Entomon 41(2): 91-98 (2016)
Article No. ent. 41203

Repellent activity of plant essential oil extracts against malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae).

Wondmeneh Jemberie*, Alebachew Tadie, Abiyu Enyew, Amsalu Debebe, Nagappan Raja

Department of Biology, College of Natural and Computational Sciences, University of Gondar, Ethiopia. Post Box 196, Gondar, Ethiopia.E-mail: wondmeneh12@gmail.com

ABSTRACT: Repellent activity of essential oils extracted from the leaves of *Otostegia integrifolia* and *Stephania abyssinica*, roots of *Echinops kebericho* and seeds of *Millettia ferruginea* and *Datura stramonium* were tested against malaria vector, *Anopheles arabienses*. The repellent activity was determined at 125, 250, 500 and 1000 ppm concentration by human volunteer. The dorsal side of each human arm 153.86 cm^2 of the skin was exposed for twenty minutes by covering the remaining area with rubber glove. The control and treated arm were introduced simultaneously into mosquito cage under laboratory condition maintained at 27 ± 1°C, 65–70% RH. Among the four different concentrations tested, maximum repellent activity was observed at 1000 ppm of *O. integrifolia*, *S. Abyssinica and M. ferruginea* and also *E. kebericho* has strong repellent properties in all concentrations.*O. integrifolia, S. abyssinica, M. ferruginea* and *E. kebericho* may contain repellent chemicals which can be used for the development of safer mosquito repellent product. © 2016 Association for Advancement of Entomology

KEY WORDS: Repellent activity, volatiles, malaria vector, *Anopheles*

INTRODUCTION

Mosquitoes are responsible for transmission of malaria which is one of the important and fatal diseases worldwide (Yohannes and Boele, 2011). In sub-Saharan Africa, children under the age of five years and pregnant women are highly affected by malaria (Morlais *et al.,* 2005). In Ethiopia, 68 per cent of the populations live in malaria prone areas covering almost 75 per cent of the land (FDROEMOH, 2006; PMI, 2010).

The diverse eco-climatic condition in Ethiopia is much favourable for malaria transmission pattern seasonal and unstable. The widely distributed malaria vector in Ethiopia includes *Anopheles arabiensis, Anopheles pharoensis, Anopheles funestus* and *Anopheles nili.* These vector breeds in small, temporary, sunlight pools and in low land as well as highland areas up to 2000 m. a. s. l. (Nyanjom *et al.,* 2003; Ashenafi Woime, 2008).

According to the World Health Organization, mosquito control using insecticides is the most efficient means for short term being widely exploited in the treatment of bed nets and indoor residual spraying (Yakob *et al.,* 2011; Bigoga *et al.,* 2012). Chemical control of mosquitoes is highly complicated because of persistent chemical insecticides lead to environmental pollution, killing non-target organism and insecticides resistance development among the vector populations, especially in the *Anopheles gambiae* complex (UNICEF, 2000). Despite, considerable effort is

* Author for correspondence

made through control programs to curb the disease burden; it is still remains a major public health problem in many countries including Ethiopia. These problems have warranted the need for search and development of alternative eco-friendly strategies.

Bioactive compounds from plants are eco-friendly, environmentally safe, biodegradable and cost effective without altering natural habitat (Redwane *et al.,* 2002; Mittal and Subbarao, 2003). The larvicidal and repellent effects of essential oils extract from various plants are reported to be effective against different mosquito species (Duangkamon *et al.,* 2011; Raghavendra *et al.,* 2011). Secondary metabolites of bioactive plants reported to inhibit insects' development and production behaviour repellence (Viglianco *et al.,* 2006), anti-feeding effect (Eriksson *et al.,* 2008), growth regulation (Wheeler and Isman, 2001), feeding deterrence (Koul, 2004), and oviposition deterrence (Banchio *et al.,* 2003). The plant secondary metabolites are evaluated against insects as volatile chemicals (Bobadilla *et al.,* 2005), essential oils (Pérez-Pacheco *et al.,* 2004) and powders (Silva *et al.,* 2003). Mudalungu *et al.* (2013) reported potential larvicidal activity of both essential oil and non-volatile compounds from *Fagaropsis angolensis* leaves against *A. gambiae* larvae. The larvicidal and repellent effects of the essential oils from the seeds and leaves of *Chenopodium ambrosoides* was reported against larvae and adults of *A. gambiaes* mosquitoes (Bigoga *et al.,* 2013). There is no report on repellent properties *O. integrifolia, S. abyssinica, M. ferruginea, D. stramonium* and *E. kebericho* against *A. arabiensis* mosquitoes. These valuable medicinal plants are widely distributed and abundance in Ethiopia. Therefore, present study was initiated to evaluate repellent properties of selected plant essential oil extracts against malaria vector, *A. arabiensis.*

MATERIALS AND METHODS

The laboratory study was conducted from February 2015 to June 2015 at General Entomology laboratory, Department of Biology, College of Natural and Computational Sciences, University of Gondar. The study area is located in the North West of Ethiopia with 12^o 36" N latitude and 37^o 28" E longitude with an elevation of 2133 meter above sea-level.

a) Anopheles arabiensis culture establishment

Eggs of *Anopheles arabiensis* were collected from pastor campus, Addis Ababa University and reared in the laboratory at 27 ± 1° C, 65–70% RH and 12:12 h light: dark cycle. Once the larvae reached the pupal stage they were transferrd to adult emergence cage. The adult mosquitoes emerged from the pupa were provided *ad libitum* access of 10 percent sugar solution (w/v) and kept in Bugdorm cages (30 cm x 30 cm x 30 cm). The starved female adults were allowed to feed blood meal from the arms of human volunteers. Blood fed female mosquitoes were allowed to oviposit in 30 ml cups filled with 15 ml of distilled water. The cup edges were covered with a filter paper for egg deposition. Filter papers that contain eggs were transferred to plastic trays (25 cm x 25 cm x 7 cm) filled with distilled water. After egg hatching larvae were provided with finely powdered Tetramin® fish food. The mosquitoes cultures were maintained continuously to get adequate adult female mosquitoes to conduct repellent bioassay.

b) Extraction of essential oils

Otostegia intergrifolia and *S. abyssinica* leaves, *E. kebericho* root and *D. stramonium* and *Millettia ferruginea* seeds were collected during autumn season from Kola Deba region, Ethiopia. The plant parts were thoroughly cleaned with water and shade dried by spreading on a clean and well-ventilated surface. After drying, plant parts were grounded by using electric blender in order to get fine powder for essential oil extraction. Two hundred grams of powder from each plant sample was mixed with 1000 ml of distilled water in a conical flask and subjected to essential oil extraction by hydro-distillation method at 100° C using Clevenger apparatus for 3 h. The essential oils were separated from water, dried over anhydrous sodium sulphate and stored at 4° C for further experimentation (Mudalungu *et al.,* 2013).

Figure 1. Medicinal plants and parts used for essential oil extraction

c) Preparation oil concentration

Stock solution of 10,000 ppm concentration was prepared by adding 1 ml of pure essential oil mixed with 1 ml of acetone and make up to 100 ml in 250 ml conical flask by adding distilled water. Four concentrations viz., 1000, 500, 250 and 125 ppm were prepared through dilution of the stock solution with distilled water. Three replicate for each concentration were made for repellent response of mosquitoes against non-blood fed of female *A. arabienses*. In addition, control contains 1 ml of 100 percent acetone and the amount of distilled water varied according to parallel concentration prepared (Xue *et al*., 2001).

d) Experimental design

Repellent properties of essential oils against mosquitoes were conducted in dark room by maintaining at 27 ± 1^{o} C and relative humidity of 60-70 percent. The whole arm of volunteer was covered with glove except specific area with the diameter of 7 cm removed at the back of palm. The exposed part of arm was applied four drops of essential oils in one hand as a treatment and four drops diluted acetone on the other hand as a control. Both arms simultaneously inserted in to mosquito cage for 20 minute and monitored for number of mosquitoes landed on treatment and control arms.

e) Data analysis

The percentage repellence index (R) was estimated by using the formula $R = (C-T)/C \times 100\%$, where C and T are the data of mosquitoes landed on the control and treated arms, respectively (Chio and Yang, 2008). The SPSS Version 20 software was used to calculate LC_{50} values and LC_{90} values and 95 per cent of upper and lower confidence limit (UCL). The results were subjected to Chi-square analysis for statistical significant at 5 percent level ($p<0.05$).

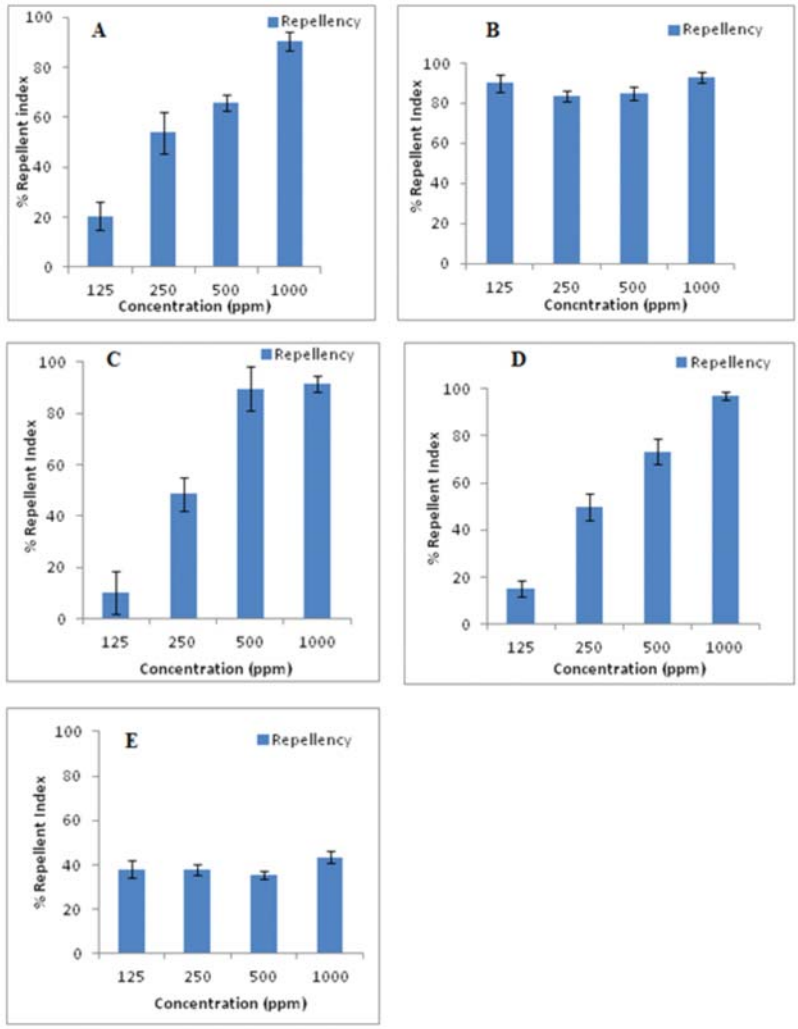

Figure 2. Mean percentage repellent activity of plant essential oils against *Anopheles arabiensis*, A. *Otostegia integrifolia,* B. *Echinops kebericho,* C. *Stephania abyssinica,* D. *Millettia ferruginea,* E. *Datura stramonium*.

RESULTS

a) Repellent activity of *Otostegia integrifolia* essential oil against *A. arabienses*

Mean percentage repellent response of non-blood fed female *A. arabiensis* exposed to essential oil of *O. integrifolia* treated arms of human volunteers after 20 minutes was presented in Fig 2A. Results revealed that the mean percentage repellent activity ranged from 20.54 ± 5.82 to 90.47 ± 3.63 at 125 and 1000 ppm respectively. The calculated LC_{50} and LC_{90} value was 1.886 ppm and 4.865 ppm respectively. The chi-square analysis results showed statistical difference at 5% level (χ^2 = 74.180; p< 0.05). The calculated range of 95 percent lower and upper confidence limit of LC_{50} and LC_{90} value was 1.630 - 2.139 ppm and 3.986 - 6.644 ppm respectively.

b) Repellent activity of *Echinops kebericho* essential oil against *A. arabienses*

Mean percentage repellent response of non-blood fed female *A. arabiensis* landed on essential oil of *E. kebericho* treated human volunteer after 20 minutes was presented in Fig 2B. Results revealed that the mean percentage of repellent activities ranged from 90.31 ± 4.34 - 93.16 ± 2.62 was observed at 125 and 1000 ppm respectively. The calculated LC_{50} and LC_{90} value was 0.28 and 0.71 ppm respectively. The chi-square analysis results showed statistical significant difference at 5% level (χ^2 = 71.58; P< 0.05).

c) Repellent activity of *Stephania abyssinica* essential oil against *A. arabienses*

Mean percentage repellent response of non-blood fed female *A. arabiensis* landed on essential oil of *S. abyssinica* treated human volunteer arms after 20 minutes was presented in Fig 2C. Results revealed that the mean percentage repellent activity ranged from10.27 ± 4.09 – 87.73 ± 4.46 at 125 and 1000 ppm respectively. The calculated LC_{50} and LC_{90} value was 1.89 and 3.52 ppm respectively. The chi-square analysis results showed statistical difference at 5% level (χ^2 = 263.80; p< 0.05). The calculated range of 95% lower and upper confidence limit of LC_{50} and LC_{90} value was 1.52 – 2.26 and 2.87 – 5.09 ppm respectively.

d) Repellent activity of *Millettia ferruginea* essential oil against *A. arabienses*

Mean percentage repellent response of non-blood fed female *A. arabiensis* landed on essential oil of *M. ferruginea* treated human volunteer arms after 20 minutes was presented in Fig. 2D. Results revealed that the mean percentage of repellent activities ranged from 15.33 ± 3.40 – 97.12 ± 1.67 at 125 and 1000 ppm respectively. The calculated LC_{50} and LC_{90} value was 1.89 and 3.85 ppm respectively. The chi-square analysis results showed statistical significant difference at 5% level (χ^2 = 82.05; p< 0.05). The calculated range of 95 percent lower and upper confidence limit of LC_{50} and LC_{90} value was 1.67 – 2.11 and 3.31 – 4.78 ppm respectively.

e) Repellent activity of *Datura stramonium* essential oil against *A. arabienses*

Mean percentage repellent response of non-blood fed female *A. arabiensis* landed on essential oil of *D. stramonium* treated human volunteer arms after 20 minutes was presented in Fig. 2E. Results revealed that the mean percentage repellent activity ranged from 38.10 ± 3.68 – 43.47 ± 2.64 at 125 and 1000 ppm respectively. The calculated LC_{50} and LC_{90} value was 13.07 and 26.42 ppm respectively. The chi-square analysis results did not show statistical difference at 5 percent level (χ^2 = 19.16; P>0.05).

DISCUSSION

Bioactive products of plant have been used to control mosquitoes for a very long time. Natural plant products reported to be effective against the mosquito vector species and considered as plausible alternatives to synthetic chemical insecticides. Secondary metabolite produced by diverse plant species contains unique biological principles, such as toxin for physiological activities and attractant or deterrents for behavioural response of insect (Arivoli *et al.*, 2011; Muthu *et al.,* 2012). Essential oil extract from leaves, flowers seeds and roots of various plants not only exhibit inhibitory activity against bacteria, fungi and termites but also showed strong mosquito repellent and larvicidal activities (Sosan *et al.,* 2001; Cheng *et al.,* 2004). Larvicidal and repellence effects of essential oils from various botanicals against different mosquito species were reported (Bigoga *et al*., 2013).

The local communities in Ethiopia traditionally adapt various methods to repel the insects/ mosquitoes. Application of smoke by burning the plant parts is one of the most common practices. In addition, spraying extracts of *O. integrifolia* and *E. kebericho* after crushing and grinding; hanging and sprinkling on the floor as a protestant against mosquito bites by believing repellent properties (Kidane *et al*., 2013). Previous studies, confirmed that extracts of smoke from burning leaves were repellent to host-seeking non-blood-fed female *A. arabiensis* (Due *et al*., 2011). Moreover, Karunamoorthi *et al*. (2008) observed that *O. integrifolia* were burnt to repel mosquitoes, have also demonstrated a large reduction in the number of mosquitoes landing. There are also many other examples of burning leaves to decrease the number of mosquitoes in the house, some of which have also resulted in the reduction of other arthropod vector densities indoors, such as the sand fly and black fly (Moore and Debboun, 2006 and Biran *et al*., 2007).

The present results confirmed repellent activities of *O. integrifolia, E. kebericho, S. abyssinica* and *M. ferruginea* essential oils against *A. arabiensis*. Mean percentage repellence of essential oil was 90.47, 93.16, 87.75 and 97.12 percent at 1000 ppm after 20 minutes exposure period in *O. integrifolia, E. kebericho, S. abyssinica* and *M. ferruginea* respectively. The smoke from *Otostegia integrifolia* leaves was previously suggested as a strong mosquito repellent in controlled semi-field studies using volatiles expelled through heating the leaves on metal plate (Seyoum *et al*., 2003 ; Due *et al.,* 2011). The leaves of many plant species reported to contain repellent compounds (Carroll and Loye, 2006). The smoke produced by burning dry roots of *E. kebericho* act directly as a natural insect repellent to provide protection against mosquitoes and other harmful arthropods (Fokialakis *et al*., 2006; Tariku *et al.*, 2011). These essential oils are used currently in many commercially available products like; perfume, soap and deodorant, appear to be within the repellent activity against mosquito species tested in the laboratory (Carroll and Loye, 2006). The result of this study is an indication that the essential oil of tested five species of plants has potential repellent action against *A. arabiensis*. These essential oils can be utilized for the development of mosquito repellent products.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the University of Gondar for financial assistance (project number 372-04-01; fund number 6223).

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(Received 09 December 2015; Accepted 22 February 2016; Published 30 June 2016)