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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 powerdered 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 differnt concentrations for 48 h, and the hatch rate was calculated to evaluate the ovicidal activity. Those exposed to actone 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 a good deterrent agent. **Conclusions:** The ethanolic extract of *V. zizanioides* roots may be used an alternative pesticide to control *A. stephensi* at the early stage of life history, possibly due to the presence of various active chemical compounds.

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-oraganisms 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,

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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 repellent 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 tribolatum* (*S. tribolatum*)], 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. tribolatum* 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 cellected at different breeding habitats in and around Coimbatore, India with an O-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 ℃, 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 & Mulla[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 deterrent 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 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) ℃ 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:

Where, N_T is the total number of eggs in the test solution and N_S 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

3.1. Ovicidal activity of ethanol root extract of *V. zizanioides* against *A. stephensi* eggs

The ovicidal behavior of the ethanol root extract of *V. zizanioides* was observed in the *A. stephensi* eggs. The total number of eggs used in the study was 100 for control as well as for the test, and the percentage of egg hatchability in control (acetone mixed with distilled water) was (99.2±0.4)%. The eggs of *A. stephensi* treated with different concentrations of the leaf extract failed to hatch. The percentage of egg hatchability was decreased with the increasing concentration of the extract. With the extract concentration of 125 ppm, the percentage of hatchability was very high recorded as (80.4±1.2)% and nil hatchability was recorded when the concentration was increased to 375 ppm. All the values are represented as means±standard error of five values. Hence, by increasing the concentration from 125 ppm to 375 ppm, the percentage of hatchability was recorded to be as follow: (80.4±1.2)%, (71.2±0.7)%, (65.6±0.9)%, (53.2±0.6)%, (38.4±0.7)% and NH (Nil hatchability/100% mortality) respectively.

3.2. Oviposition deterrent activity of ethanol root extract of *V. zizanioides* against gravid female *A. stephensi*

Table 1 shows the oviposition deterrent activity of ethanol root extract of *V. zizanioides* against the gravid female *A. stephensi*. In the laboratory oviposition deterrent test, the root extract of *V. zizanioides* at each concnentration greatly reduced the number of eggs deposited by the gravid *A. stephensi*. The acetone in distilled water serving as a control received only a small number of eggs. The percentage of egg laying was very high in the control when compared to that after the extract treatment. The deterrent activity was calculated as ER. The extract at the highest concentration allayed egg laying with an ER of 78.9%, and that at lower concentrations also had deterrent activity of 53.7% ER. The OAI was also calculated and the negative values of OAI indicated the extract was a highly deterrent agent. The test was replicated five times.

Table 1
Oviposition deterrent effect of *V. zizanioides* ethanol root extract against gravid female *A. stephensi*.

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 deterrency 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 larvicidal 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, epizizanal, khusimol, 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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