J. Bot. **46**(1): 27-33, 2017 (March)

MEDICINAL PLANT EXTRACTS: CONTROL STRATEGY AGAINST DENGUE MOSQUITOES

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Key words: Dengue mosquito, Larvicidal, Pupicidal, Plant extracts

Abstract

Study was made to know the bioactivity of five medicinal plants, *viz.*, eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), ginger (*Zingiber officinale*), basil (*Ocimum basilicum*) and peppermint (*Mentha piperita*) against *Aedes aegypti* mosquito. Mosquitoes were reared in laboratory. The extracts were examined for their larvicidal and pupicidal activities with different concentrations (*viz,* 100, 200, 300 and 400 ppm) for each plant extract after different time intervals (*viz.* 8, 16, 24 and 48 hrs) using WHO protocol. Greater death rate was seen in early life stages than older ones. Maximum mortality (99%) was observed with eucalyptus and mint oil in early instar larvae (1st and 2nd) and least with ginger extracts. Least LC_{50} was recorded 102 ppm for 48 hrs in case of early instar larvae from eucalyptus extract while highest was 601 ppm for peppermint extract in case of pupae. Ginger oil showed superiority amongst the remaining extracts in case of LT_{50} values.

Introduction

Mosquitoes, as vectors, are responsible for many fatal diseases like malaria, dengue, filariasis, yellow fever etc. (Remia and Logaswamy 2010). These diseases cause millions of deaths every year. However, no country of the world is exceptional from having vector borne diseases (Ravikumar *et al*. 2011). Nowadays many severe forms of dengue are increasing like dengue hemorrhagic fever, dengue shock syndrome and central nervous system involvement (Choochote *et al*. 2004). *Aedes albopictus* and *Aedes aegypti* act as vectors of dengue and yellow fever, and usually affect children (Sulaiman *et al.* 2006). Former species is mostly found in fields or forests and it prefers to live in shady areas while latter mosquito species prefers residential areas. Over population and poor sanitation are the main factors in the spread of these vectors (Akram *et al*. 2010).

In the past, insecticides were used to control mosquitoes, but they had developed resistance against the synthetic insecticides (Abu Bakar *et al*. 2009). Insecticides are not environment friendly because they affect/kill non target organisms and pollute the environment. These synthetic insecticides leave toxic residues on food products and cannot be easily biodegradable (Remia and Logaswamy 2010). Due to the side effects of insecticides, alternative control methods are used (Sumroiphon *et al*. 2006).

An alternative to the synthetic insecticides or along with other insecticides under the integrated vector control program, phytochemicals acquired from plants with confirmed mosquito control can be used (Kumar *et al*. 2011). Their larvicidal and repellent actions have been reported against many mosquito vector species. Previous workers used plants belonging to the families Asteraceae, Labiatae, Myrtaceae, Meliaceae, Rutaceae, Piperaceae etc. for extracting potential toxic components from them and applied against household insects like mosquitoes (Hafeez *et al*.

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2010). They studied their effects on single larval instar or pupae of *Aedes albopictus* and no work was done on whole life stages (i.e., all larval instars and pupae of the mosquitoes). Due to this reason, this study was attempted and the effects of different concentrations of extracts from the plants of eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), ginger (*Zingiber officinale*), basil (*Ocimum basilicum*) and peppermint (*Mentha piperita*) were bioassayed on all larval instars and pupae of *Aedes aegypti.*

Materials and Methods

This study was carried out in the laboratory of the department of Zoology, Government College University, Faisalabad in 2015. Adult mosquitoes were collected from the forest at Gutwala Park, Faisalabad with the help of an aspirator and were transferred into rearing cages placed in the laboratory where the mosquitoes were identified as *Aedes aegypti* with the help of identification key (Barraud 1934, Qasim *et al*. 2014). The female mosquitoes were fed with the blood of white rat and the males on 10% sugar solution. The eggs laid on the water in the cages were collected and shifted in plastic trays with fresh tap water for hatching. The larvae were fed with fish diet. Early instar (1st and 2nd), late instar (3rd and 4th) larvae and pupae were used for the study as suggested by Kumar *et al*. (2011). The toxicants were extracted in the form of oil from different selected plant materials which were collected from the plants in the premises of the Government College University, Faisalabad (Table 1).

Table 1. 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

After washing with water and drying at room temperature in shade, the plant materials were oven dried at 60^{o}C for 48 hrs (Hafeez *et al*. 2010). Then these materials were ground with the help of an electrical grinder (Kenwood BL-480) and the powder was stored in plastic bottles for oil extraction. Essential oils were extracted from 25 g of each plant material with the help of a Soxhlet apparatus (Cheng *et al*. 2009b). Petroleum ether (250 ml) was used as an organic solvent to extract oil through the Soxhlet apparatus for 8 to 24 hrs (Bagavan *et al*. 2009). After vacuum evaporation, the extracts were used to formulate 100, 200, 300 and 400 ppm concentrated solutions by dissolving in 0.5, 1.0, 1.5 and 2.0 µl petroleum ether for larvicidal and pupicidal activities. The bioassays were carried out under laboratory conditions (26 ± 1)^{0}C and (75 ± 5)% RH in accordance with WHO technique for mosquito with the transparency adjustment (WHO 2009). From the extracted oils four different concentrations (*viz.* 100, 200, 300 and 400 ppm) including a control were used. Each concentration was replicated three times. From the average of three replicates percentage mortality was calculated by using the formula suggested by Sumroiphon *et al*. (2006). As soon as possible the dead larvae were removed from the beaker to prevent the other larvae from being affected. The percentage mortality was calculated by the following formula;

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

The mortality of the larvae at different concentrations of the extracts was calculated after the exposure of 8, 16, 24 and 48 hrs. After calculating the average larval death, the data were subjected to Probit analysis for scheming LC_{50}, LT_{50}, at 95 per cent fiducial limits and Chi-square values were calculated at $p < 0.05$ (Cheng *et al*. 2009b).

Results and Discussion

The data regarding mortality percentage could be noticed from the Fig. 1 in that about 5% mortality was observed in control treatments in case of all immature stages. Highest mortality (up to 100%) was observed in case of early instar larvae (1st and 2nd) and least (60%) was observed in case of pupae and late instar larvae (3rd and 4th) at all plant extracts. Highest mortality (75 - 100%) was observed in case of eucalyptus and least (65 - 86%) was observed in case of ginger for all immature life stages of *Ae. aegypti* as shown in the Fig. 1. The present study showed that the higher concentrations (300 and 400 ppm) resulted in highest mortality than lower concentrations (100 and 200 ppm) within time intervals of 24 and 48 hrs exposure. These results are in line with the findings of Ansari *et al.* (2000) who reported 90% mortality of *Aedes* mosquito larvae after 24 hrs exposure to peppermint (*Mentha piperita*) oil. These results are also at par with previous workers (Jang *et al*. 2002) who reported that partly purified plant extracts of Brazilian plants had great worth in controlling the mosquitoes, *Ae. aegypti* and *Culex pipiens pallens*.

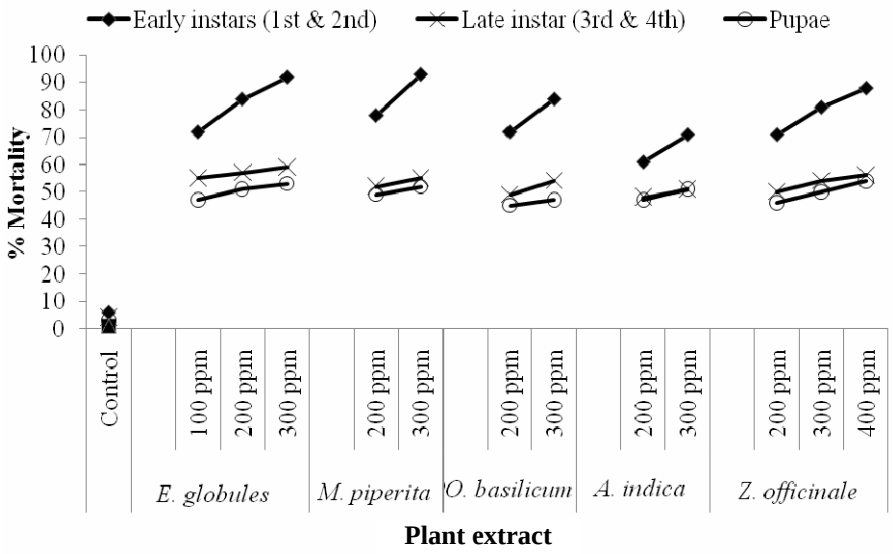

Fig 1. Mortality (%) of different life stages of *Aedes* mosquitoes after 24 hrs with different concentrations of five plant essential oils.

Moreover, medicinal plants not only contain some bioactive compounds that are proved lethal to the aquatic life stages of mosquito, but also are easily biodegradable in the environment (Abdel-Ghaffar *et al*. 2009, Apel *et al*. 2009). About 5% mortality was also observed in case of control treatments, this showed that the solvent also had a great effect on the mortality of mosquitoes (Fig. 1). These findings are in agreement with the other scientists who used different solvents (acetone, chloroform, ethyl acetate, hexane and methanol) for extraction of plant oils and different mortality percentages were observed against different life stages of mosquitoes (Anees 2008).

Table 2. Time mortality response of *Aedes aegypti* larvae and pupae against different plant extracts.

Plants	Larval instar tested	24 hrs lethal concentration (ppm)		48 hrs lethal concentration (ppm)	
		LC_{50}	p value	LC_{50}	p value
Eucalyptus (*E. globules*)	Early instar (1st and 2nd)	164	0.041	102	0.006
	Late instar (3rd and 4th)	325	0.020	276	0.007
	Pupae	370	0.019	329	0.004
Neem (*A. indica*)	Early instar (1st and 2nd)	209	0.049	156	0.042
	Late instar (3rd and 4th)	405	0.005	378	0.051
	Pupae	450	0.002	423	0.043
Peppermint (*M. piperita*)	Early instar (1st and 2nd)	178	0.004	153	0.029
	Late instar (3rd and 4th)	478	0.012	567	0.029
	Pupae	524	0.029	601	0.030
Basil (*O. basilicum*)	Early instar (1st and 2nd)	241	0.003	250	0.002
	Late instar (3rd and 4th)	501	0.003	489	0.001
	Pupae	567	0.004	541	0.001
Ginger (*Z. officinale*)	Early instar (1st and 2nd)	266	0.071	301	0.003
	Late instar (3rd and 4th)	489	0.013	531	0.002
	Pupae	543	0.015	534	0.002

Lethal concentration to kill the 50% population (LC_{50}) of *Ae. aegypti* larvae (early and late instars) and pupae is shown in the Table 2. The results showed that the best plant extracts after 24 hrs was *E. globules* with least LC_{50} value (164 ppm) for early instar larvae and the least effective plant extract was *Z. officinale* with the highest LC_{50} value (266 ppm). The least value of LC_{50} (102 ppm) for 48 hrs was recorded in case of *E. globules* with p-value 0.006 and highest LC_{50} value was (301 ppm) for ginger extract with p-values 0.003 in case of early instar larvae. In case of late instar larvae, the value of LC_{50} (325 ppm) was least from eucalyptus extract, but the highest LC_{50} (501 ppm) in case of basil for 24 hrs. The LC_{50} values for all immature stages of this mosquito were relatively lower for eucalyptus and relatively higher for ginger than other plant extracts as shown in the Table 2.

Tables 3 revealed time mortality (LT_{50}) data of *Aedes* which killed 50% of test mosquito's immature stages. The data displayed superiority of ginger oil amongst the remaining extracts at all recorded concentrations (100, 200, 300 and 400 ppm). Basil plant extracts took remarkably less time at 400 ppm (2.6 hrs) for early instar larvae and this time interval increased with decrease in concentrations while the eucalyptus oil took longer time than all other tested oils for early instar larvae as shown in the Table 2. For late instar larvae, eucalyptus extract showed least time (26 hrs) to kill 50 % population of immature stages at higher concentration (400 ppm) and ginger oil took longer time (35 hrs) at 300 ppm.

In terms of lethal time to kill 50% population of immature stages at lower concentration (100 ppm), eucalyptus extracts took maximum time, i.e. 23, 38.2 and 93 hrs (almost 4 days), followed by basil (17, 45 and 82 hrs) while ginger took least time (15, 36.7 and 45 hrs) to cause 50% mortality in case of early (1st and 2nd), late (3rd and 4th) instar larvae and pupae (Table 3). From the Table 4, we can see that 300 ppm took least time (7.1 hrs) to kill the 50% population of early instar larvae with 0.01 p values from peppermint extract. Eucalyptus oil took the maximum time (10.8 hrs) to kill the 50% of the early instar larvae with p-value 0.45. In case of pupae, 300 ppm

Table 3. Time mortality response of *Aedes aegypti* larvae and pupae against different plant extracts.

Plant extract	Life stages	100 ppm concentration				200 ppm concentration			
		LT_{50}	Slope ± SE	χ^2	p	LT_{50}	Slope ± SE	χ^2	p
Eucalyptus (*E. globules*)	Early instars	23	1.80 ± 0.15	7.92	0.02	14	1.30 ± 0.12	31.4	0.00
	Late instars	38.2	1.9 ± 0.15	15.2	0.00	37.8	0.87 ± 0.13	9.7	0.00
	Pupae	93	0.71 ± 0.17	9.8	0.00	49	0.78 ± 0.15	15.7	0.00
Neem (*A. indica*)	Early instars	17	1.6 ± 0.15	11.3	0.00	2.6	0.59 ± 0.09	35.7	0.00
	Late instars	43	1.08 ± 0.15	11.5	0.00	41	0.84 ± 0.15	10.6	0.01
	Pupae	78	0.69 ± 0.16	13.1	0.00	51	0.81 ± 0.14	12.1	0.00
Peppermint (*M. piperita*)	Early instars	18	1.7 ± 0.15	11.3	0.00	18	1.7 ± 0.15	11.3	0.00
	Late instars	47	1.02 ± 0.15	12.8	0.00	46	0.92 ± 0.15	12.7	0.00
	Pupae	85	0.70 ± 0.16	15.1	0.00	61	0.71 ± 0.15	15.1	0.00
Basil (*O. basilicum*)	Early instars	17	1.6 ± 0.15	10.3	0.00	2.6	0.59 ± 0.09	37.7	0.00
	Late instars	45	1.01 ± 0.15	12.5	0.00	43	0.85 ± 0.15	11.6	0.01
	Pupae	82	0.70 ± 0.16	13.1	0.00	56	0.71 ± 0.15	14.0	0.00
Ginger (*Z. officinale*)	Early instars	15	1.3 ± 0.13	5.6	0.59	13	1.07 ± 0.11	7.9	0.01
	Late instars	36.7	0.94 ± 0.18	4.84	0.09	36	0.87 ± 0.17	3.8	0.14
	Pupae	45	0.89 ± 0.15	5.6	0.05	42	0.78 ± 0.13	3.0	0.42

Table 4. Time mortality response of *Aedes aegypti* larvae and pupae against different plant extracts.

Plant extract	Life stages	300 ppm concentration				400 ppm concentration			
		LT_{50}	Slope ± SE	χ^2	p	LT_{50}	Slope ± SE	χ^2	p
Eucalyptus (*E. globules*)	Early instars	10.8	1.32 ± 0.17	0.01	0.45	5.6	0.93 ± 0.14	12.4	0.00
	Late instars	33.5	0.89 ± 0.12	1.4	0.00	26	1.02 ± 0.17	11.0	0.00
	Pupae	47	0.79 ± 0.13	1.0	0.00	29.5	1.5 ± 0.16	11.1	0.00
Neem (*A. indica*)	Early instars	7.3	3.11 ± 0.15	2.3	0.05	7	1.1 ± 0.12	5.2	0.02
	Late instars	33	0.74 ± 0.13	8.1	0.01	26.7	0.83 ± 0.2	5.9	0.03
	Pupae	49	0.73 ± 0.13	9.6	0.01	31	1.4 ± 0.13	6.9	0.01
Peppermint (*M. piperita*)	Early instars	7.1	3.16 ± 0.10	1.5	0.06	8	1.2 ± 0.12	6.1	0.03
	Late instars	37	0.78 ± 0.13	7.0	0.01	28	0.91 ± 0.20	6.3	0.04
	Pupae	52	0.88 ± 0.14	11.6	0.00	32	1.6 ± 0.15	7.9	0.01
Basil (*O. basilicum*)	Early instars	7.2	3.10 ± 0.10	1.4	0.05	4.9	1.1 ± 0.12	5.9	0.02
	Late instars	35	0.77 ± 0.12	9.0	0.01	27	0.90 ± 0.20	6.1	0.03
	Pupae	53	0.83 ± 0.14	13.6	0.01	29.9	1.5 ± 0.17	7.0	0.01
Ginger (*Z. officinale*)	Early instars	9	0.78 ± 0.10	0.8	0.62	8.2	0.85 ± 0.16	10.2	0.00
	Late instars	35	0.75 ± 0.14	4.7	0.25	27.5	1.04 ± 0.22	4.6	0.10
	Pupae	38	0.59 ± 0.11	2.5	0.08	37	0.43 ± 0.12	2.6	0.22

took the least time (38 hrs) interval to kill the 50% population with p-value equal to 0.08 that was highly significant but the highest time interval was (53 hrs) by the use of basil oil concentration with p-value 0.01. From the data it could be observed that eucalyptus took least time for all larval instars and pupae than all other tested plant extracts. Hidayatulfathi *et al.* (2004) also evaluated methanol extracts of some Malaysian plants as a larvicide against mosquitoes, *Ae. aegypti*. The methanol extract of *Eucalyptus globules* showed a high degree of toxicity to all mosquito species (*Anopheles maculatus* Theobald, *Culex quinquefasciatus* Say, *Ae. aegypti* L. and *Ae. albopictus* Skuse) with LC_{50} of 39.15 to 58.29 µg/ml. The results of present study are in agreement with those Hidayatulfathi *et al.* (2004) who also studied comparative efficacy of *E. globules* and *Z. officinale* against mosquito and concluded that the mortality percentage increased with an increase in concentration and exposure time. These oils exhibit larvicidal activities due to multiactions against vectors (mosquitoes) like toxicity, antifeedant action, growth regulators, antimitotic effects, oviposition repellency and cuticular damage to prevent them from moulting (Mulla and Su 1999). These actions are due to presence of different chemicals present in these oils like limonoids and azadirachtin in neem oil (Ndione *et al*. 2007), α-terpinene in eucalyptus, menthone and menthyl esters in peppermint (Robert 2001), limonene and a number of oxygenated mono-terpenes such as camphor, 1,8-cineole, borneol, and bornyl acetate (Govindarajan *et al.* 2013) in basal and mono and sesquiterpenoids in ginger (Ali *et al.* 2008). It can be concluded that medicinal plant extracts have a great potential to control different life stages of *Aedes aegypti* and could be used as a part of vector management program.

The medicinal plant extracts have good larvicidal and pupicidal activity against *Aedes aegypti* immature stages under lab conditions. So, the authors suggest that these plant extracts should be studied in the field for the control of dengue mosquito.

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(Manuscript received on 26 April, 2015; revised on 10 October, 2016)