Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Effect of leaf essential oil of *Coccinia indica* on egg hatchability and different larval instars of malarial mosquito *Anopheles stephensi*

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ARTICLE INFO

Article history:
Received 1 June 2011
Received in revised form 15 July 2011
Accepted 15 August 2011
Available online 20 December 2011

Keywords:
Anopheles stephensi
Coccinia indica
Essential oil
Larvicidal activity
Egg hatching inhibition

ABSTRACT

Objective: To assess the larvicidal and egg hatching inhibition property of the leaf essential oil of *Coccinia indica* (*C. indica*) against *Anopheles stephensi* (*An. stephensi*). **Methods:** The larvicidal potential of *C.indica* leaf essential oil was evaluated against 1st, 2nd, 3rd and 4th instars larvae of *An. stephensi* using WHO protocol. The 24h LC_{50} and LC_{90} values of the essential oil were determined following probit analysis. The egg hatching inhibition activity was also tested at 10, 20, 40, and 60 mg/L. The IC_{50} value of essential oil was determined against eggs of *An. stephensi*. **Results:** The essential oil extracted from *C. indica* possessed excellent larvicidal and egg hatching inhibition activity against *An. stephensi*. The bioassays showed LC_{50}–LC_{90} of 54.3–140.3, 65.5–155.6, 86.8–180.7 and 95.3–192.6 for 1st, 2nd, 3rd, and 4th larval instars, respectively. The 50% egg hatching inhibition concentration (IC_{50}) was noted at 16.5 mg/L. **Conclusions:** The present finding suggest that the *C. indica* leaf essential oil provided an excellent potential for controlling *An. stephensi* mosquito at earlier stage of their life cycle.

1. Introduction

Mosquito transmitted diseases such as malaria, yellow fever, dengue, filariasis, chikungunya and encephalitis are important health problems in developing countries, particularly in the tropical region[1]. Every year a large part of the world's population is affected by mosquito–borne diseases, for example malaria, which affects 500 million people and kills 2.5 million people annually, primarily children[2]. Malaria is by far the most important mosquito–borne disease which is endemic in more than 100 countries. In spite of major efforts undertaken for its control, through drug treatment and vector control, an increase in malaria incidence has occurred in the last 30 years, due to poor socio–economic conditions and development of drug and insecticide resistance in parasites and vectors, respectively[3].

Anopheles stephensi Liston (Diptera) (*An. stephensi*) is the primary vector of malaria in India and other west Asian countries, and improved methods of control are urgently needed[4]. Currently, most insecticides are non–selective and can be harmful to other beneficial organisms and to the environment in the form of biomagnifications. Further the indiscriminate use of insecticides is creating insecticides resistance among the mosquitoes[5]. The researchers therefore have diverted their attention towards plant extracts, which are ecofriendly and cost effective. The present experimental plant *C.indica* growing wild throughout India and also cultivated in various parts of India. It belongs to the Family Cucurbitaceae. The whole plant is traditionally used for various medicinal purposes. Leaves of this plant are used in Indian folk medicine for treatment of diabetes, ulcers, inflammation, fever, asthma and cough[6].

Most mosquito control programs target the larval and embryonic stages at their breeding sites with larvicides and ovicides, respectively[7]. Since adulticides may reduce the adult population only temporarily[8]. Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the embryo and larvae. During the immature stage, mosquitoes are relatively immobile;

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remaining more concentrated in breeding site than they are in the adult stage[9]. The present study aimed to assess the bio efficacy of *C. indica* leaf essential oil against embryonic and larval stages of *An. stephensi*.

2. Materials and methods

2.1. Mosquito culture

An. stephensi eggs were collected from breeding sites located in and around the A.V.C. College campus. Larvae were reared in plastic trays filled with filtered tap water and fed dog biscuits and yeast powder in the ratio of 3:1. Pupae were transferred from the trays to a cup containing tap water and placed in screened glass cages [(45×38×38) cm] where the adult emerged. Adults were continuously provided with 10% sucrose solution and were periodically blood-fed on restrained 5-7-week-old chicks. Mosquito culture and bioassay were carried out at (27±2) ℃, 75%-85% relative humidity with 14:10 light and dark photoperiod cycle.

2.2. Preparation of essential oil

The leaves of *C. indica* were collected from the premises of A.V.C.College. Essential oil was obtained by the hydro distillation of 3 kg fresh leaves in a Clevenger apparatus for 8h. The oil layer was separated from the aqueous phase using a separating funnel. The resulting essential oil was dried over anhydrous sodium sulphate and stored in an amber-coloured bottle at 8 ℃ for further analysis.

2.3. Larvicidal activity

Larvicidal activity was carried out by following the standard procedure[10]. Based on the wide range and narrow range tests, essential oil was tested at 50, 100, 150, and 200 mg/L. Essential oil was first dissolved in 1 mL DMSO, and then diluted in 249 mL of filtered tap water to obtain each of the desired concentrations. Twenty five 1st, 2nd, 3rd and 4th instars larvae were introduced to each of the test concentration as well as control separately. For each concentration, four replicates were run at a time. The larval mortality was recorded after 24h of exposure, during which no food was given to the larvae. The control was prepared using 1 mL of DMSO in 249 mL of water.

2.4. Egg hatching inhibition assay

Inhibition test was performed with a total of 200 eggs were individually released in to 100 mL of disposable plastic cups containing 50ml of diluted concentrations of 10, 20, 40 and 60 mg/L for 3 h treatment. After treatment, eggs were individually transferred to distilled water for hatching assessment. Each treatment was done with four replicates. The control was prepared using 1ml of DMSO in 49 mL of water. The percentage hatching was assessed 120 h after treatment by the following formula.

$$\frac{\text{Number of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

2.5. Statistical analysis

The observed percent mortality was adjusted for the control mortality, using Abbot's formula[11], and then subjected to propit analysis[12] for find out lethal concentrations of 50% and 90% mortality. One way variance analysis (ANOVA) was performed followed by Tukey's test using SPSS software. P value less than 0.05 were considered to indicate statistical significance.

3. Results

The hydro distillation of fresh leaves of *C. indica* yielded 3.1 mL/kg essential oil. The oil was less dense than water and was a light yellow colour. The essential oil was easily soluble in dimethyl sulfoxide (DMSO). Larvicidal activity of essential oil of *C. indica* against 1st, 2nd, 3rd and 4th instars larvae of *An. stephensi* was presented in Table 1. At 24h duration, the mortality range of 1st, 2nd, 3rd and 4th instars larvae were 48.3%-100.0%, 42.5%-100.0%, 37.8%-98.8% and 31.3-92.8%, respectively, at concentration range of 50 to 200 mg/L. However, 1.3%- 2.5% mortality was observed with control. At concentration of 200 mg/L, the 1st and 2nd instars larvae showed irregular movement and all larvae were died immediately after exposure to the treatment. A positive correlation was observed between the essential oil concentration and the per cent mortality, the rate of mortality being directly proportional to concentration.

Table 1

Larvicidal efficacy of *C. indica* essential oil against different instars of *An. stephensi*.

Concentration(mg/L)	Larval mortality (%)			
	1st instar	2nd instar	3rd instar	4th instar
50	48.3±1.4	42.5±2.1	37.8±1.9	31.3±1.7
100	73.5±2.3	66.8±1.7	59.5±1.7	52.3±1.4
150	94.3±2.2	87.3±1.4	79.5±2.2	71.5±1.4
200	100.0±0.0	100.0±0.0	98.8±2.1	92.8±1.9
Control	2.3±0.5	2.5±0.5	1.3±0.3	1.3±0.3

Each value (mean±SEM) represents mean of four replicates value. Values were significantly different from the control at $P< 0.05$ level (Tukey's test).

The 50% mortality (LC_{50}) was shown at 54.3, 65.5, 86.8 and 95.3 mg/L for 1st, 2nd, 3rd and 4th instars larvae, respectively. The LC_{90} values (90% mortality) were shown at 140.3, 155.6, 180.7 and 192.6 mg/L for 1st, 2nd, 3rd and 4th instars larvae, respectively. From the LC_{50} and LC_{90} values, it was evident that 1st and 2nd instars were more susceptible than 3rd instar and the later was more susceptible than 4th instar. The result of egg hatching inhibition of essential oil, at the dosage of 10, 20, 40 and 60 mg/L showed 55.5%, 40.7%, 18.5% and 3.7% egg hatching, respectively. The 50% egg hatching inhibition concentration (IC_{50}) was noted at 16.5 mg/L. It was evident that IC_{50} value was less than LC_{50} and LC_{90} values for the different instars larvae. The lower concentration of the essential oil was required for the egg hatching inhibition than larvicidal.

4. Discussion

Plant essential oils are complex mixture of mainly terpenoids, phenols, oxides, ethers, alcohols, esters, aldehydes and ketones[13]. These constitute effective alternatives to synthetic pesticides without producing adverse effects on the environment[14]. Moreover, the interest in essential oils has regained momentum during the last decade, and primarily due to their fumigant and contact insecticidal activities[15]. The LC_{50} values of the *C. indica* essential oil are comparable with our previous studies. *Clausena dentata* leaf essential oil exhibit LC_{50} value at 140.2 mg/L against 3rd instar larvae of *Aedes aegypti*[16]. The ethanolic leaf extract of *Cassia obtusifolia* had significant larvicidal effect, LC_{50} and LC_{90} values were 52.2 and 108.7 mg/L, respectively, against 3rd instar larva of *An. stephensi*[17]. Also the different larval instars susceptibility result agree with the finding of previous study[9] who had reported that 2nd instar larvae of *Anopheles arabiensis* was more susceptible than 3rd instar, and the later was more susceptible than 4th instar larva to the leaf extract of *Calotropis procera*, with LC_{50}-LC_{90} of 273.53-783.43 mg/L, 366.44-1018.59 mg/L and 454.99-1224.62 mg/L for 2nd, 3rd, and 4th larval instars, respectively. The LC_{50} and LC_{90} values of present work are larval age dependent. This may support the ideas of previous work[18] who noted that insect age and physiological status of larvae plays an important role in influencing susceptibility.

Concerning the effect on egg hatchability, *C.indica* essential oil showed potent egg hatching inhibition activity, which will be an added benefit to larvicidal activity of *C. indica* essential oil. The present results are comparable with our earlier studies of *Solanum trilobatum* leaf extract showed ovicidal activity against egg rafts with age group ranging from 0-18h of *Culex quinquefasciatus* and *Culex tritaeniorhynchus*[19] and also leaf extract of *Chenopodium ambrosioides* exhibit ovicidal activity against egg raft with different age groups of *Culex quinquefasciatus*[20]. In our present study, the mode of action of egg hatching inhibition and larvicidal activity of essential oil was not studied, but previous studies have shown that the egg shell is made of several layers to protect the embryo, as well as chitin[21]. The action of lipophilic substances upon eggs may be by causing indurations of the egg shell and interference in water and gas exchange[22] or by penetration into the egg and impeding enzymatic reactions as well as hormonal activities which disturb the embryogenesis process[23]. The mode of action of larvicidal may be due to either essential oils increase the tendency of tracheal flooding and chemical toxicity in mosquito larvae[24-35] or compound in essential oil interfered with proton transfer in mitochondria leading to larval mortality[36].

In this study it was observed that, leaf essential oil of *C. indica* has showed larvicidal and egg hatching inhibition activity against the *An. stephensi*. The biological activity of this essential oil due to various compounds in essential oil, these compounds may jointly or independently contribute to produce larvicidal and egg hatching inhibition activity. According to earlier authors report[37, 38], one plant species may possess substances with a wide range of activities, e.g. Neem (Azadirachta indica) products showed antifeedant, oviposition deterrence, repellency, growth disruption, sterility and larvicidal action against insects. In this way, biological activities and local availability of *C. indica* might form a new arsenal for vector management, especially in areas where mosquitoes have developed resistance to conventional insecticides.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the Professor and Head of the Department of Zoology, AVC College for facilities provided.

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