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ORIGINAL PAPER

Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae)

Kandaswamy Kalaivani · Sengottayan Senthil-Nathan · Arunachalam Ganesan Murugesan

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Abstract The larvicidal activity of hydrodistillate extracts from *Mentha piperita* L. *Ocimum basilicum* L. *Curcuma longa* L. and *Zingiber officinale* L. were investigated against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae).The results indicated that the mortality rates at 80, 100, 200 and 400 ppm of *M. piperita, Z. officinale*, *C. longa* and *O. basilicum* concentrations were highest amongst all concentrations of the crude extracts tested against all the larval instars and pupae of *A. aegy*pti. Result of log probit analysis (at 95% confidence level) revealed that lethal concentration LC_{50} and LC_{90} values were 47.54 and 86.54 ppm for *M. piperita*, 40.5 and 85.53 ppm for *Z. officinale*, 115.6 and 193.3 ppm for *C. longa* and 148.5 and 325.7 ppm for *O. basilicum*, respectively. All of the tested oils proved to have strong larvicidal activity (doses from 5 to 350 ppm) against *A. aegypti* fourth instars, with the most potent oil being *M. piperita* extract, followed by *Z. officinale*, *C. longa* and *O. basilicum*. In general, early instars were more susceptible than the late instars and pupae. The results achieved suggest that, in addition to their medicinal activities, Lamiaceae and Zingiberaceae plant extracts may also serve as a natural larvicidal agent.

Introduction

In last two decades, the use of chemical insecticides in mosquito control method has resulted in instability of the environment, mosquito resistance, mosquito resurgences and toxic to non-target organisms including natural enemies in the agriculture ecosystem (Greenwood and Mutabingwa 2002). Hence, it has now become important to find an alternative means of mosquito control method, which can eliminate the use of chemical pesticides.

Naturally available microbial pesticides, including plant derivatives, are receiving increased revelation in scientific community, and they may serve as alternatives to chemical pesticides and as key components of integrated vector control (Lacey and Orr 1994). Botanical pesticides and essential oils are the essential alternatives for chemical as they possess an array of chemicals that includes larvicidal, adulticidal and repellency activities against medically important vectors that transmit disease to humans (Shaalan et al. 2005).

Mosquitoes are one of the most important insect pests that affect the health and well being of humans and domestic animals worldwide. Female mosquitoes require a blood meal for egg production, and they produce a painful bite as they feed. While feeding, they can transmit a number of disease-causing organisms to humans and animals. The diseases these organisms cause includes: encephalitis, dengue fever, filariasis, yellow fever and malaria (Lounibos 2002). There are some 3,300 species of mosquitoes belonging to 41 genera, all contained in the family Culicidae. This family is divided into three subfamilies including Toxorhynchitinae, Anophelinae (anophelines) and Culicinae (culicines) (Molyneux 1994; Service 1996)

The most important pest and vector species belong to the genera *Anopheles*, *Culex*, *Aedes*, *Ochlerotatus*, *Psorophora*, *Haemagogus* and *Sabethes*. *Anopheles* species, as well as transmitting malaria, are vectors of filariasis (*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*) and a few

K. Kalaivani · S. Senthil-Nathan (✉) · A. G. Murugesan
Sri Paramakalyani Centre for Excellence in Environmental Sciences (SPKCEES), Manonmaniam Sundaranar University, Alwarkurichi-627 412,
Tirunelveli, Tamil Nadu, India
e-mail: senthil@msuniv.ac.in

S. Senthil-Nathan
e-mail: senthilkalaidr@hotmail.com

arboviruses. Certain *Culex* species transmit *W. bancrofti* and a variety of arboviruses (Horsfall 1972; Molyneux 1994).

Aedes species are important vectors of yellow fever, dengue, encephalitis viruses and many other arboviruses, and in a few restricted areas they are also vectors of *W. bancrofti* and *B. malayi*. Species in the very closely related genus *Ochlerotatus* also transmit filariasis and encephalitis viruses. *Mansonia* species transmit *B. malayi* and sometimes *W. bancrofti* and a few arboviruses. Dengue fever continues in persistent epidemic afflicting millions and causing thousands of deaths annually which is transmitted by *Aedes aegypti* (Service 1996.)

Recent research on insecticidal action of plant materials especially secondary metabolites and essential oils resulted that they are eco-friendly, biodegradable and species specific (Senthil-Nathan 2007; Senthil-Nathan et al. 2006a,b, 2008; Rattan 2010). Essential oils can be used as an alternative to synthetic insecticides for vector control programmes. Essential oils are natural volatile substances found in a variety of plants. When isolated from plants, essential oils are not usually extracted as chemically pure substances but consist of mixtures of many compounds. It is well known that plant-derived natural products are extensively used as biologically active compounds (Zebitz 1984). Among them, essential oils were the first preservatives used by man, originally in their natural state within plant tissues and then as oils obtained by water distillation (Bakkali et al. 2008). Essential oils composed by isoprenoid compounds, mainly mono- and sesquiterpenes are the carriers of the smell found in the aromatic plants (Franzios et al. 1997). Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavour enhancers in many food products, as odorants in fragrances and as insecticides (Zhu et al. 2001).

Larvicidal and adulticidal activities of plant essential oils have been descried against *Culex*, *Aedes*, and *Anopheles* mosquito species. Most of the study has focused on lethal concentration and mortality against single instar but their action on total life cycle including pupa is still an obstacle. Hence, an attempt has been made to find out the effect of *Mentha piperita*, *Ocimum basilicum*, *Curcuma longa* and *Zingiber officinale* on total larval instar and pupa of *A. aegypti*.

Materials and methods

Mosquito culture

A. aegypti culture has been maintained in the Biopesticides and Environmental Toxicology Laboratory (BET Lab), SPK Centre for Excellence in Environmental Sciences since at least 2007, without exposure to pesticides. They were maintained at 27±2°C and 75–85% RH under a 14:10 L/D photoperiod. Larvae were fed a diet of Brewers yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (23×23×32 cm) where adults emerged. Adults were maintained in 30×30×30-cm glass cages. Adults were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day 5, post-emergences adults were deprived of sugar for 12 h, then provided with a mouse placed in resting cages overnight for blood feeding by females. Adult mosquitoes were maintained under the same environmental conditions as the larvae.

Plant extracts

Fresh leaves of *M. piperita*, *O. basilicum* and the rhizomes of *Z. officinale* and *C. longa* were picked from the garden of this Centre, and the leaves were collected (250 g) during morning hours. To extract and quantify the volatile oil, a weight of 250 g of fresh herb and rhizomes were separately subjected to hydro-distillation for over 3 h using a modified Clevenger apparatus according to Guenther (1955). The volume of the extracted essential oil was determined and recorded on the basis of the herb fresh weight. The essential oil was extracted with petroleum ether, which was then evaporated in vacuum in a rotary evaporator (Buchi, Switzerland). The oil was then stored at −5°C until needed.

Bioassays and larval mortality

Bioassays were performed in first to fourth instars of *A. aegypti* using concentration from 2.5 to 350 ppm. Petroleum ether served as a control. A minimum of ten larvae/concentration were used for all the experiments, which were replicated five times. The lethal concentrations (both LC_{50} and LC_{90}) were calculated using probit analysis (Finney 1971).

For mortality studies, ten larvae each of first, second, third and fourth instars and pupae were introduced in 250-ml glass beakers containing various concentrations (2.5 to 400 ppm) of the essential oils supplemented with 50 mg/l of yeast extract. A control was also maintained. The treatments were replicated five times and each replicate set contained one control (Senthil-Nathan et al. 2005). The percentage mortality was calculated by using the formula (1) and corrections for

mortality when necessary were done by using Abbott's (1925) formula (2)

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

$$\text{Corrected percentage of mortality} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in C after treatment}}\right) \times 100 \quad (2)$$

Where: n = number of larvae, T = treated, C = control

Statistical analysis

Data from mortality experiments were subjected to analysis of variance (ANOVA of arcsine, logarithmic and square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test (P=0.05) (Snedecor and Cochran 1989). The relationship between probit and log concentrations were established as probit equations and probit regression lines were drown for each of larval stage.

Results

Exposure of essential oil in the mosquito larval diet increased mortality in all larval instars. The effect on larval mortality was concentration dependent. The LC_{50} and LC_{90} values of essential oils after 24 and 48 h against the larvae are shown in Figs. 1, 2, 3 and 4. Essential oils were potent in all experiments with least LC_{50}. It is clearly pointed out that the high concentration of the respective essential oils of plants produced high mortality in the initial larval stages.

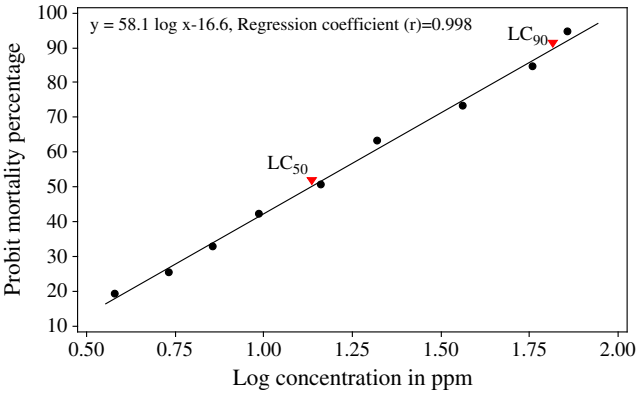

Fig. 2 Lethal concentrations (LC_{50} and LC_{90}) of *Z. officinale* against the *A. aegypti*

The most potent oil was *M. piperita* oil extract with an LC_{50} and an LC_{90} of 47.54 and 86.54 ppm, respectively, after 24 and 48 h. This was closely followed by *Z. officinale* oil extract which showed an LC_{50} and an LC_{90} of 40.5 and 85.53 ppm after 24 and 48 h, respectively. *C. longa* oil extract had an LC_{50} 115.6 ppm and an LC_{90} of 193.3 ppm, respectively, after 24 h; while, the least potent among the four tested oils was *O. basilicum* leaf oil extract, with an LC_{50} and LC_{90} of 148.5 and 325.7 ppm, respectively, after 24 h. Therefore, essential oils from *M. piperita* and *Z. officinale* appear to have strong larvicidal activity against the larvae of *A. aegypti* as their LC_{50} values were below 50 ppm.

The survival of the larvae were significantly reduced by all the oil formulation treatments with 80, 100, 200 and 400 ppm of *M. piperita*, *Z. officinale*, *C. longa* and *O. basilicum* (P<0.05). The tests also showed that larval

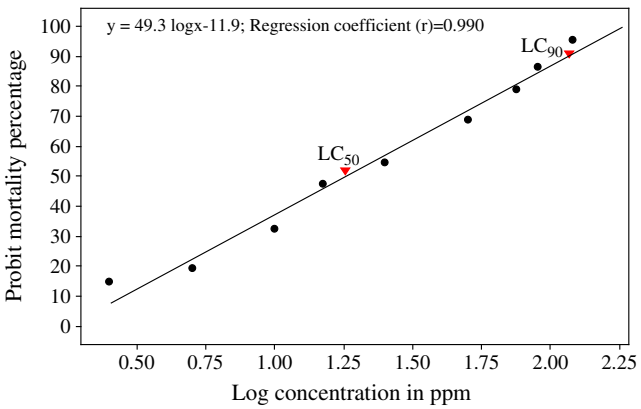

Fig. 1 Lethal concentrations (LC_{50} and LC_{90}) of *M. piperita* against the *A. aegypti*

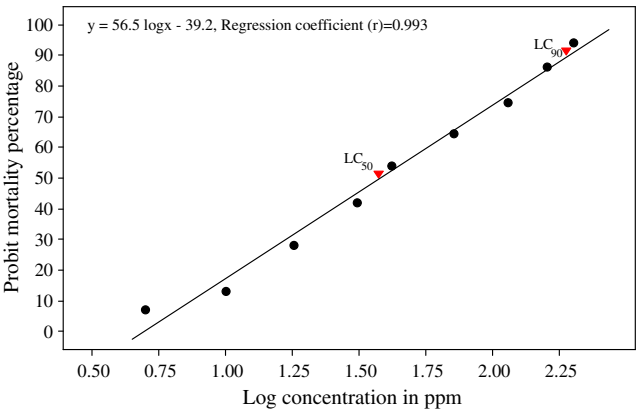

Fig. 3 Lethal concentrations (LC_{50} and LC_{90}) of *C. longa* against the *A. aegypti*

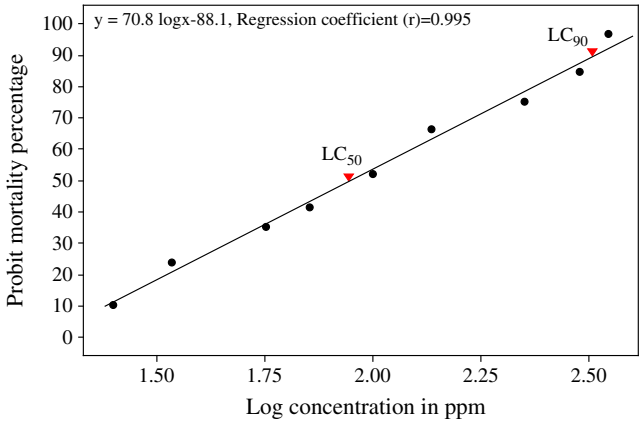

Fig. 4 Lethal concentrations (LC_{50} and LC_{90}) of *O. basilicum* against the *A. aegypti*

development times were significantly prolonged at concentrations equal to or higher than 80 ppm of the all oil formulations. In addition, pupation was significantly inhibited at concentrations higher than 80 ppm ($P<0.05$).

Figures 5, 6, 7 and 8 show the impacts of the four oil formulations on the mortality of *A. aegypti*. Mentha oil was highly larvicidal at high concentrations (80 ppm), but this activity declined progressively as the dose decreased (Fig. 5). At concentrations above 60 ppm of the mentha oil formulation, over 80% of the observed mortality occurred within the first 24 h, ($F=32.55$; $df=4$; $P<0.001$ for first instar, $F=28.02$; $df=4$; $P<0.001$ for second instars and $F=43.63$; $df=4$; $P<0.001$ for third instars larvae) while at lower concentrations the rate of mortality was very slow and some larvae lived as long as 5 to 6 days before they either pupated or died.

Thus, lethal effects on early larval instars appear to greatly reduce survival of later instars. First instar larvae were most susceptible in bioassay experiments with the lowest lethal concentrations Figs. 5, 6, 7 and 8).

Zinger oil exhibit the 100% mortality for all the four instars such as first instars ($F=31.32$; $df=4$; $P<0.001$), second instar ($F=35.69$; $df=4$; $P<0.001$), third instars ($F=36.96$; $df=4$; $P<0.001$), fourth instars ($F=37.56$; $df=4$; $P<0.001$) and pupal stage ($F=35.88$; $df=4$; $P<0.001$) at 100 ppm, which effectively control the *A. aegypti* than the *O. basilicum* and *C. lango* oil extract at 24 h.

C. longa oil extract exhibit 100% mortality in first instars ($F=38.44$; $df=4$; $P<0.001$) and second instars ($F=$

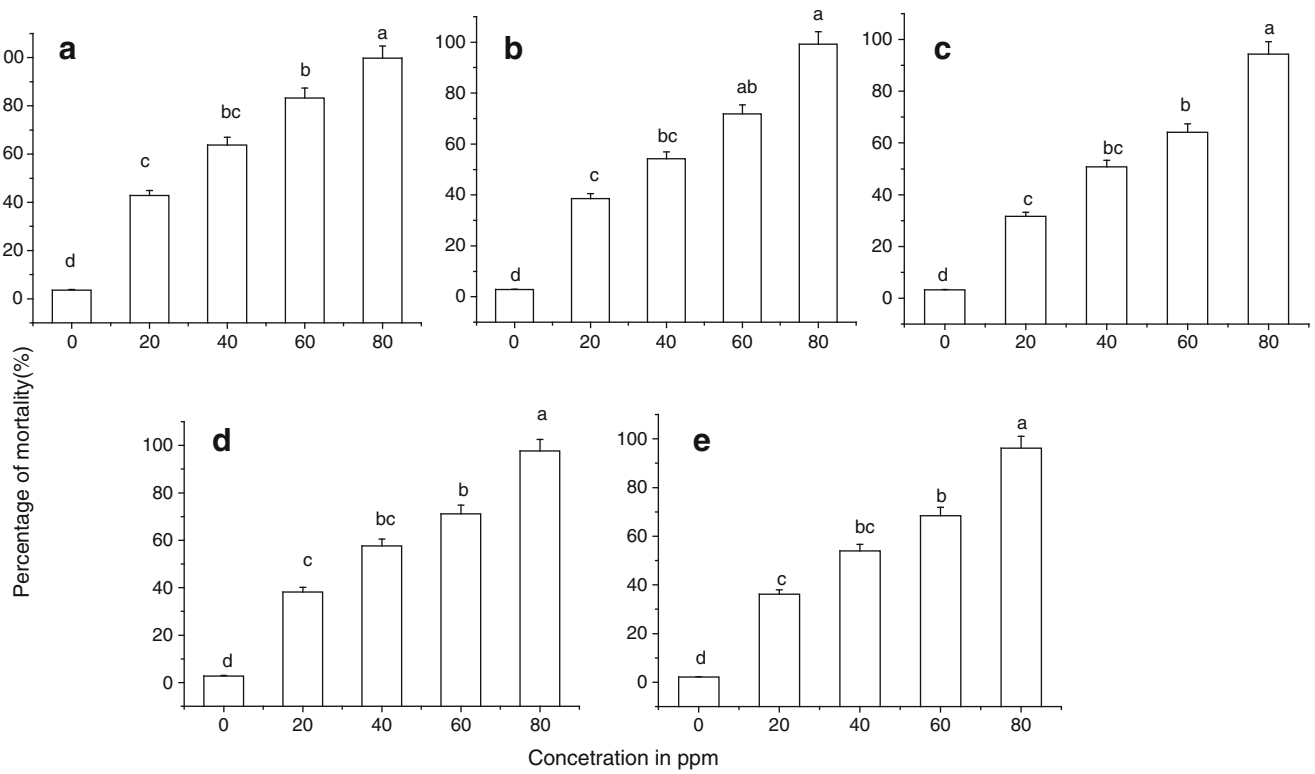

Fig. 5 Percentage mortality of *A. aegypti* after treatment with essential oil from *M. piperita*. Means (±standard error (SEM)) followed by the *same letters above bars* indicate no significant difference ($P<0.05$) in a Tukey's test (**a** first instar, **b** seconnd instar, **c** third instar, **d** fourth instar and **e** pupae)

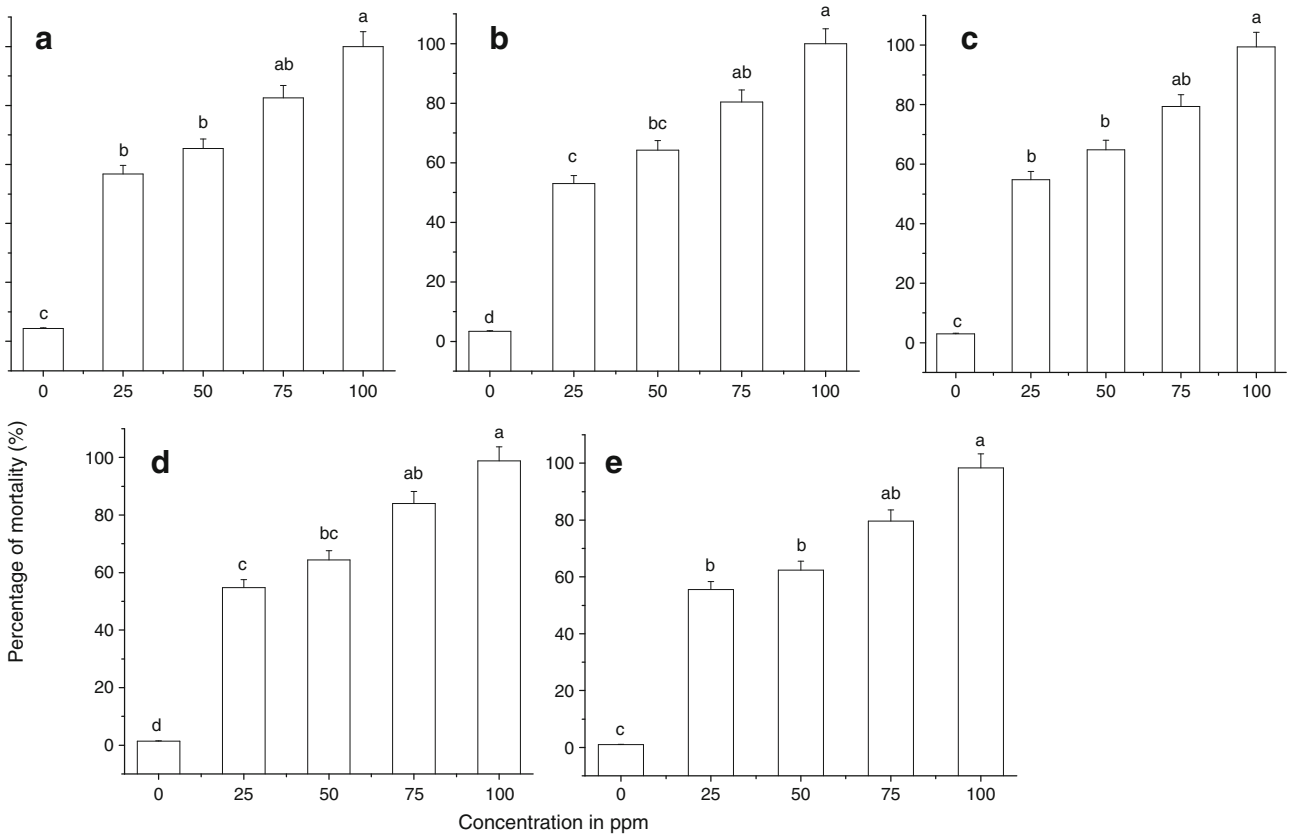

Fig. 6 Percentage mortality of *A. aegypti* after treatment with essential oil from *Z. officinale*. Means (±SEM) followed by the *same letters above bars* indicate no significant difference ($P<0.05$) in a Tukey's test (**a** first instar, **b** seconnd instar, **c** third instar, **d** fourth instar and **e** pupae)

37.02; $df=4$; $P<0.001$), whereas the third ($F=32.55$; $df=4$; $P<0.001$) and fourth instars ($F=34.62$; $df=4$; $P<0.001$) and pupal stage ($F=30.88$; $df=4$; $P<0.01$) shows above 95% mortality at 200 ppm, the mortality varied significantly with various concentration in ppm. The dose was 4.5 times higher concentration than the mentha oil formulation, and 2.2 times higher concentration than the zinger oil formulation which showed an LC_{50} of 27.5 and 39.5 ppm, respectively.

Figure 8 shows that the mortality rate of the *O. basilicum* oil formulation was approximately five times higher than that of the mentha oil formulation. At 400 ppm the *O. basilicum* formulation produced 98.5% mortality in first instar ($F=32.35$; $df=4$; $P<0.001$), 97.3% mortality in second instar ($F=29.10$; $df=4$; $P<0.001$), 97.5% in third instar ($F=26.98$; $df=4$; $P<0.001$), 95.7% in fourth instar ($F=38.29$; $df=4$; $P<0.001$) and 96.5% in pupal ($F=32.65$; $df=4$; $P<0.001$) stage, respectively.

Discussion

The oil extract obtained from the *M. piperita*, *Z. officinale*, *C. longa* and *O. basilicum* were an effective larvicide agent against the *A. aegypti* larvae; it was highly toxic to mosquito larvae and inhibited the development of pupae. The high rates of larval mortality observed at higher concentrations (80, 100, 200 and 400 ppm of *M. piperita*, *Z. officinale*, *C. longa* and *O. basilicum* oil extract, respectively) within a 48-h exposure indicate the high toxicity of the product.

The partially purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts (Jang et al. 2002; Tripathi et al. 2002). Essential oils extracted from the plants may be an alternative source of mosquito larval control agents since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management

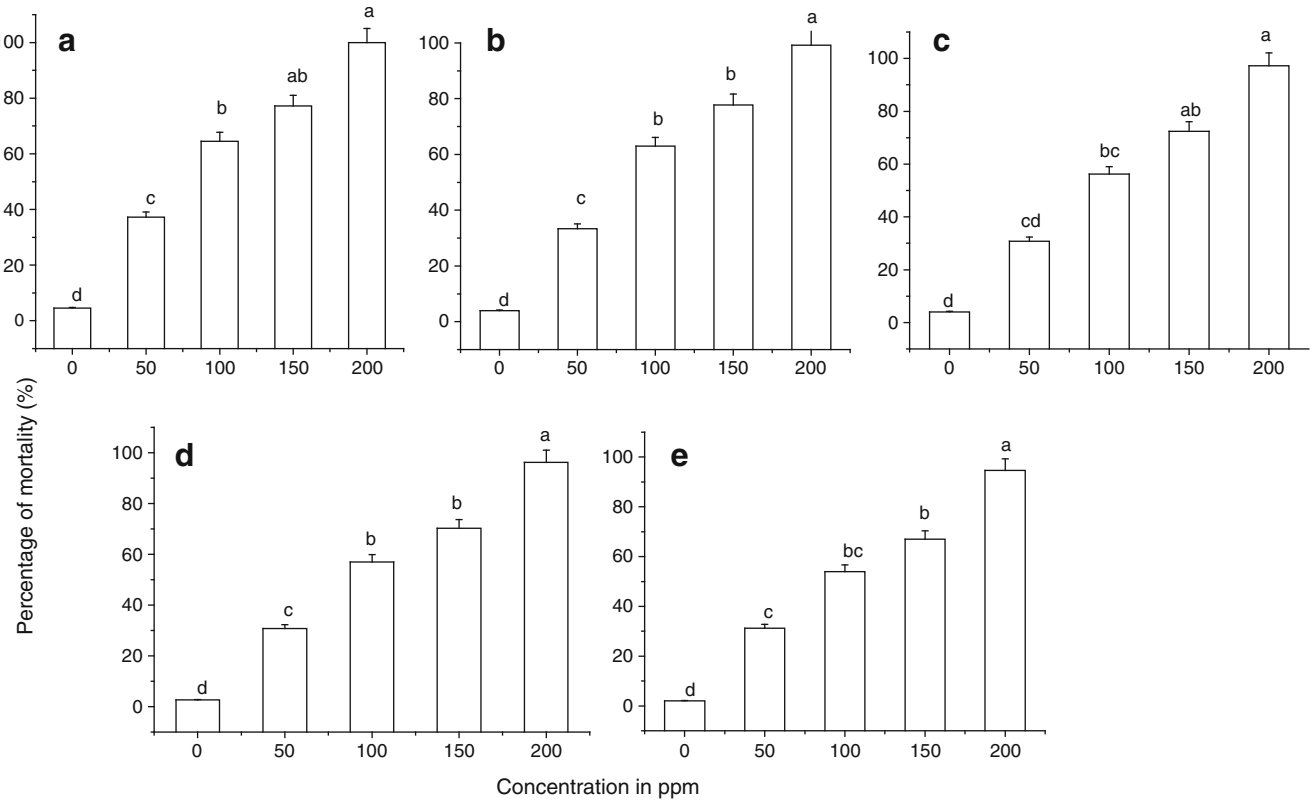

Fig. 7 Percentage mortality of *A. aegypti* after treatment with essential oil from *C. longa*. Means (±SEM) followed by the *same letters above bars* indicate no significant difference ($P<0.05$) in a Tukey's test (**a** first instar, **b** seconnd instar, **c** third instar, **d** fourth instar and **e** pupae)

programmes. In fact, many researchers have reported on the effectiveness of plant essential oils against mosquito larvae and human parasites, and the recent examples are studied by Chantraine et al. (1998), Amer and Mehlhorn (2006), Aivazi and Vijayan (2008), Abdel-Ghaffar et al. (2009) and Apel et al. (2009). Senthil-Nathan et al. (2005) described that the plant-based compounds from neem oil such as limonoids may be an effective alternative to conventional synthetic insecticides for the control of *Anopheles stephensi*. In addition, Ansari et al. (2000) found that application of *M. piperita* oil at 3 ml/m^2 of water surface area resulted in 100% mortality within 24 h for *Culex quinquefasciatus*, 90% for *A. aegypti* and 85% for *A. stephensi*. Furthermore, Anees (2008) have reported that the acetone, chloroform, ethyl acetate, hexane and methanol leaf and flower extracts of *O. sanctum* were studied against the fourth instar larvae of *A. stephensi* and *C. quinquefasciatus*. The highest larval mortality was found in chloroform and hexane extract of *O. sanctum* against the larvae of *A. aegypti* and *C. quinquefasciatus*, respectively.

Our study revealed that the *Z. officinale* oil extract shows the highest mortality for all the stages. It was also proved by Pushpanathan et al. (2008). They observed the larval mortality within 24 h after treatment with *Z. officinale* oil extract at 50.78 ppm. Furthermore, Lin et al. (2010) found that the pure secondary metabolites from *Z. officinale* including shogaol, gingerol, gingerol and shogaol have larvicidal activity against the parasitic round worm, *Angiostrongylus cantonensis* (Chen). The growth regulatory effect in lower dose is the most important physiological effect of essential oil from the leaves and rhizomes of *C. longa* L. The rhizome oil was more toxic to the mosquito larvae, exhibiting 100% mortality at 192 ppm with an LC_{50} of 192 ppm. The observed toxicities were also found to be concentration dependent. It was also proved by Ajaiyeoba et al. (2008) that essential rhizome oil from *C. long* was most potent larvicide against the *Anopheles gambiae* with an LC_{50} of 0.017 mg/ml. Furthermore, Tripathi et al. (2002) studied that *C. longa* leaf oil possesses toxic, antifeedant, oviposition-deterrent and ovicidal activity against *Rhyzopertha dominica* F. (lesser grain borer), *Sitophilus oryzae* L. (rice weevil) and *Tribolium castaneum* Herbst (red flour beetle). The larvicidal mode of action of essential oils was investigated by Corbet et al. (1995) who distinguished the susceptibility of mosquito larvae and pupae to surface

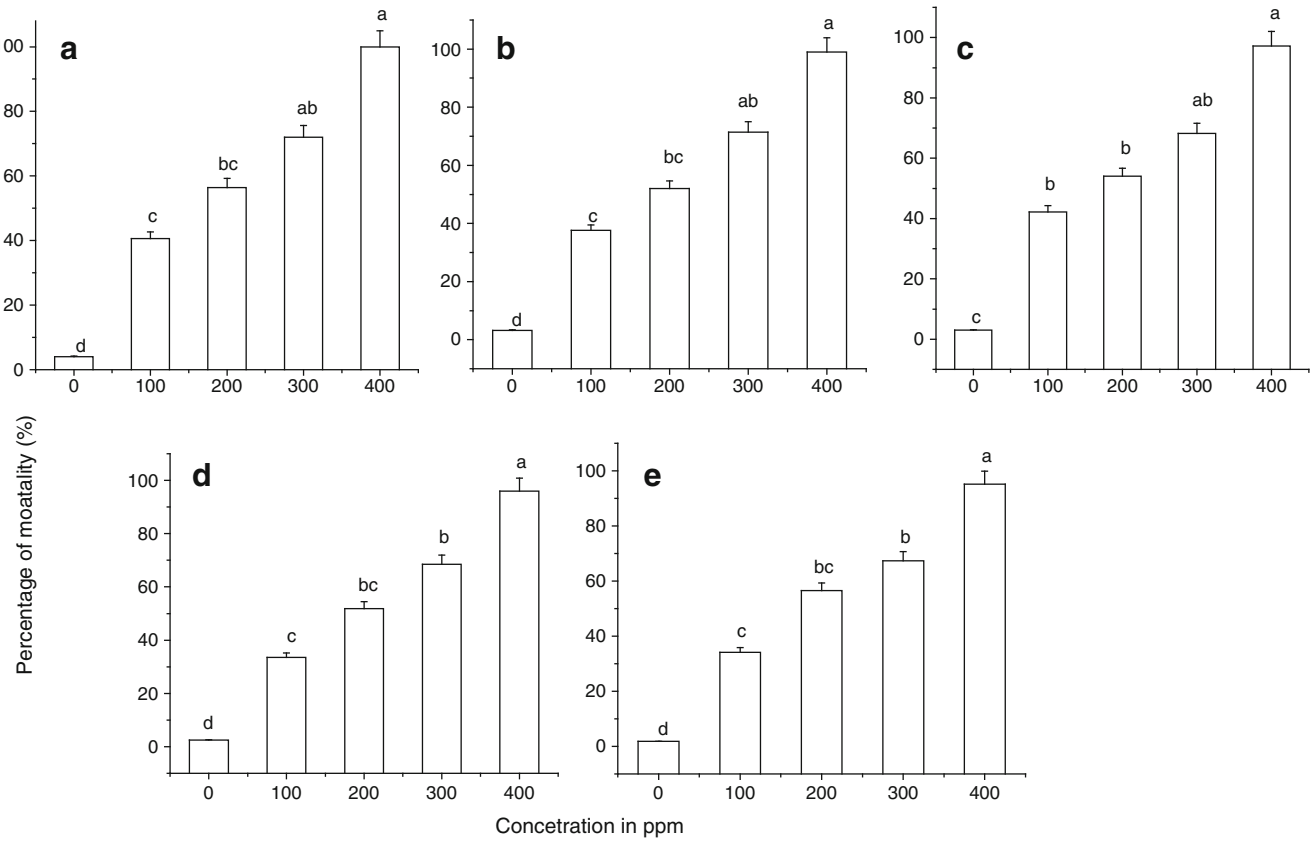

Fig. 8 Percentage mortality of *A. aegypti* after treatment with essential oil from *O. basilicum*. Means (±SEM) followed by the *same letters above bars* indicate no significant difference ($P<0.05$) in a Tukey's test (**a** first instar, **b** seconnd instar, **c** third instar, **d** fourth instar and **e** pupae)

materials entering their tracheal system, observing that essential oils increased the tendency to tracheal flooding and chemical toxicity.

An assessment of the results presented here with the result from various other studies on the efficacy of different essential oil products is difficult. There are various differences with the prior studies, notably because of differences in the source of products, concentrations of the secondary metabolites of the products, types of mosquitoes tested, and parts of the plant from which the products were extracted (Okumu et al. 2007; Knio et al. 2008).

The results of this study will add to a great reduction in the application of synthetic insecticides, which in turn raise the opportunity for eco-friendly control of various vectors by botanical pesticides. Since these are often active against a limited number of species including specific target insects, inexpensive, biodegradable and highly suitable for use in mosquito control programme (Alkofahi et al. 1989; Senthil-Nathan et al. 2006a, b), they could lead to develop possible safer insect control agents. Plant allelochemicals may be fairly useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Senthil-Nathan et al. 2005).

Conclusions

The present study demonstrates that essential oils from the leaves and rhizome of *M. piperita*, *Z. officinale*, *C. longa* and *O. basilicum* have strong larvicide potential against the *A. aegypti*. Application of these oils could be very useful to reduce the larvae of *A. aegypti* breeding in wide variety of containers, ranging from watering cans and discarded plastic bags to ground depressions and blocked roof gutters. This would offer an eco-friendly and less expensive way to reduce the problem of the *A. aegypti*, especially that all of the examined plants are commonly available and used in India. Elaborate studies on mode of action in mosquito physiology and synergism with microbial insecticides under field conditions are in progress.

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