WATER EXTRACTS OF HARGAL PLANT (*Solenostemma argel*, Del Hyne) AND USHER (*Calotropis procera* Ail) LEAVES AS NATURAL INSECTICIDES AGAINST MOSQUITO LARVAE

By

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ABSTRACT:

This study was carried out to evaluate the insecticidal efficacy of water extracts of Hargal plant, *Solenostemma argel* Del Hyne, and Usher, *Calotropis procera* Ail, leaves against the larvae of two mosquito species, *Anopheles arabiensis* and *Culex quinquefasciatus*. The synthetic larvicide, Abate® (Temphos), was used as a standard. Statistical analysis showed significant differences between the tested concentrations of each plant, with activity increases progressively with dosage rates. The highest concentrations of the two plants gave the best and comparable results with that of standard insecticide, Temphos. Hargal extract showed the best LD₅₀ against *Cx. quinquefasciatus* (0.006 ml/L) and *An. arabiensis* (0.140 ml/L), as compared with Usher extract (0.108 and 0.263 ml/L, respectively).

The current results were considered promising to proceed in studying the bioactive plants which represent an environmentally sound alternative for the synthetic larvicides.

KEYWORDS: *Solenostemma argel*, *Calotropis procera*, *Anopheles arabiensis*, *Culex quinquefasciatus*, water extract

ملخص:

أجريت هذه الدراسة لتقييم فعالية المحلول المائي لنباتي الحرجل والعش ضد بركات نوعين من البعوض، الأنوافلس و الكيولكس. استخدم مبيد أبيت (تيمفوس) كمبيد قياسي. أظهر التحليل الإحصائي
INTRODUCTION

Malaria and other vector-borne diseases contribute to the major disease burden in tropical and sub-tropical regions. One of the methods which are used to control these diseases is to control their vectors in order to interrupt the cycle of disease transmission. In the past, extensive applications of synthetic organic chemical insecticides in public health have resulted in development of insecticide resistance in some important vectors of malaria, filariasis and dengue fever (WHO, 1992). During the last decades, various studies on natural plant products against mosquito vectors reported their effectiveness as possible alternatives to synthetic chemical insecticides (Schumutterer et al., 1995 and Pathak et al, 2000). However, several studies executed worldwide have shown promising results from different active plants. Sukumar et al (1991) reported 99 families, 276 genera and 346 plant species to have insecticidal properties. Also, the interest has revived recently in Sudan for using natural products for the control of pests and disease vectors (e.g., Siddig, 1991; Abdel Gadir, 1993; El-kamali, 2001; Osman, 2003; Ali, 2004 and Edriss et al, 2008). Accordingly, the present study was conducted to investigate the insecticidal potentiality of aqueous extracts from two indigenous plants in Sudan, Hargal, Solenostemma argel Del Hyne, and Usher, Calotropis procera Ail, for the larval control of two important mosquito species, Anopheles arabiensis (Patton) and Culex quinquefasciatus Say.
MATERIALS AND METHODS

Collection and breeding of mosquito larvae: *Anopheles arabiensis*

Batches of *An. arabiensis* eggs were kindly provided by the Malaria Training Center in Sennar. The eggs were incubated in a laboratory at the National Administration of Malaria, Bilharzia and Leishmania Control in Khartoum. The eggs were placed in white Enamel dishes filled with water up to 4 inches until hatching. After hatching, the larvae were fed with fish food until pupation. The obtained pupae were collected in a wire net cage and placed in an insectary, where suitable conditions for adults were provided by means of controlled temperature at 22 °C and 80% R.H., in addition to an electric bulb put on for 12 hours during the day. Sugar dissolved in tap water was placed in small bottles, each provided with a thin cotton robe extending from the top to the base side. Adult mosquitoes feed on the sugar solution by sucking on the tip of the wet robe. Adult females were also provided with blood meals, by inserting a bare human hand in the cage for adult feeding. Petri-dishes covered with wet cotton and filter papers were placed inside the cages for egg laying.

*Culex quenquifasciatus*

Rafts of *Cx. quenquifasciatus* eggs were collected regularly from several ditches around Shambat area. Collection was made early in the morning using small ladles, and poured in big plastic bucket. Materials collected were taken to the laboratory and distributed in white metal dishes containing dechlorinated tap water. After hatching, first instar larvae were fed on small amounts of wheat flour until reaching the third instar.

Preparation of plant extracts:

Hargal water extract

Fresh shoots of Hargal plants were obtained from local market and ground using an electric blender (Moulinex). The powder obtained was passed through a 1mm mesh sieve and stored in glass jars covered with plastic covers and left at room conditions. For preparation of the extract, 20 gm of the powder was macerated in distilled water and shacked by a magnetic stirrer for 6 hours. The extract was filtered through a cotton cloth after 24 hours. The volume was
adjusted to 400 ml with distilled water for a stock concentration of 50 mg/L. and serial dilutions were made from the stock solution.

**Usher water extract:**
Fresh leaves of Usher plant were collected from the Faculty fields at Shambat, and left to dry for one week at room temperature. Dried leaves were firstly crushed by hand, then ground by an electric blender. The obtained powder was passed through a 1mm mesh sieve and stored in tightly covered glass jars. For preparation of the water extract, 20gm of the powder was mixed with 400ml of distilled water in a conical flask and filtered by a filter paper. The filtrate was kept in a tightly closed glass jars wrapped with aluminium foil and kept in the refrigerator at 5 °C as a stock solution (50 mg/L). Several dilutions were prepared from the stock solution as before.

**Bioassay:**
**Testing larvicidal activity of Hargal and Usher water extract**
Fifteen, 3rd or early 4th instar larvae of each mosquito species were used per replicate in this experiment. The larvae were put in dishes, each containing 250 ml of tap water. Five concentrations of each extract: (1, 0.5, 0.25, 0.125, and 0.0625 %), in addition to the standard larvicide, Abate®, as a standard, were applied in three replications. Tap water only was used in the control treatment. No food was provided for the larvae. The mortality effects of treatments were checked after 24 hours.

**Statistical analysis:**
Data were transformed using Arc Sine and ANOVA was done according to the Completely Randomized design. Duncan’s Multiple Range Test was used for means separation Also, the probit analysis was applied (e.g., Finney, 1971; Busvine, 1971 and Sokal & Rohlf, 1973) to calculate the LD₅₀ for the different extracts.

**RESULTS**
**Effects of Hargal and Usher water extracts on mosquito larvae**
The water extracts of the two plants caused a significant increase in percentage mortality of the larvae of the two mosquito species (p< 0.01), compared to the control (Tables 1 and 2).
The highest concentrations of the two plant extracts were as effective as that of the standard larvicide, Temphos, which caused 100% mortality of mosquito larvae after 24 hours. Application of the probit analysis for the results of the bioassays of Hargal extract showed LD_{50} of 0.006 % and 0.140% for the *Culex* (Figure 1) and *Anopheles* (Figure 2) larvae, respectively. With Usher extract, the probit analysis showed LD_{50} of 0.108 % and 0.263 % for the *Culex* (Figure 3) and *Anopheles* (Figure 4) larvae, respectively.

Table 1. Effect of Hargal plant water extract on the larvae of *Culex quinquefasciatus* and *Anopheles arabiensis* (Mortality counts made after 24hrs.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Culex</em> larvae (% Mortality)</th>
<th><em>Anopheles</em> larvae (% Mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0625 % water extract</td>
<td>89.1 (70.74) b</td>
<td>28.9 (32.49) d</td>
</tr>
<tr>
<td>0.1250 % water extract</td>
<td>95.6 (77.87) ab</td>
<td>46.7 (43.08) c</td>
</tr>
<tr>
<td>0.250 % water extract</td>
<td>99.2 (85.00) ab</td>
<td>53.4 (46.92) c</td>
</tr>
<tr>
<td>0.5 % water extract</td>
<td>99.2 (85.00) ab</td>
<td>91.3 (72.87) b</td>
</tr>
<tr>
<td>1 % water extract</td>
<td>100 (90.00) a</td>
<td>100 (90.00) a</td>
</tr>
<tr>
<td>Temphos</td>
<td>100 (90.00) a</td>
<td>100 (90.00) a</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>0.0 (0.57) c</td>
<td>0.0 (0.57) e</td>
</tr>
<tr>
<td>SE±</td>
<td>3.68**</td>
<td>1.52**</td>
</tr>
</tbody>
</table>

** Significant difference at (P<0.01)

*Figures followed by the same letter(s) are not significantly different at 0.05 levels (Duncan’s Multiple Range Test).

*Figures between brackets were arcsine transformation for percent.

Fig 1: Dose- Mortality response lines of *Solenostemma argel* against *Culex sp.* larvae.
Fig (2): Dose- Mortality Response Lines of *Solenostemma argel* against *Anopheles sp.* larvae.

Table 2. Effect of Usher leaves water extracts on the larvae of *Culex quinquefasciatus* and *Anopheles arabiensis*. (Mortality counts made after 24hrs.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Culex larvae (% Mortality)</th>
<th>Anopheles larvae (% Mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0625 % water extract</td>
<td>38.7 (37.86) b</td>
<td>3.1 (10.19) d e</td>
</tr>
<tr>
<td>0.1250 % water extract</td>
<td>41.4 (40.02) b</td>
<td>8.7 (17.13) c d</td>
</tr>
<tr>
<td>0.250 % water extract</td>
<td>76.1 (60.75) b</td>
<td>15.4 (23.11) c</td>
</tr>
<tr>
<td>0.5 % water extract</td>
<td>100 (90.00) a</td>
<td>60.1 (50.81) b</td>
</tr>
<tr>
<td>1 % water extract</td>
<td>100 (90.00) a</td>
<td>100 (90.00) a</td>
</tr>
<tr>
<td>Temphos</td>
<td>100 (90.00) a</td>
<td>100 (90.00) a</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>0.0 (0.57) c</td>
<td>0.0 (0.57) e</td>
</tr>
<tr>
<td>SE ±</td>
<td>5.03**</td>
<td>2.26**</td>
</tr>
</tbody>
</table>

*Significant difference at (P< 0.01)

*Figures followed by the same letter(s) are not significantly different at 0.05 levels (Duncan’s Multiple Range Test).

*Figures between brackets were arcsine transformation for percent.
Fig (3): Dose-Mortality Response Lines of *Calotropis procera* against *Culex sp.* larvae.

Fig (4): Dose-Mortality Response Lines of *Calotropis procera* against *Anopheles sp.* larvae.
DISCUSSION

During the last three decades, pest control methods were directed to the use of insecticides of plant origin. This trend appeared as a result of the accumulated side effects and environmental contamination from a long term extensive application of toxic synthetic insecticides. The good example of these botanical insecticides was those derived from the neem tree (*Azadirachta indica*) and tried against more than 400 species of pests (Schumutterer *et al.*, 1995). Also, Stoll (2000) mentioned about 65 plant species that showed insecticidal activity against a large number of insect pests. However, several studies were carried out in Sudan using plant extracts against a number of insect pests of agricultural or medical importance (e.g., Siddig, 1991; Ahmed, 1993; El-Kamali, 2001; Osman, 2003, and Ali, 2004).

In this study, water extracts of two indigenous plants, Hargal and Usher, were tested as larvicides against two prevailing mosquito species in Sudan, *Culex quinquefasciatus* and *Anopheles arabiensis*. The results showed that, the highest concentration (1% w/v) of Hargal shoot water extract was the best significant treatment in controlling the *Culex* and *Anopheles* larvae according to the tested concentrations. Similar results were obtained with the highest rate of Usher leaves water extract against the two mosquito species.

The probit analysis revealed that, Hargal water extract showed an LD$_{50}$ of 0.006 mg/L for *Culex* larvae. El-Kamali (2001) applied water extracts from the stems, roots, fruits and flowers of Hargal against *Culex* larvae, and obtained LD$_{50}$ of 1, 0.8, 0.5 and 0.25 mg/L, respectively. It seems from these contrasting figures that, application of Hargal extract of the whole plant (as applied in this study) is more potent as a biocide than the extract of each part alone. Probit analysis also showed an LD$_{50}$ of 0.14 mg/L of Hargal extract against *Anopheles* larvae.

Concerning the effects of Usher water extract on both mosquito species, the probit analysis showed LD$_{50}$s of 0.108 mg/L and 0.263 mg/L for *Culex* and *Anopheles* larvae, respectively. These results are in the same trend with those of Ali (2004), who obtained LD$_{50}$ of 122.29 mg/L and 166.71 mg/L for *Culex* and *Anopheles* larvae, respectively. On the other hand, the present results disagree with those of Osman (2003) who found LC$_{50}$s of 0.929 g/L and 1.367 g/L of Usher leaves water extract against the larvae of *Culex* and *Anopheles*, respectively.
Osman, (2003) also showed that, Usher fruits water extract had almost the same efficiency against both mosquito larvae. The results of the present study clearly showed that, both Hargal and Usher water extracts were effective against both mosquito species and may represent a good friendly alternative to synthetic larvicides.

REFERENCES


