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Toxicity of Ethanolic Extract of Leaves of *Calotropis procera* Aiton (Ushar) (Gentianales: Apocynaceae) Against the Larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae)

Taha Mansour Elhag Hammed

Department of Life Sciences, Faculty of Education, Nile Valley University

Corresponding author: tahamansour1977@gmail.com

Abstract

The *Calotropis procera* plant (Ushar) which is used in this study, is available in nature, and considered as a promising resource of larvicide. The objectives of this study are to evaluate $LC_{50\%}$ and $LC_{90\%}$ values under laboratory condition after exposure (for 24, 48 and 72 hours) of ethanolic extract of *C. procera* (Ushar) leaves against the larvae of *Cx. quinquefasciatus.* The LC_{50} values (50% mortality) were 360 and 198 ppm for 24 and 48 or 72 hours of exposure, respectively. The LC_{90} values (90% mortality) were estimated to be 881ppm at 24 hours and 479 ppm for 24 and 48 or 72 hours of exposure to *C. procera* leaves extract.

***Keyword*:** *Calotropis procera*, *Culex quinquefasciatus*, larvae, Toxicity

سمية المستخلص الإيثيلي لأوراق نبات العشر *C. procera* ضد يرقات بعوضة الكيولكس خماسية الخطوط

طه منصور الحاج حمد

قسم علوم الحياة، كلية التربية، جامعة وادي النيل، السودان

المستخلص

نبات العشر *Calotropis procera* المستخدم في هده الدراسة متوفر في الطبيعة ويعد من المصادر الواعدة للمبيدات اليرقية .هدفت هذه الدراسة لتقييم سمية المستخلص الإيثيلي لأوراق نبات العشر *C. procera* ضد يرقات بعوضة الكيولكس خماسية الخطوط *Culex quinquefasciatus* في ظروف المعمل. عُبِر عن السمية هنا بـLC_{50} (التركيز الذي يقتل 50% من اليرقات) وLC_{90} (التركيز الذي يقتل 90% من اليرقات). كانت قيم LC_{50} و LC_{90} محسوبة بالجزء من المليون ppm للمستخلص الإيثيلي لأوراق العُشر *C. procera* (LC_{50} =360 and 198 ppm; LC_{90} =881 and 479 ppm)بعد فترة تعرض لمدة 24و48 أو 72 ساعة في ظروف المعمل علي الترتيب.

كلمات مفتاحية: نبات العشر، بعوضة الكيولكس خماسية الخطوط، اليرقات، السمية

Introduction

Culex quinquefasiatus (mosquito) is of cosmopolitan distribution, and it transmits human filariasis a major public health problem in many tropical countries including Sudan, it infects more than two million individual worlds annually (Gosh *et al*., 2008). The adult females have anthropophilic and zoophilic tendencies. It is a potential vector of *Dirofilaria immitis* and arboviruses like West Nile virus (WNV), Rift Valley fever virus, avian pox and protozoa like *Plasmodium relictum* that causes bird malaria and Chikungunya virus (Bhattacharya and Basu, 2016). El-Rayah (2007) reviewed that about 45 species of *Culex* mosquitos are recorded by Lewis (1954) in Sudan including the old name of *Cx. quinquefasciatus* (*Cx. pipiens fatigans* Wiedman). The *Cx. quinquefasciatus* is also known as domestic annoying mosquito especially in urban areas. It is the most prominent species of mosquitoes group in Khartoum, and Sudan (El-Rayah, 2007). The control of *Culex* is normally based on the prevention of breeding. Since such intervention is impossible or economically unfeasible, larvicides can be used (Rozendaal, 1997). Larval stages of mosquitoes are targeted in control strategies, because the larva is relatively immobile, and more concentrated than the adult stage (Karunamoorthi *et al*, 2008; Ali and EL-Rabaa, 2010). Synthetic insecticides pose a high residual toxicity which poison live-stock and human beings (Karunamoorthi *et al*., 2008). Consequently, researchers are currently investigating various plant extracts to be used as insecticides for controlling larvae of mosquitoes, as they are suspected to be environment friendly, biodegradable, and safer than synthetic larvicides (Cetin *et al*., 2006).

More than 2000 plants are known to possess larvicidal activity in their secondary metabolite compounds of plants, such as saponin (Wiesman and Chapagain, 2006), phenolics, isoflavonoids, essential oils, alkaloids, and tannin (Gosh *et al.*, 2008). Phytochemicals may serve as a suitable alternative to synthetic insecticides. In Sudan, Ali (1987) reported larvicidal activity of *Calotropis procera* (*Usher*). Many plants extracts are applied as a phyto-control of mosquito's larvae such as *C. procera*, a common semi desert weed which are widely distributed in Sudan (Ali and EL- Rabaa, 2010)

The objectives of this study are to evaluate toxicity effect represented as $LC_{50\%}$ and $LC_{90\%}$ values under laboratory condition of ethanolic extract of *C. procera* (Uschar) leaves against the larvae of *Cx. quinquefasciatus.*

Materials and methods

Mosquito rearing

Egg rafts of *Culex* mosquitoes were collected from their breeding sites, and then transported to the laboratory into plastic vials (7cm in diameter and 7cm deep) containing de-chlorinated (three days old) tap water; Under, such conditions hatching takes place after approximately 24 to 48 hours. The larvae were kept in clean plastic container containing distilled water; they were fed on autoclaved wheat flour. Pupae were transferred in small plastic cups and kept into mosquito breeding cages 60 × 60 × 60 cm. High relative humidity was ensured inside the cage (60±10%) by means of a wet towel fixed over the top of cage. The cage was provided with a wick containing 10 percent sucrose for male. Females were given the chance to feed on blood of a back plucked pigeon once daily (Elhag, 2010). To guarantee genetic homogeneity, all larvae used in the present investigation were older than the fourth filial generation (F4).

Collection and preparation of plants materials

Fresh, healthy, leaves of the Ushar *C. procera* were clipped from wild growth populations surrounding of the Faculty of Education, Nile Valley University during November-2018, and transported to the laboratory, washed, plotted in newspapers, and left for two weeks to dry in the shade. The dry leaves were powdered and sieved, and kept in plastic bags until needed (Tahir *et al*., 2013).

Extraction of plants materials

Extractions of substances from the dry leaves were done using absolute ethanol (analytical grade). When needed, about 50 grams of the dry leaves were mixed with 300 ml ethanol. The blend was kept cold for a week in a refrigerator. The solution was then filtered with muslin cloth first and then by filter paper, filtrate was kept in a previously weighed 500 ml glass beaker. The solvent was completely evaporated in water bath (70^0C) as described by Elimam (2007); Elhag (2010) and Shahi *et al*. (2010). The thick pasty extracts were left in a coverless beaker at room temperature (30 ± 5^0C) to get a dry extract. A strict weighing procedure with a digital balance

accurate to the fourth decimal was followed to determine the amount of dry extract obtained from a known weight of dry leaves.

Preparation of stock solutions of the plant extracts

Stock solutions were prepared from crude extract dry materials by adding a suitable amount of distilled water to yield a known concentration 1% (*w/v*), thus 10000 parts per million (ppm). The prepared mixtures were kept in an airtight brown vial as stock solution and refrigerated until used for bioassays to prepare the test concentrations. The following simple equation was used to determine the total volume of distilled water needed to obtain the required concentration of the stock solution:

$$S = (D \times 100)/ C$$

Where:
S: the required ml of distilled water; *D*: dry weight of plants materials extract in grams or volume of latex; *C*: concentration of stock solution required (%).

Bioassay for larvicidal activity

Following the conventional methods recommended by WHO (2005), five concentrations (5000 ppm, 2500 ppm, 1250 ppm, 620 ppm and 310 ppm) of plant extract were used to catch a trend rundown mortality between 0 % to 100% in mosquito larvae. Every concentration was tested with a total of 100 late third instars laboratory reared larvae executed as five replicates, each with 20 larvae. The tests were done in 200 ml glass jar containing 50 ml of the test solution. The control was set up with 50 ml of distilled water. The test larvae were not fed during the test. Mortality was recorded after 24, 48, and 72 hours for each concentration.

Statistical analysis

To find out whether the applied doses of toxicants are mathematically related to the intensity of response and to evaluate the strength of the relationship, the quantitative data obtained were subjected to Pearson correlation analysis (R Coefficient correlation). The concentration and mortality percentage were found to form a linear relationship. The regression equation ($y = a + bx$) was calculated by regression analysis. LC_{50}, LC_{90} and 95% confidence limits intervals were calculated from a log dosage-probit mortality equation (A double transformation regression probit analysis).

Results

Correlation between the concentrations of the tested plants extracts and the mortality response of *Culex quinquefasciatus* larvae

The Pearson's correlation R analysis of results showed the presence of high significantly positive correlation between concentrations of plant extract and mortality responses. The results showed concentration dependency, as concentration increased, percentage mortality increased. High

positive significance Pearson's correlation has been detected between concentrations of ethanolic extract of *C. procera* leaves and mortality responses of the larvae of *Cx. quinquefasciatus* at 0.998, 0.864, and0.864 for 24, 48 and 72 hours of exposure under laboratory conditions, respectively (Table 1 and Figure 1).

Toxicity of ethanolic extract of *Calotropis procera* (Ushar) leaves against the larvae of *Culex quinquefasciatus* under laboratory conditions

The extract possesses high level of toxicity against the larvae of mosquitoes *Cx. quinquefasciatus*. The LC_{50} values (50% mortality) were 360 and 198 ppm for 24 and 48 or 72 hours of exposure, respectively. The LC_{90} values (90% mortality) were estimated to be 881ppm at 24 hours and 479 ppm for 24 and 48 or 72 hours of exposure. Among the three time of exposure for the two tested form it was observed that, 72 hours of exposure was gained the lowest LC_{50}, and LC_{90} value; while 48 hours of exposure comes second followed by 24 hours of exposure (Table 1 and Figure 1).

Discussion

Although chemical control is an effective control for pests because it is practical and rapid in action, but the uses of synthetic insecticides has led to environmental pollution it has many disadvantages such as residual effects, and they also kill non- targeted insects. Mosquitoes also showed resistance to the insecticides with passage of time (Khan *et al.,* 2015). The plant used in this study *C. procera* is promising natural source of larvicides, it is simple and safe to the layman, not hazardous to the environment, and biodegradable (Olofintoye *et al.,* 2011).

The statistical analysis of the results obtained in the present study showed dose dependency, as concentration of extract increased, mortality of mosquito larvae increased. The leaves extracts of the milkweed *C. procera* show oviposition deterrant, larvicidal and ovicidal activities against mosquito (Girdhar and Pkarnd, 1984; Singh *et al.,* 2005; Sripongpun, 2008 and Kabir *et al.,* 2010).

Table (1): Correlation between different concentrations of *Calotropis procera* (Ushar) leaves extract and mortality of *Culex quinquefasciatus* larvae under laboratory conditions.

Duration of exposure /hours	Lethality /ppm (95% Confidence interval)		R	Sig
	LC_{50}	LC_{90}		
24	360 (230- 490)	881 (760- 1002)	0.998	0.095
48	198 (000- 396)	479 (361- 597)	0.864	0.155
72	198 (000- 396)	479 (361- 597)	0.845	0.155

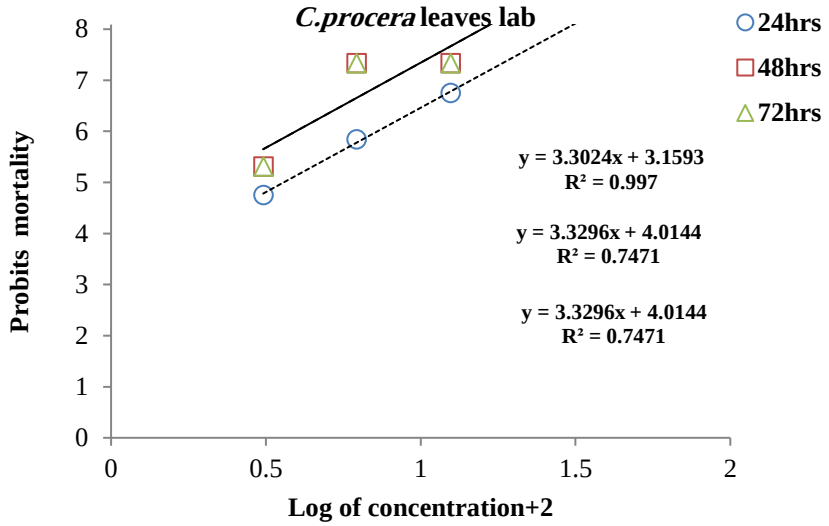

Figure 1. Concentration /response regression line of ethanolic extract of *C. procera* (Ushar) leaves against *Culex quinquefasciatus* larvae

In general, the present results showed time dependency, as duration of exposure increased, mortality of mosquito larvae increased. It can be observed that, 72 hours of exposure gave the lowest LC_{50}, value; while 48 hours of exposure comes second followed by 24 hours of exposure. This agreed with the findings of Singh *et al*. (2015) who noticed that, the toxicity potential of the *C. procera* leaf extract has increased after prolonged periods of exposure of the larvae of *Aedes aegypti*, the LC50 decreased by 2.3%. The influence of exposure time on larval mortality may be due to the amount of active ingredients consumed. The results coincided with those of Elimam (2007), who found that the LC_{50} values in the case of *C. procera* leaf were 187.93, 218.27 and 264.85 ppm for 2nd, 3rd and 4th instars larvae of *Cx. Quinquefasciatus*, respectively. The LC_{90} values (90% mortality) were shown at 433.51, 538.27 and 769.13 ppm for 2nd, 3rd and 4th instars larvae, respectively of *Cx. quinquefasciatus*. The results are in conformity with that reported by Kumar *et al*. (2012) who obtained LC_{50} values of 137.9 ppm against *Culex gelidus* and 110.05 ppm against *Cx. triataeniorhynchus* when assayed with aqueous extract of *Calotropis gigantica*. However, results disagreed with those of Osman (2003) who found LC_{50} of 0.929 g/L of Usher leaves water extract against the larvae of *Culex*, and those of Ali (2004), who obtained LD_{50} of 122.29 mg/L for *Culex* larvae. Singh *et al.* (2015), found that, *C. procera* leaves hexane extract LC50 and LC90 values were 78.39 and 100.60ppm, respectively when conducted against *Aedes aegypti* larvae. Further, Usher leaf water extract LC_{50} recorded 108 mg/l by Hag El Tayeb *et al*. (2009). The effects of ingestion in poisoning of larvae, is in consistent with the results of Elimam (2007) who revealed that a non-feeding pupal stage was not affected till a concentration of 10000 ppm of *C. procera* extract.

From the results of this study it can be concluded that ethanolic extracts of *Calotropis procera* possess good larvicidal activity against *Culex quinquefasciatus* larvae and considered as a promising source of larvicides, because it is available in nature, its application is simple and safe to the layman not hazardous to the environment, cheap, and biodegradable. Larval sites of

Cx. quinquefasciatus are well known to be frequently heavily polluted, and it is important to know whether a larvicide is efficient in laboratory and would be feasible in polluted water which needs further studies.

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