Journal of Science and Technology 12 (03) December 2011
ISSN 1605 – 427X

www.sustech.edu

Larvicidal Effects of Some Plant Extracts Against *Anopheles arabiensis* Patton Larvae (Diptera : Culicidae)

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ABSTRACT: Laboratory experiments were conducted to evaluate the larvicidal activity of ethanol extracts from leaves of three plants: castor bean (*Ricinus communis*), vinca (*Vinca rosea*) and lantana (*Lantana camara*) under 5 concentrations as 250- 3000 ppm against the 3rd instar larvae of the main mosquito vector of malaria in Sudan *Anopheles arabiensis*. The different larval mortality percentages were recorded after 24 hours. The larvicidal effect of each plant extract was compared with the standard larvicide Temephos (Abate®). The three tested plant extracts in their different concentrations have shown larvicidal effects on *An. arabiensis* larvae. Statistical analysis showed no significant differences between the higher concentration of each of the three plant ethanol extracts and the standard larvicide Temephos and that all concentrations recorded 100 % mortality of the tested larvae. The results indicated that, castor extract had the greatest larvicidal effect against *An. arabiensis* larvae with the lowest LC_{50} (282.7060 ppm) followed by vinca and lantana extracts respectively. This study suggests that, the leaf extracts of the three plant species should be considered as promising larvicides against *An. arabiensis* larvae.

KEYWORDS: *Anopheles arabiensis, Lanatana camara, plant extracts, Ricinus communis,* Temphos*, Vinca rosea*

INTRODUCTION:

Mosquitoes are by far the most important of the bloodsucking arthropods, giving annoyance to and causing diseases in humans, other mammals, and birds (Goddard, 2007). About 3500 species of mosquitoes have been described worldwide. Relatively few of them are significant vectors of human diseases; however, the mosquito-transmitted disease problem worldwide is quite severe (Goddard, 2007). They are important vectors of malaria, various forms of filariasis and numerous arboviruses, the best known being dengue, yellow fever and West Nile virus (Service, 2008).Early efforts to control arthropod pests and disease vectors relied primarily on the application of broad-spectrum pesticides. This approach often caused undesirable side effects, including environmental pollution, the development of insecticide resistance and sometimes sudden increases in old, or even new and worse, pests (Youdeowei and Service, 1983).

Insecticide resistance is now a major problem facing malaria vector control programs in most African countries with all three main vectors

species, *An. gambiae, An. arabiensis* (of the *An. gambiae* complex), and *An. funestus*, showing resistance to one or more of the insecticide classes used in vector control (Coetzee, 2004). So, due to the dramatic increase in resistance of mosquitoes to familiar chemicals in the absence of new compounds, better alternative means of control are sought (Tripathi, *et. al*., 2003). Natural plant poisons have at least a reasonable advantage over synthetic molecules in terms of ecological safety. Their development as successful pests and vector control agents can also be economically feasible, especially if the sources of material are abundant plants e.g. common weeds, prolific herbs, shrubs, and trees having a wide and rich distribution (Sharma *et al*., 1981).

The main objectives of this study were to evaluate the efficacy of ethanol leaf extracts of *Ricinus communis* L. (Castor), *Vinca rosea* L. (Vinca) and *Lantana camara* L. (Lantana), (which have a wide distribution in the Sudan) against the larvae of *Anopheles arabiensis* as compared to the standard larvicide temephos (Abate ®).

MATERIALS and METHODS:

Anopheles arabiensis culture:

An. arabiensis larvae used in this work were kindly supplied by the insectary of The Tropical Medicine Research Institute, Khartoum. Adults were kept in cages (30 x 30 x 30 cm), fed with 10% glucosesolution, in addition to an animal blood meal given to the females twice per week. A petri dish lined with moist cotton piece and covered with filter paper was put inside each cage for eggs laying. The obtained eggs were transferred to trays (40 x 28 x 8 cm) half-filled with water. The hatching larvae were fed on a small amount of fish food daily. When the pupae were formed no food was supplied and the trays were covered with mosquito nets. The emerged adults were transferred to another cages by an aspirator.

Preparation of the plant materials:

Freshly developed leaves of castor bean plant (*Ricinus communis* L.), vinca (*Vinca rosea* L.) and Lantana (*Lantana camara* L.) were obtained from Shambat area. Leaves were washed in running water and spread on papers to dry under shade for 10 days. Then the dried leaves were ground to a fine powder by means of an electric blender. Each of the leaves powders was then kept at room temperature in labeled plastic bags until used.

Extraction of the plant materials:

The extraction processes were conducted at the Environment and Natural Resources Research Institute (ENRRI), National Research Center (NRC).

Twenty five grams of each of the previously prepared plant powder were wrapped separately in filter paper, placed in thimble units of soxhlet extractor, each unit was connected to a flask containing 250 ml ethanol (95%) as solvent. The extraction process continued for 24 hours. The obtained extracts were stored in a refrigerator until used for experimentation.

Larval bioassay:

All treatments were conducted at the insectary of the Khartoum Malaria-Free Initiative. Stock solutions (10%) from ethanol extracts of the plant leaves (castor, vinca and lantana) were prepared for each plant separately. From these stock solut-

ions Further dilutions were made and after preliminary tests the final concentrations used in the experiments were 3000, 2000, 1000, 500 and 250 ppm.

The standard larvicide Temephos (Abate®) was used at a rate of 0.025 ppm. One liter of each plant extract and Temephos concentration was prepared and it was distributed in four 500 ml capacity plastic bowls (replicates) 250 ml per bowl. The untreated water control was prepared with the same previous method. All concentrations of the three botanical extracts and the standard larvicide Temephos were evaluated for mosquito larvicidal activity according to WHO (1969) method. 20 of 3rd instar larvae were placed in each bowl (replicate). The exposure period was 24 hours during which no food was offered to the larvae. Mortality was recorded after the 24 hours, by counting the completely dead or moribund larvae together with the larvae that failed to reach the surface of the solution.

Statistical analysis:

An MSTAT-C software (version: 2.10) was used for analysis of data by using One way ANOVA analysis method and mean separation was achieved using Duncan's Multiple Range Test. For data transformation, the arcsine was applied. Also SPSS programme (version: 10.0) was used for Probit analysis, as described by Finney (1971) and the concentrations were transformed to logarithm (Log-concentration) and the lethal concentrations (LC_{50} and LC_{95}) were calculated.

RESULTS:

Tables 1-4 and figure 1 show the mortality after 24 hours caused by each plant extract and the standard larvicide Temephos on *Anopheles arabiensis* in chronological order.

All the data showed that there was a highly significant difference between treatments and control. The mortality progressively increased with increasing extract concentrations.

Larvicidal effect of castor leaves extract:

In table 1 the 5 concentrations of castor leaves extract caused larval mortality of 36.25%, 95%, 100%, 100% and 100% respectively. The LC_{50} and LC_{95} calculated were 282.7060 and 501.2372 ppm (Table 4).

Larvicidal effect of Vinca leaves extract:

In table 2 the 5 concentrations of vinca leaves extract caused larval mortality of 12.5%, 55%, 90%, 100% and 100% respectively. The LC_{50} and LC_{95} calculated were 471.6689 and 1166.0812 ppm (Table 4).

Larvicidal effect of Lantana leaves extract:

Also in table 3 the 5 concentrations of lantana leaves extract showed larval mortality of 17.5%, 65%, 72.5%, 95% and 100%, respectively.

The LC_{50} and LC_{95} were 477.5257 and 1905.1891 ppm (Table 4).

Table 1. Mean percentage mortality of Anopheles arabiensis caused by Castor.

Treatments (ppm)	% **Mortality**
250	32.5 (34.60) d
500	80 (64.50) c
1000	92.5 (74.32) b
2000	100 (98.75) a
3000	100 (98.75) a
Water control	0 (1.25) e
Temephos (0.025)	100 (98.75) a
C.V %	8.02%
L.S.D	1.599
SE ±	6.27

*Means within a column followed by the same letter(s) are not significantly different at P=0.05 (Duncan's Multiple Range Test). *Means between brackets were transformed to the arc sine √Percentage.

Table 2. Mean percentage mortality of *Anopheles arabiensis* caused by Vinca.

Treatments (ppm)	% **Mortality**
250	12.5 (19.74) d
500	55 (47.91) c
1000	90 (76.33) b
2000	100 (98.75) a
3000	100 (98.75) a
Water control	1.25 (4.168) e
Temephos (0.025)	100 (98.75) a
C.V %	11.33
L.S.D	10.58
SE ±	7.22

*Means within a column followed by the same letter(s) are not significantly different at P=0.05 (Duncan's Multiple Range Test). *Means between brackets were transformed to the arc sine √Percentage.

Table 3. Mean percentage mortality of *Anopheles arabiensis* larvae caused by Lantana .

Treatments (ppm)	% **Mortality**
250	17.5 (24.45) d
500	65 (53.88) c
1000	72.5 (58.43) c
2000	95 (81.12) b
3000	100 (98.75) a
Water control	0 (1.25) e
Temephos (0.025)	100 (98.75) a
C.V %	9.44
L.S.D	8.264
SE ±	6.65

*Means within a column followed by the same letter(s) are not significantly different at P=0.05 (Duncan's Multiple Range Test). *Means between brackets were transformed to the Arc sine √Percentage.

Table 4. Probit regression line parameters response of Anopheles arabiensis larvae to different plant extracts.

Treatments	Intercept	S.E.	Chi Square	D. F.	Probability	LD_{50}	LD_{95}
Castor	-16.2121	2.2343	12.290	18	0.832	282.7060	501.2372
Vinca	-11.1875	1.0775	18.878	18	0.399	471.6689	1166.081
Lantana	-7.33276	0.7081	31.084	18	0.028	477.5257	1905.1891

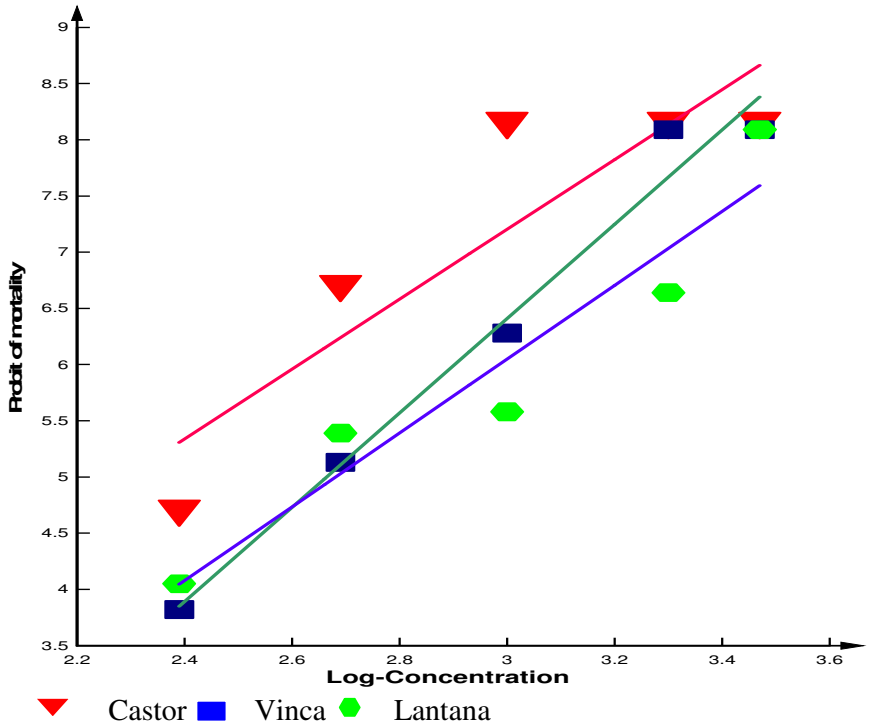

Figure 1: Probit regression lines for mortality of *Anopheles arabiensis* larvae treated with different plant extracts.

DISCUSSION:

Pesticides play significant roles in agriculture and public health programmes. However, increased use and abuse of pesticides have caused great environmental and public health concerns.

Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, degradable, and are readily available in many areas of the world (Sivagnaname and Kalyanasundaram, 2004). Many plant extracts are known to be toxic to different species of mosquitoes and could be used to control the diseases they transmit (Willcox *et al*., 2004). According to Bowers *et al*., (1995) the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. Since Sudan is considered rich in its natural flora (El-Ghazali *et al*., 1997), additional research is

needed to investigate the efficacy of these plants in vector control program. In the same line the present study was conducted to evaluate the efficacy of some plants against *An. arabiensis* larvae.

The results of the test shown in tables 1-3 and figure 1, indicatedted that all concentrations of the three plant extracts demonstrated larviciding effects against *An. arabiensis* larvae.

Also the results of the study showed that there were no significant differences between the higher concentrations of each plant extract and the larvicide Temephos against *An. arabiensis* larvae.

Moreover castor leaves extract was absolutely the best to cause mortality when compared with vinca and lantana treatments, because it exhibits the highest mortality percentages at all concentrations and lowest LC_{50} with *An. arabiensis* larvae. The present results of castor extract are consistent with those Aouinty *et al*., (2006) who stated that aqueous extracts of *Ricinus communis* leaves and *Tetraclinis articulata* wood showed strong toxic activity against 2nd and 4th instars larvae of Culicidae; *Culex pipiens* (Linné), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen).

The larvicidal activity shown by *R. commuinis* is probably due to the presence of the alkaloid, ricinine and the ricin protein which are toxic substances. The leaves contain the alkaloid ricinine (Harborne and Baxter, 2001).

As indicated, the different concentrations of vinca leaves extract had larvicidal effect against *An. arabiensis* larvae. This is in line with the findings of Kehail (2004) who reported that a range of 5 to 65% mortalities with a mean of 28.3 % was recorded for *An. arabiensis* larval population treated with 3g of *Vinca rosea* leaves powder per liter water. This aqueous extract exhibited these effects for around five days only.

Nelson *et al*., (2006) reported that the whole *V. rosea* plant is poisonous. So the larvicidal properties exhibited by vinca extract in this study, might be related to presence of vinca alkaloids toxins in the plant.

The results of lantana leaves extract also proved that they have larvicidal properties against *An. arabiensis* larvae. These findings agreed with Nath *et al*., (2006) who found that leaf extract of *L. camara* showed larvicidal (LC_{90}) activity against *C. quinquefasciatus* and *Aedes albopictus*. Another study by Innocent *et al*., (2008) showed the effect of the root barks extracts of *Lantana viburnoides* sp *viburnoides* var *kisi* against late 3rd or early 4th instar larvae of *Anopheles gambiae s.s.* They reported that extracts could serve as a source of larvicides for managing various mosquito habitats in the field even in their semipurified form. Similarly, the presence of lantadene triterpenoids and furanon-aphthaquinones in *Lantana* sp may serve as an indicator for the plant's mosquito larvicidal properties.

Results of probit regression analysis (Table 4) demonstrated the LC_{50}s of different plant extracts. It showed the same trend of the mortality results,

when castor extract exerted the lowest LC_{50}s against *An. arabiensis* (LC_{50}=282.7060 ppm) compared to the other plant extracts.

These results suggest that the leaves extracts of the three plant species (castor, vinca and lantana) are promising as larvicides against *An. arabiensis*, so further studies are needed to determine the active ingredients in these phytochemicals and to know their mode of action, toxicity, and stability and to study their impacts on human health and non target organisms in mosquito feeding habitats.

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