Effect of *Vetiveria zizanioides* L. Root extracts on the malarial vector, *Anopheles stephensi* Liston

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**Abstract**

**Objective:** To evaluate the ovicidal and oviposition deterrent potential of the ethanolic extract from *Vetiveria zizanioides* (*V. zizanioides*) roots against the malarial vector, *Anopheles stephensi* (*A. stephensi*). **Methods:** The dried clean *V. zizanioides* roots were powdered and extracted with ethanol for 8 h in a soxhlet apparatus. After evaporation, the residue was dissolved in acetone. One hundred freshly laid eggs of *A. stephensi* were exposed to the extract at different concentrations for 48 h, and the hatch rate was calculated to evaluate the ovicidal activity. Those exposed to acetone aqueous solution were used as control. The egg laying behavior of gravid female *A. stephensi* was also observed using oviposition deterrent test. Effective repellency (ER) was used to evaluate the oviposition deterrent activity. **Results:** Exposure to the crude ethanol extract of *V. zizanioides* reduced the hatchability rate of *A. stephensi* eggs, and zero hatchability was exerted at 375 ppm. In the oviposition deterrent test, the extract alleviated the egg laying with an ER of 78.9% at the highest concentration of 375 ppm and even 53.7% at the lowest concentration of 125 ppm. Moreover, the negative values of oviposition active index also suggests the extract was an alternative pesticide to control *A. stephensi* at the early stage of life history, possibly due to the presence of various active chemical compounds.

**Key words:** *Vetiveria zizanioides*, *Anopheles stephensi*, Ovicidal activity, Oviposition deterrent, Effective repellency, Oviposition active index

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1. Introduction

Mosquitoes are vectors of etiologic agents of malaria, filariasis and viral diseases. *Anopheles stephensi* Liston (Diptera: Culicidae) is the primary vector of malaria in India and other West Asian countries and improved methods of control are urgently needed[1]. Recent studies stimulated the investigation of insecticidal properties of plant derived from microbes or botanicals and concluded that they are environmentally safe, degradable and target specific[2]. Botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented non-toxic effects on non-target organisms[3]. The plant world comprises a rich storehouse of phytochemicals, which are widely used to prepare synthetic insecticides. The continuous use of synthetic insecticides causes side effects on non-target organisms and insecticide resistance in mosquitoes[4].

*Vetiveria zizanioides* (*V. zizanioides*) L. is a tall, tufted, perennial, scented grass, with a straight stem, long narrow leaves and a lacework root system that is abundant, complex, and extensive. It offers an inexpensive yet effective and eco-friendly tool to combat soil erosion. The roots have been used in Asia for centuries for their fragrance, and are woven into aromatic matting and screens. The roots of some cultivars and ecotypes possess essential oil that has been utilized as fragrant material since ancient times. Water quality signifies the absence of contaminants, which are waste products, pollutants and nutrients. The plant extracts, *e.g.*, vetiver extract, have many special characteristics that lend support for its uses in solving the water problem. In the case of human health, when dealing with the contamination of water, prevention is better than cure[5]. The plant also contains active ingredients used in traditional medicine and as a botanical pesticide. Secondary metabolites of plants, many of which are produced for protection against micro–or organisms and insect predators, are natural candidates for the development of new products to combat *A. stephensi*. Several studies have focused on the activities of larvicides, adulticides, repellents and ovipositional attractants[6–8]. Bagavan et al had reported that the hexane, chloroform, ethyl acetate,
acetone and methanol extracts of G. superba leaves were
tested against the fourth instar larvae of A. subpictus and C. tritaeniorhynchus[9]. Murugan et al. studied the larval toxicity and
smoke repellant potential of methanol extract of O. basilicum against different instar (I, II, III and IV) larvae and
pupae of A. aegypti[10]. The larvicidal and adult emergence
inhibition activities of Ricinus communis (R. communis)
seed extract against A. stephensi, Culex quinquefasciatus (C. quinquefasciatus) and Aedes albopictus (A. albopictus) were
evaluated[11]. Senthil kumar et al. had reported the larvicidal
and adulticidal activities of leaves extract of R. communis
against A. stephensi[12]. Thoothuvalai [Solanum trilobatum
(S. trilobatum), a thorny shrub widely spread in India,
has been screened for its ovicidal and larvicidal activities
against Culex mosquitoes[13]. The oviposition deterrent and
skin repellent activities of S. trilobatum were tested against
A. stephensi[14]. Essential oil extracted by steam distillation
from the leaves of Tridax procumbens (T. procumbens) (coat
buttons) was evaluated for their topical repellency effects on
A. stephensi in mosquito cages[15].

However, from ancient times, these plants including V. zizanioides have also been used as raw materials for
cosmetics, pharmaceuticals, botanical pesticides, disinfectants, insect repellents, herbal teas, herbal drinks,
etc. As far as our literature survey could ascertain, no
information was available on the ovicidal and oviposition
deterrent activities of the experimental plants given here.
Hence, we have undertaken the following objectives of the
study to evaluate the ovicidal potential of the ethanolic
extract from V. zizanioides roots against the malarial vector,
A. stephensi.

2. Materials and methods

2.1. Collection of eggs

The eggs of A. stephensi were collected from the National
Institute for Communicable Diseases (NICD), Mettupalayam,
Coimbatore, Tamil Nadu, India without exposure to any
insecticide and were also collected at different breeding
habitats in and around Coimbatore, India with an 0–type
brush. The eggs were then brought to the laboratory and
transferred to 18 cm x 13 cm x 4 cm size enamel trays
containing 500 ml of water and kept for larval hatching. They
were hatched, reared and maintained for many generations
in the laboratory. The eggs and larvae obtained from this
stock were used for different experiments.

2.2. Maintenance of adult mosquitoes

The pupae were collected from culture trays and transferred
to glass beakers containing 500 ml of water with a sucker.
The glass beakers were kept in a 90 cm x 90 cm x 90 cm size
mosquito cage for adult emergence. The cage was made up
of wooden frames and covered with polythene sheets on four
sides (two laterals, one back and one upper) and the front
part was covered with a muslin cloth. The bottom of the
cage was fitted with strong cardboard. The freshly emerged
adults were maintained in the conditions of 27.2 °C, 75%–85%
RH, under 14L:10D photoperiod cycles. The adults were fed
with 10% sugar solution for three days before an animal were
provided for blood feeding.

2.3. Blood feeding of adult A. stephensi and egg laying

The females were fed by hand at 6:00 p.m every alternate
day. Feeding mosquitoes on human arm for experimental
purposes was suggested by Judson[16] and Briegel[17]. Both
females and males were provided with a 10% glucose solution
on cotton wicks as described by Villani et al.[18]. The cotton
was always kept moist with the solution and changed every
day. Theoder and Parsons noticed that glucose as well as
ordinary sugar appeared equally attractive to mosquitoes[19].
An egg trap (cup) which was lined with filter paper and
contained pure water was always placed at a corner of the
cage. This arrangement made collection of eggs easier.

2.4. Collection of plant and preparation of phyto extract

V. zizanioides was collected from the area around Bharathiar
University, Coimbatore. The plants were cleaned
and the roots were shadily dried. The dried materials were
powdered by an electrical blender. From the sample, 100 g
of the plant material was extracted with 300 ml of ethanol
for 8 h in a soxhlet apparatus. The extracts were evaporated
to dryness in a rotary vacuum evaporator to yield 122 mg
of pale brownish residue. One gram of the residue was
dissolved in 100 ml of acetone (stock solution) from which
different concentrations, i.e., 125, 175, 225, 275 and 325 ppm,
were prepared.

2.5. Ovicidal bioassay

The method of Su & Mullal[20] was followed to test the
ovicidal activity. The leaf extract was diluted in the
respective solvent to achieve different concentrations. One
hundred freshly laid eggs of A. stephensi were exposed to
each concentration of ethanol extract of V. zizanioides until
they hatched or died. Each concentration was replicated six
times. Eggs exposed to acetone in water served as control.
After the treatment, the eggs from each concentration were
transferred to distilled water in a cup and counted under
a microscope for hatching assessment. The hatch rate was
assessed after 48 h post treatment by the following formula:
2.6. Oviposition deterrence test

The oviposition deterrence test for *A. stephensi* was performed using the method of Xue et al.[21]. After 4 days of blood feeding, 50 gravid females at 10 days old were transferred to each mosquito cage (45 cm x 38 cm x 38 cm) covered with a plastic screen, with a glass top and a muslin sleeve for access. A 10% sucrose solution was available at all times. Serial dilutions of the leaf extract were made in ethanol. Enamel bowls containing 100 ml of rainwater were added to the leaf extract to obtain test solutions of 125, 175, 225, 275 and 325 ppm. Two enamel bowls holding 100 ml of rainwater were placed in opposite corners of each cage, one treated with the test material, and the other with a solvent control (1% ethanol). The positions of the bowls were alternated between the different replicates so as to nullify any effect of position on oviposition. Three replicates for each concentration were run, with cages placed side by side for each bioassay. All experiments were run at an ambient temperature of (27±2) °C with relative humidity of 70%-80%. After 24 h, the number of eggs laid in the treated and control bowls was recorded. The percentage of effective repellency (ER) for each leaf extract concentration was calculated using the following formula.

2.7. Determination of oviposition activity index (OAI)

The results of the oviposition experiment were expressed as mean number of eggs and OAI which was calculated using the formula:

\[
\text{OAI} = \frac{N_t - N_c}{N_t} 
\]

Where, \( N_t \) is the total number of eggs in the test solution and \( N_c \) is the total number of eggs in the control solution. Index values lie within the range of +1 to −1. Positive values indicate that more eggs were deposited in the test cups than in the control cups and that the test solutions were attractive. Conversely, negative values indicate that more eggs were deposited in the control cups than in the test cups and that the test solutions were deterrents.

3. Results

Table 1

| Extract concentration (ppm) | Number of eggs in bowl | Effective repellency (%) | OAI |
|-----------------------------|------------------------|--------------------------|-----|
|                             | Treated | Control |                     |     |
| 125                         | 89.2±1.4 | 192.8±1.8 | 53.7 | −0.36 |
| 175                         | 96.4±1.2 | 216.2±2.1 | 55.4 | −0.38 |
| 225                         | 86.6±1.1 | 210.8±1.7 | 58.9 | −0.41 |
| 275                         | 72.8±0.9 | 190.6±1.8 | 61.8 | −0.44 |
| 325                         | 72.2±0.9 | 280.4±2.3 | 74.3 | −0.59 |
| 375                         | 78.8±1.2 | 375.2±2.5 | 78.9 | −0.65 |

4. Discussion

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide
must not cause high mortality in target organisms in order to be acceptable[22]. The extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of A. stephensi eggs to the leaf extracts of various solvents not only elicited egg mortality but also delayed hatchability to larval stages[23]. The ovicidal activity indicated an important finding that the larvae which hatched out of the treated eggs were succumbed to death within an hour or two. In the present study, we sought to determine whether an ethanol extract from V. zizanioides could be used for mosquito control. We observed a functional response of the ovicidal and oviposition deterrent activity exhibited by the ethanolic extract of V. zizanioides. In the case of ovicidal activity, exposure to the freshly laid eggs was more effective than that to the older eggs. It has been shown that the age of the embryos at the time of treatment played a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, Dimilin to C. quinquefasciatus[24]. Similarly, the oviposition deterrent activity, ovicidal and gravid mortality effects of ethanolic extract of Andrographis paniculata Nees against the malarial vector A. stephensi Liston was evaluated by Kuppusamy et al.[25]. Larvicidal and oviposition activity of Cassia obtusifolia Linn (Leguminosae) leaf extract against A. stephensi Liston was also evaluated[26]. The full oviposition detergency was obtained with Melia azedarach leaf extract at 1 g/L against A. aegypti[27]. Similarly, the aqueous and hydro–alcoholic extracts of Melia azedarach L. (Meliaceae) leaves and seeds were tested to explore the in vitro ovicidal and larvalvicidal activity against Haemonchus contortus (Strongylida)[28], and the results were comparable with our results. Additionally, through screening several plants for their larvicidal activity, Sharma et al found that Artimisia annua was the most toxic against anopheles with an LC_{50} of 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively[29]. In addition, the larvicidal effects of Momordica charantia fruit on A. stephensi (LC_{50} of 66.05 ppm) and C. quinquefasciatus (LC_{50} of 96.11 ppm) were also investigated[30].

The biological activity of the plant extract might be due to a variety of compounds in V. zizanioides roots, including phenolics, terpenoids and alkaloids. These compounds may jointly or independently contribute to cause oviposition deterrent and ovicidal activity against A. stephensi[31]. The main chemical compounds in the roots are zizanal, epizizan, khussimol, α–vetivone and β–vetivone. The direct and indirect contributions of such compounds to treatment efficacy through reducing larval feeding and fitness need to be properly understood in order to guide the use of botanical insecticides for the management of A. stephensi. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future[32]. Since A. stephensi breeds in drinking water tank, many of the plant extracts are subject to risk factors in mosquito control[33]. The plant extracts which are highly toxic against A. stephensi are also toxic to human beings[34]. In the present study, V. zizanioides root extract showed good effect on A. stephensi and it was non–toxic to human beings. Many previous studies proved that the extract of V. zizanioides acts as a water purifying agent. V. zizanioides can also be used a herbal drink. Its roots are used to prepare Sharbat (sherbet) or soft drink during summer or to perfume drinking water[35]. Paul and Hart studied the effect of vetiver for the waste water treatment and believed that the vetiver roots can be used as a natural water purifying agent in household as well as in the community systems[36]. Hence, V. zizanioides can be considered as a water purifying agent as well as a potent biopesticide.

Conflict of interest statement
We declare that we have no conflict of interest.

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References
[1] Burfield T, Reekie SL. Mosquitoes, malaria and essential oils. Int J Aroma 2005; 15: 30–41.
[2] Ascher KRS, Schmutterer H, Zehitz CPW, Naqvi SNH. The Persian lilac or chinaberry tree: Melia azedarach L. In: Schmutterer H ed. The Neem Tree: source of unique natural products for pest management, medicine, industry and other purposes. Weinheim: Vancouver Coastal Health; 1995, p. 605–642.
[3] Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of Cassia fistula Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitol Res 2008; 102: 289–292.
[4] Kelm MA, Nair MG, Schutzki RA. Mosquitoicidal compounds from Magnolia salicifolia. Int J Pharmacognosy 1997; 35: 84–90.
[5] Chomchalow N. The Role of Vetiver in Controlling Water Quantity and Treating Water Quality: An Overview with Special Reference to Thailand. 2003. [Online] Available from: http://www.vetiver.
from different parts of the mangrove tree Rhizophora mucronata (Rhizophoraceae) Lam. Against three arthropods. *African J Se Tech* 2001; 2(2): 44–49.

[23] Rajkumar S, Jebanesan A, Nagarajan R. Effect of leaf essential oil of Coccinia indica on egg hatchability and different larval instars of malarial mosquito *Anopheles stephensi*. *Asian Pac J Trop Med 2011*; 4(12): 948–951.

[24] Miura T, Schafer CH, Takahashi RM, Mulligan FS. Effects of insect growth inhibitor, dimilin on hatching of mosquito eggs. *J Econ Ent* 1976; 69: 655–658.

[25] Kuppusamy C, Murugan K. Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* Nees against the malarial vector *Anopheles stephensi* Liston (Diptera:Culicidae). *Entomol Res 2008*; 38: 119–125.

[26] Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res 2009*; 104: 337–340.

[27] Cortia C, Almiron W, Valladares G, Carpinella C, Ludueña F, Defago M, Palacios S. Larvicidal and oviposition deterrent effects of fruit and leaf extracts from *Melia azedarach* L. on *Aedes aegypti* (L.) (Diptera: Culicidae). *Bioresource Technol 2008*; 99: 3066–3070.

[28] Kamaraj C, Rahuman AA, Bagavan A, Mohamed JM, Elango G, Rajakumar G et al. Ovicidal and larvicidal activity of crude extracts of *Melia azedarach* against *Haemochus contortus* (Strongylida). *Parasitol Res 2010*; 106: 1071–1077.

[29] Sharma P, Mohan L, Srivastava CN. Phytoextract–induced developmental deformities in malaria vector. *Bioresource Technol 2006*; 97: 1599–1604.

[30] Singh RK, Dhiman RC, Mittal PK. Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). *J Vector Borne Dis 2006*; 43: 88–91.

[31] Medhi SM, Reza S, Mahnaz K, Reza Aam, Albas H, Faemeh M et al. Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi*. *Asian Pac J Trop Med 2010*; 3(11): 841–845.

[32] Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J Fontana JD. Larvicidal action of ethanolic extracts from *Anopheles gambiae* and *Aedes aegypti*. *J Econ Entomol 2011*; 103(6): 1699–1704.

[33] Ahmad N, Fazal H, Abbasi BH, Iqbal M. In vitro larvicidal potential against *Anopheles stephensi* and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorus*. *Asian J Trop Med 2011*; 4:3: 169–175.

[34] Prabhukar K, Murugan K, Naresh Kumar A, Ramasubramanlan N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed 2011*; 1(2): 124–129.

[35] Rao RR, Suseela MR. *Vetiveria zizanioides* (linn.). Nash A multipurpose eco-friendly grass of India National Botanical Research Institute Lucknow, India. [Online] Available from: http://www.vetiver.org/TVN_vetiver_water.pdf.

[36] Paul Truong N, Hart B. Vetiver system for wastewater management. International Freshwater Conference. Bonn December 2003; Germany.