

## Larvicidal and pupicidal action of medicinal plant extracts against dengue mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae)

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### ABSTRACT

The efficacy of essential oils from some medicinal plants as a larvicidal and pupicidal agent was tested against *Aedes albopictus*. The oils were extracted from different plant parts (leaves, branches and rhizomes) with Soxhlet apparatus using acetone as a solvent. The mortality was determined after 8, 16, 24 and 48 hours with different concentrations (100, 200, 300 and 400 ppm) along with a control treatment. Data was analysed by Probit analysis to calculate LC<sub>50</sub> and LT<sub>50</sub>. Results revealed that early immature stages were more susceptible than later ones. Ginger was more effective with the lowest LC<sub>50</sub> values after 8 and 16 hours against all tested life stages followed by peppermint, basil, eucalyptus and neem. In addition, basil was efficacious after 24 and 48 hours. Therefore, we conclude that these oils can be used in combination for the management of mosquito immature stages.

**Key words:** Dengue mosquitoes, Larvicidal, Plant oils, Pupicidal.

### INTRODUCTION

Some mosquito borne diseases such as dengue and West Nile virus have neither vaccine nor treatment, and the best solution is to control the vector mosquitoes (*Aedes albopictus* and *Ae. aegypti*) by using source reduction, synthetic chemicals, biological or plant extracts (Ravikumar *et al.*, 2011). Due to harmful effects of chemicals, mosquito resurgences and development of mosquito resistance (Naz *et al.*, 2014), the trend is now to gradually shift towards using the plant extracts because they contain a number of bioactive compounds (Akram *et al.*, 2010) that are biodegradable, species specific and hence safe for the environment and other non-target organisms (Bokhari *et al.*, 2014). Moreover, medicinal plants are cheaply available and rich source of bioactive compounds like insect growth regulators, antifeedents, repellent, larvicidal, ovicidal and reduction of fecundity and fertility (Borah *et al.*, 2010). Many plant extracts have been studied as larvicides, pupicides or repellents against the mosquitoes (Ravikumar *et al.*, 2011; Nasir *et al.*, 2015a). The earlier biologists focused their study against a single life stage (Hafeez *et al.*, 2011) and a little study was carried out on all life stages of mosquitoes (Nasir *et al.*, 2015b; Nasir *et al.*, 2015c). The purpose of this study was to test the larvicidal and pupicidal efficacy of some medicinal plants, i.e., eucalyptus (*Eucalyptus globules*),

neem (*Azadirachta indica*), peppermint (*Mentha piperita*), basil (*Ocimum basilicum*) and ginger (*Zingiber officinale*) against all immature life stages (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> larval instars and pupae) of *Ae. albopictus*.

### MATERIALS AND METHODS

Adult mosquitoes were collected using an aspirator from a forest area (Gutwala forest) near Faisalabad, Punjab (31° 28' 41" N, 73° 12' 30" E and 185m above the sea level) as shown in Fig. 1. They were transported to the Department of Zoology, Government College University, Faisalabad for identification and rearing in plastic cages (60x60x60 cm). The females laid eggs after feeding on the blood of white rat. The eggs hatched out in fresh water at 26±1°C and 75±5% RH in the laboratory. Fish diet (plant based powder form commercially available) was added in the hatching tray for feeding the larvae. The 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instar larvae and pupae were used for the bioassay (Kumar *et al.*, 2011). Plant extracts were obtained from eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), ginger (*Zingiber officinale*), basil (*Ocimum basilicum*) and peppermint (*Mentha piperita*) with Soxhlet apparatus and using acetone as solvent. The extracts were tested for their larvicidal (against 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instar larvae) and pupicidal (against pupae) activity with different concentrations (100, 200, 300 and 400 ppm) after different time intervals (8, 16, 24 and 48 hrs) using WHO protocol

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**Fig 1:** Map showing the site of mosquito collection

(WHO, 2009). The study was repeated for three times with a control treatment (Sumroiphon *et al.*, 2006). Twenty larvae/pupae were used for each treatment. After calculating percent mortality, the data was subjected to probit analysis for  $LC_{50}$  and  $LT_{50}$  values (Finney, 1971).

## RESULTS AND DISCUSSION

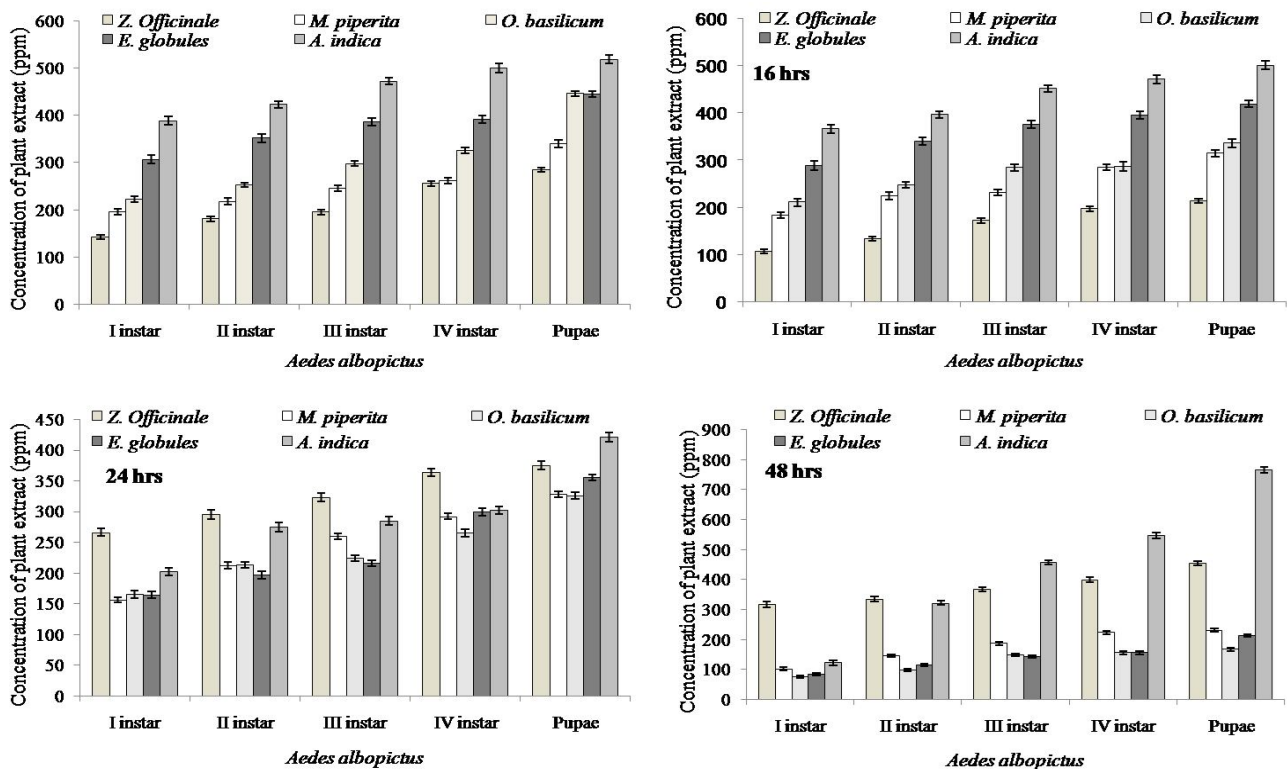
The highest mortality (100 %) was seen in 1<sup>st</sup> instar larvae and lowest (20 %) in 4<sup>th</sup> instar larvae and pupae. Some mortality (5%) was also seen in the control treatment. The eucalyptus oil provided highest mortality (40-100 %) depending on concentration and larval instar while ginger provided lowest mortality to all immature stages of *Aedes albopictus* Skuse. The results showed that the most effective plant extracts after 8 hours were from *Z. officinale* with lowest  $LC_{50}$  value (142 ppm) for 1<sup>st</sup> instar larvae and the least effective from *A. indica* with the highest value (389 ppm). In case of 2<sup>nd</sup> instar larvae, the lowest  $LC_{50}$  values were (197 and 98 ppm) to kill the 50% population from eucalyptus and basil extracts. The highest  $LC_{50}$  values were (295 and 334 ppm) from ginger extract for 24 and 48 hours, respectively. The  $LC_{50}$  values for all immature stages of this mosquito were relatively low for basil and relatively high for neem than other plant extracts as shown in Fig. 2. The data showed that the values of  $LT_{50}$  were less for early larval stages (1<sup>st</sup> and 2<sup>nd</sup> instars) and more time was needed to kill the 50% population of later immature stages (3<sup>rd</sup>, 4<sup>th</sup> instars and pupae) as shown in Table 1.

The immature stages of mosquitoes are most susceptible for control because these are aquatic that makes it easy to target them in this habitat. The use of conventional pesticides causes many health issues for the human beings. Botanicals especially aromatic plants are more promising in this regard because these are rich source of many bioactive compounds. In the present study, 5 medicinal plants were studied for their effects against different life stages of mosquito. The present study showed that the higher concentrations (300 and 400 ppm) resulted in the highest mortality than lower concentrations (100 and 200 ppm) among larger time intervals, i.e., 24 and 48 hrs. These results are in agreement with previous workers (Jang *et al.*, 2002) who proved that partly purified plant extracts had great worth in controlling the *Aedes* and *Culex* mosquitoes.

Moreover, medicinal plants not only contain some bioactive compounds that are proven to be lethal for aquatic life stages of mosquitoes but also are easily biodegradable in the environment (Kovendan *et al.*, 2008). This study showed that these medicinal plant extracts (*Zingiber officinale* & *Mentha piperita*) have good larvicidal and pupicidal activity against the *Ae. albopictus* immature stages under laboratory conditions. *Zingiber officinale* showed excellent results for controlling different life stages of *Aedes* mosquitoes in the present study. These results are at par with Pushpanathan *et al.* (2008). Therefore, we suggest that these plant extracts should be studied in the field for the control of dengue mosquito. The solvent used for oil extraction also had strong effects on the efficacy of plant oils against immature stages of mosquitoes (Anees, 2008). Our experiment also showed that the solvent had a valuable effect on the mortality of different immature stages of mosquitoes (up to 5% mortality after 48 hrs in case of 1<sup>st</sup> instar larvae in the control treatments and more than 3.5% in 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instars and pupae). The variability in the efficacy of plant oils against different mosquito species are common due to variations in quality and quantity of active compounds (Amer and Mehlhorn, 2006a), solvent used, age of larvae and concentration used. So, isolation and purification of bioactive compounds could be the next step for the efficient use of plant extracts as mosquitocide. These plant extracts should be applied in the field under natural conditions with a sprayer to check their efficacy because Prabhu *et al.* (2011) reported more than 90 % mortality against mosquito (*Anopheles*) larvae after about 3 days.

**Table 1:** Time mortality response of *Aedes albopictus* larvae and pupae against different plant extracts.

| Plant extract                             | Life stages            | 300 ppm concentration |            |      | 400 ppm concentration |            |      |
|---|------------------------|-----------------------|------------|------|-----------------------|------------|------|
|   |                        | LT <sub>50</sub>      | Slope±SE   | P    | LT <sub>50</sub>      | Slope±SE   | P    |
| Eucalyptus ( <i>Eucalyptus globules</i> ) | 1 <sup>st</sup> instar | 8.6                   | 1.31 ±0.16 | 0.5  | 3.5                   | 0.95 ±0.13 | 0.00 |
|   | 2 <sup>nd</sup> instar | 17.5                  | 1.3 ±0.12  | 0.00 | 11.9                  | 1.1 ±0.15  | 0.00 |
|   | 3 <sup>rd</sup> instar | 31.3                  | 0.88 ±0.13 | 0.00 | 24                    | 1.04 ±0.19 | 0.00 |
|   | 4 <sup>th</sup> instar | 39                    | 0.99 ±0.14 | 0.58 | 26.2                  | 0.89 ±0.21 | 0.02 |
|   | Pupae                  | 45                    | 0.77 ±0.14 | 0.00 | 28                    | 1.6 ±0.14  | 0.00 |
| Neem ( <i>Azadirachta indica</i> )        | 1 <sup>st</sup> instar | 5.1                   | 3.12 ±3.15 | 0.05 | 5                     | 1.2±0.13   | 0.03 |
|   | 2 <sup>nd</sup> instar | 18.7                  | 1.15 ±0.12 | 0.00 | 16.7                  | 1.13 ±0.12 | 0.00 |
|   | 3 <sup>rd</sup> instar | 31                    | 0.73 ±0.13 | 0.01 | 22.4                  | 0.84 ±0.21 | 0.03 |
|   | 4 <sup>th</sup> instar | 42                    | 0.73±0.14  | 0.04 | 28.9                  | 0.82±0.17  | 0.15 |
|   | Pupae                  | 47                    | 0.72 ±0.13 | 0.01 | 29                    | 1.5±0.14   | 0.01 |
| Peppermint ( <i>Mentha piperita</i> )     | 1 <sup>st</sup> instar | 5.1                   | 3.13 ±3.15 | 0.07 | 6                     | 1.1 ±0.13  | 0.04 |
|   | 2 <sup>nd</sup> instar | 20.7                  | 1.18 ±0.12 | 0.00 | 20.7                  | 1.18 ±0.12 | 0.00 |
|   | 3 <sup>rd</sup> instar | 35                    | 0.79 ±0.13 | 0.01 | 26                    | 0.93 ±0.21 | 0.04 |
|   | 4 <sup>th</sup> instar | 45                    | 0.7 ±0.15  | 0.04 | 29                    | 0.82 ±0.19 | 0.18 |
|   | Pupae                  | 51                    | 0.86 ±0.15 | 0.00 | 30                    | 1.7 ±0.16  | 0.01 |
| Basil ( <i>Ocimum basilicum</i> )         | 1 <sup>st</sup> instar | 5.2                   | 3.12 ±3.15 | 0.06 | 2.9                   | 1.2±0.13   | 0.03 |
|   | 2 <sup>nd</sup> instar | 19.7                  | 1.16 ±0.12 | 0.00 | 19.7                  | 1.14 ±0.12 | 0.00 |
|   | 3 <sup>rd</sup> instar | 32                    | 0.76 ±0.13 | 0.01 | 23                    | 0.91 ±0.21 | 0.04 |
|   | 4 <sup>th</sup> instar | 64                    | 0.71 ±0.15 | 0.04 | 27                    | 0.81±0.19  | 0.16 |
|   | Pupae                  | 50                    | 0.82 ±0.15 | 0.01 | 28.2                  | 1.6±0.16   | 0.01 |
| Ginger ( <i>Zingiber officinale</i> )     | 1 <sup>st</sup> instar | 7                     | 0.79 ±0.10 | 0.66 | 6.2                   | 0.88 ±0.17 | 0.00 |
|   | 2 <sup>nd</sup> instar | 23                    | 0.73 ±0.13 | 0.00 | 17                    | 1.09 ±0.19 | 0.02 |
|   | 3 <sup>rd</sup> instar | 33                    | 0.74 ±0.15 | 0.25 | 25.5                  | 1.04 ±0.23 | 0.10 |
|   | 4 <sup>th</sup> instar | 35                    | 0.96 ±0.16 | 0.13 | 34                    | 0.66± 0.21 | 0.02 |
|   | Pupae                  | 36                    | 0.58 ±0.12 | 0.08 | 35                    | 0.45 ±0.13 | 0.23 |



**Fig 2:** LC<sub>50</sub> of five plant extracts against different immature stages of *Ae. albopictus* at different time intervals

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