Research Article

Toxicity and Oviposition Deterrent Activities of Thyme Essential Oils against *Anopheles arabiensis*

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Background. Malaria is one of the deadliest mosquito-borne diseases in sub-Saharan Africa and Ethiopia. Owing to their costs and environmental issues, synthetic insecticides are poor choices to control mosquitoes. Plant-based products can be considered as safe and biodegradable alternatives. The present study aimed to test the toxicity and oviposition deterrent activities of *Thymus serrulatus* and *Thymus schimperi* essential oils (EOs) against *Anopheles arabiensis*. Methods. Thyme EOs were extracted by hydrodistillation using the Clevenger-type apparatus. They were named Tar, Ala, and Yil after the areas of thyme collection Tärmarber, Alamata, and Yilmama Densa, respectively. Laboratory-based tests were used to determine the larvicidal, adulticidal, oviposition deterrent, and half lethal dose (LD₅₀) of each EO. Results. The concentrations of 100 μL/L and 50 μL/L resulted in complete mortalities of larvae and adults, respectively, for all the three EOs considered. The EOs exhibited high repellency with oviposition activity index of −1 (OAI = −1) at concentrations of 50 μL/L (Tar), 100 μL/L (Ala), and 200 μL/L (Yil). Conclusions. The EOs of *T. serrulatus* and *T. schimperi* were effective against larvae and adult mosquitoes at small doses and resulted in oviposition deterrence at doses from 50 to 200 μL/L. Thus, these EOs are promising mosquitoicides and oviposition deterents. But, further tests both in the presence of already known and effective deterrents and field trials are required.

1. Introduction

Mosquitoes transmit different diseases including malaria [1–3]. Malaria, the most deadly mosquito-borne disease [4], kills an estimated amount of more than 600,000 people every year, mainly children under five years of age in sub-Saharan Africa [4]. By 2015, the share of Ethiopia to the world malaria cases was six percent [5]. The emergence of resistant strains of *Plasmodium falciparum* and lack of an effective malaria vaccine makes vector control an important way to interrupt the cycle of disease transmission [4].

Synthetic insecticides such as organochlorine and organophosphate compounds are major tools in mosquito control [6]. Nevertheless, lack of novel insecticides, high cost of synthetic insecticides, and concern for environmental sustainability make synthetic insecticides poor choices [1–3, 7–11]. Consequently, there is a rise in search of alternative vector control strategies [6, 9]. Plant-derived compounds have a good perspective as alternatives to fight mosquito-borne diseases [9]. *Thymus* species are among many plants with mosquitocidal activities [9].

Studies show that different thyme species have larvicidal, adulticidal, and oviposition deterrent activities against insects. The essential oils (EOs) of *Thymus broussonetii* and *Thymus marocanus* had larvicidal activities against *C. pipiens* larvae [12], EOs of *T. vulgaris* L. against greater wax moth, *Galleria mellonella* L. larvae [13], and thymol and 1, 8-cineole isolated from thyme EOs against *Aedes aegypti* larvae [1]. Furthermore, the essential oils obtained from *T. vulgaris* and *T. satureioides* were effective against *Culex quinquefasciatus* Say larvae [14]; EO and components (thymol and carvacrol) of *T. vulgaris* are against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer [15].
The insecticidal activity of thyme EOs is reported by different studies. *T. serpyllum* EO against *Aedes*, *Anopheles*, and *Culex* mosquitoes [16]; *T. transscapicus* EOs against *A. stephensi* [17]; *T. cariensis* and *T. ciliatus* EOs against *Callosobruchus maculatus* (cowpea weevil) [18]; *T. capitatus*, *T. bleicherianus*, and *T. arenarius* EOs against *Sitophilus oryzae* [19]; *T. numidicus* EO against *Rhizopertha dominica* [20]; and carvacrol and thymol derived from *T. vulgaris* against *Pochazia shantungensis* [21].

The oviposition deterrent activity of thyme EOs is not well reported. *T. vulgaris* EO is reported to have oviposition deterrent activity against *Acanthoscelides obtectus* [22]. However, there are no reports about the larvicidal, adulticidal, and oviposition deterrent activities of the EOs of the two Ethiopian endemic thyme species, *T. serrulatus* and *T. schimperi*. Thus, the present study is aimed at evaluating the larvicidal, adulticidal, and oviposition deterrent activities of the EOs of these thyme species from Ethiopia against *A. arabiensis* mosquitoes.

### 2. Materials and Methods

#### 2.1. Plant Material Collection and Identification

Aerial parts of *T. serrulatus* and *T. schimperi* were collected between July and September 2013 from Alamata (lat/long dec. 13.69589, 37.0542153) (Tigray region) and Yilmana Densa (lat/long dec. 12.28943, 37.0325366) and Tarmaber (lat/long dec. 10.89386, 37.0580250) (Amhara region) in Ethiopia. These plants were collected from the wild, and they are not endangered species. Consequently, specific permission was not required for the collection and investigation of these plants. The *Thymus* species were identified by Mr. Melaku Wondafrash, a botanist working in the National Herbarium of Addis Ababa University, Addis Ababa, Ethiopia, and voucher specimens were deposited in the herbarium.

#### 2.2. Preparation of Essential Oils

The investigators added fine powder (200 g) of each plant to 2 L of distilled water (with vegetal material/extraction solvent rate = 1/10 (w/v)) in a 4 L round bottom glass flask and subjected to water distillation for three hours using a Clevenger-type apparatus in the Insect Science Laboratory of Zoological Science Department of Addis Ababa University to extract the essential oils. After extraction, the volume of the EOs was measured, dried over anhydrous sodium sulfate, stored in brown glass bottles to reduce the intensity of light, and placed at 4°C until used for the experiments.

#### 2.3. Larvicidal Bioassay

Larvae of *A. arabiensis* were reared in larval rearing trays in the insectary of the Ethiopian Public Health Institute (EPHI). Larvae were supplied daily with 0.5 g larval food made of dog biscuit and brewery yeast 3:2 (w:w ratio) [23]. The EOs of Ala, Yil, and Tar were made in concentrations of 12.5, 25, 50, 100, and 200 μL/L in acetone. The chemical composition of these EOs was previously characterized and published [24]. Ala was found to be highly thymol (65.63%), Yil (carvacrol = 80.84%), and Tar (thymol: carvacrol = 48.84%: 42.12%) chemotypes. A total of 25 third and fourth instar larvae of *A. arabiensis* were placed in each 300 ml white enamel cup containing 149 ml of distilled water [16] (Figure 1). After three hours of larval acclimatization, 1 mL of desired concentration of thyme EOs (12.5, 25, 50, 100, and 200 μL/L) was added to each beaker to make a final volume of 150 mL. Each dose of the three EOs was tested three times. An equal volume of acetone was used as a negative control. Larval mortality was recorded 24 hr postexposure. Larvae were confirmed dead when they failed to move after gentle probing them with a needle [25].

#### 2.4. Adulticidal Test

Fumigating adulticidal activity was tested by airtight fumigation in conical flasks, as described by Pavela et al. [26]. Twenty nonblood-fed females (2–6 days old) were placed in each 250 mL conical flask (Figure 2) because older blood-fed females have reduced susceptibility to insecticides [27]. A 10 μL volume of the EO solution of five doses 3.125, 6.25, 12.5, 25, and 50 μL/L in acetone was immediately dropped onto a filter paper (1 x 3 cm) with a micropipette. A cork stopper was used to seal each conical flask, and the filter paper was placed at the base of the cork. The controls were treated under the same conditions with pure acetone. The conical flasks were sealed tightly and placed in a room at 26°C for four hours [28]. Mortality was determined after 24 h of exposure after being transferred to clean cages.

#### 2.5. Oviposition Deterrent Bioassay

Fifteen gravid females (eight to ten days old, four days after blood feeding) were transferred to each mosquito cage (45 × 30 × 30 cm) (Figure 3). Serial dilutions of EOs were made in dimethylsulfoxide (DMSO). We used DMSO instead of acetone because acetone is attractant to mosquitoes and could affect our findings [29]. In the multiple concentration test, five cups, each containing 100 mL distilled water with a 9 cm piece of white filter paper for oviposition as well as EOs at concentrations of 12.5, 25, 50, 100, and 200 μL/L, were placed in each cage. The sixth cup containing DMSO served as a negative control in each cage. The positions of the plastic cups were alternated between the different replicates to nullify any effect of position on oviposition. Three replicates for each concentration were run with cages placed side by side for each bioassay. A sucrose solution (10%) was available at all times. The room was set to have a temperature of 26°C and relative humidity of 75–85%. After 24 hr, the number of eggs laid in treated and control cups was counted under a hand lens. Then, the oviposition activity index (OAI) was calculated using the following formula [7].

\[
\text{OAI} = \frac{NT - NS}{NT + NS} 
\]

where NT is the total number of eggs in the test solution and NS is the total number of eggs in the control solution. EO doses with oviposition active index of +0.3 and above were considered as attractants while those with −0.3 and below as repellents [7].
2.6. Statistical Analysis. Data for the larvicidal, adulticidal, and oviposition deterrent activities were expressed as mean ± SEM and analyzed statistically using one-way analysis of variance (ANOVA) followed by LSD post hoc multiple comparisons. The minimum level of significance was set at $P < 0.05$. Statistics was computed using SPSS programme version 20 and SAS 9.2.

3. Results

3.1. Larvicidal Activity. The 200 and 100 $\mu$l/L doses of the three tested EOs (Ala, Yil, and Tar) showed complete mortality of larvae. At 50 $\mu$l/L concentration, Yil, Tar, and Ala EOs resulted in larval mortality rates of 81%, 80%, and 37%, respectively (Figure 4). In the same way, at 25 $\mu$l/L EO
doses, larval mortalities were 17% (Tar), 3% (Yil), and 1% (Ala). At the dose of 50 µL/L, EOs of Yil and Tar killed larvae with mortality rates significantly higher than that of Ala (Table 1). The mean number of larvae that died due to the 25 µL/L of Tar was significantly higher than that of Ala and Yil EOs (Figure 4). On the other hand, the 25 µL/L doses of Ala and Yil as well as the 12.5 µL/L doses of all the three EOs did not result in larvicidal activities significantly higher than that of the negative control (acetone). The best larvicidal activity was EO of Tar followed by Yil and lastly by Ala EOs.

3.2. Adulticidal Test. The fumigation adulticidal activity of the three EOs showed dose-dependent activities against adult female A. arabiensis mosquitoes (Figure 5). For example, at 50 µL/L concentration, the EOs of Ala, Yil, and Tar resulted in 100% death of adult female mosquitoes. The death rates at 25 µL/L of the EOs of Tar, Yil, and Ala were 95, 90, and 85, respectively, reaching 100% at 50 µL/L. At the dose 12.5 µL/L, the adulticidal activity of Tar was significantly higher than that of Ala and Yil which in turn resulted in mosquitocidal activities significantly higher than that of the 6.25 and 3.125 µL/L doses (Table 1). The 6.25 µL/L doses of Yil and Tar EOs also showed adulticidal activities significantly higher than that of the 6.25 µL/L of Ala, the 3.125 µL/L of Ala, Yil, and Tar, and the negative control (acetone). Therefore, the EOs of Tar acted as adulticidal even at lower doses followed by Yil. The Ala EO was found to be the least effective of the three EOs. This shows that the adulticidal activity of the EOs was in the order of Tar > Yil > Ala (Figure 5).

3.3. Oviposition Deterrent Activity. The result of the oviposition deterrent bioassay is presented as the oviposition activity index (OAI) (Figure 6). The OAI of the EOs of Ala and Tar was below ~0.3 at all test concentrations. Similar findings were observed in Yil EO at the doses of 200, 100, and 50 µL/L. On the other hand, the control (DMSO) did not result in any oviposition deterrent effect.

4. Discussion

In the larvicidal, adulticidal, and oviposition deterrent tests, all the EOs tested (Ala, Yil, and Tar) at higher doses resulted in mosquitocidal activities. These mosquitocidal activities could be associated with the presence of EO components, mainly thymol and carvacrol [30]. The percentages of thymol and carvacrol were 65.63% and 6.68% (Ala), 6.52% and
acts as a fumigant as well. Therefore, it can be inferred that in combination with thymol its insecticidal potential decreases. This indicates the antagonism of the two phenols when used as an insecticide [37]. The results from Yil and Ala agree with these statements, in that the carvacrol type Yil is highly effective than the thymol type Ala. However, the presence of thymol and carvacrol in almost equal proportions in Tar had the best result contradicting the findings of Popovic and coworkers [37] which showed that the reduced impact of carvacrol is due to the presence of thymol. This difference may be due to the synergistic activities of these EOs [30] with other components of Tar EO. The larvicidal, adulticidal, and oviposition deterrent activities of the three EOs were very high. But, their validation is not possible because positive controls were not used for each test.

5. Conclusion

The EOs of T. serrulatus (from Ala and Yil), as well as T. shimperei (from Tar), acted as larvicidal, adulticidal, and oviposition deterrent against A. arabiensis third and fourth instar larvae, nongravid females, and gravid females, respectively. Tar was the best mosquitoicidal EO followed by Yil and Ala EOs. All the tested doses of Tar and Ala and the higher doses of Yil acted as repellents. However, at lower doses, Yil acted neither as an oviposition deterrent nor as an attractant.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References


