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Nosiba S Hamid
Center of Biosciences and Biotechnology, University of Gezira

Mutaman A Kahil
Center of Biosciences and Biotechnology, University of Gezira

Nasir A Ibrahim
Department of Biology, Faculty of Science, University of Hail – KSA

Correspondence
Nosiba S Hamid
Center of Biosciences and Biotechnology, University of Gezira

Larvicidal activity of ethanol extract of *Citrullus colocynthis* Seed and fruit pulp against *Anopheles arabiensis* and *Culex quinquefasciatus*

Nosiba S Hamid, Mutaman A Kahil and Nasir A Ibrahim

Abstract
Natural products have come from various sources like terrestrial plants, terrestrial microorganism, marine organisms, terrestrial vertebrates and invertebrates. The study was aimed to examine the effects of ethanol extracts of fruit pulp and seeds of bitter apple *Citrullus colocynthis* against the 3rd and early 4th instar of *Anopheles arabiensis* and *Culex quinquefasciatus* larvae. A set of 20 larvae of each species were added by means of dropper to a set of 250 ml test cups each containing 250 ml tap water. The ethanol extracts of *C. colocynthis* fruit pulp and seeds were tested at concentrations of 87.13, 69.74, 52.26, 34.87 and 17.39 ppm. The exposure period was 24 hour. Results revealed that, the ethanol extracts of fruit pulp and seeds illustrated considerable mortality in *A. arabiensis* and *C. quinquefasciatus*. The percentages mortality in *A. arabiensis* larvae treated with ethanol extracts of *C. colocynthis* fruit pulp ranged between 90% and 45%, and when they were treated with seed extract, mortalities ranged between 85% and 35%. Likewise, the percentages mortality in *C. quinquefasciatus* larvae after 24 hours, against ethanol extract of fruit pulp ranged between 90% and 45% and when they were treated with the ethanol extract of seed showed a ranged of 90% and 30%. The toxicity values of LC_{50} which were LC_{50} values of the ethanolic, extracts of *C. colocynthis* fruit pulp and seeds were 50.11, 30.90, 25.12, and 39.81 ppm, respectively. In conclusion, In conclusion, this study found that, the ethanol extract of *C. colocynthis* seeds showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae more than *A. arabiensis* larvae. The ethanol extract of *C. colocynthis* fruit pulp showed relatively higher larvicidal potentiality against *A. arabiensis* larvae more than *C. quinquefasciatus* larvae (not like the seed extract), also, the seed extract was more potent than the fruit pulp extract by more than two folds against both larvae. This, in turn, could provide a decision for making a recommendation to use *C. colocynthis* as a safe insecticide alternative for controlling mosquito species.

Keywords: Larvicidal activities, *C. colocynthis*, seeds, fruit pulp extracts, *Anopheles arabiensis*, *Culex quinquefasciatus*

1. Introduction
Citrullus colocynthis, also known as bitter cucumber belongs to the family of Cucurbitaceae and has a worldwide distribution, being commonly found in the sandy lands of India, Saudi Arabia, West Asia, and tropical Africa and in the Mediterranean region (Pravin *et al.,* 2013. [13] *C. colocynthis* fruits were also traditionally used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes (Jayaraman and Christina, 2013) [7]. Recently, the increased attention has been paid for using *C. colocynthis* as a natural insecticide and the biological activity of this plant has been investigated against many insect pests (Soam *et al*., 2013) [17].

Mosquitoes have an almost worldwide distribution, being found throughout the tropics and temperate regions and even well beyond the arctic circle; they are absent only from Antarctica and few islands (Service, 1993) [18]. Thirty one species of *Anopheles* were reported in Sudan but few of them are malaria vectors (Nugud *et al*., 1997) [12]. *A. arabiensis* was reported as main malaria vector in Sudan and was reported by Dukeen (1981) [4] in Khartoum State. *A. gambiae* was reported only from Southern Sudan. *A. Funestus* was reported from the South and South East of Sudan and *A. pharoensis* was reported from irrigated areas (Akood, 1980) [2]. *A. pharoensis* was reported from Khartoum State may be secondary vectors of malaria (Abd-El–Nur and Dukeen, 1992) [1]. *C. quinquefasciatus* is a dominant vector of lymphatic filariasis. This mosquito also transmits encephalitis viruses and probably West Nile Virus and other arboviruses (Russell, 1996) [15].

Plants' extract provide valuable sources of active chemicals such as alkaloids, terpenoids, cucurbitacin, glycosides, flavonoids and others that have been used as insecticide against pests including mosquito (Koul and Walia, 2009) [8].

2. Materials and Methods

2.1 Preparation of ethanol extracts

The fruit pulp and seeds of bitter apple *C. colocynthis* were collected form Wad Medani City which is located in the central part of Sudan. To prepare the extracts of *C. colocynthis*, dried fruit pulp and seeds were powdered using an electric blender, and then 6 g of the powdered sample were taken and homogenized with 100 ml of ethanol. The supernatant containing the plant extract of each part was then transferred to a measuring cylinder and then used.

2.2 Mosquito larvae

Mosquito larvae were collected from the breeding sites around Wad Medani City in plastic trays containing tap water in the Basic Sciences Laboratory, University of Gezira Sudan. Larvae were separated from their predators that accidentally collected with them, and were immediately used in the bioassay tests.

2.3 Bioassay tests

The experiments were carried out at 27 ± 2 ^{O}C and 75–85% RH under a 12L/D photo period. Larvicidal activities of the fruit pulp and seeds extracts of *C. colocynthis* were determined by following the method of WHO (1996) [20]. Twenty of the 3rd and early 4th instar larvae of *A. arabiensis* and *C. quinquefasciatus* were moved by means of dropper to test cups each containing 250 ml tap water and applied with a known concentration of each of the ethanol extract. Three replicates of each concentration for each extract of *C. colocynthis* were applied. Control batch was also designed. Larval mortality were counted after 24 hour.

2.4 Statistical analysis

The mean larval mortality after 24 hours taken as Y variable and was subjected against the corresponding concentrations (X variable) to the regression analysis by using Microsoft excel 2007. The regression lines were created to determine the lethal concentrations of 50% and 95% (LC_{50} and LC_{95}) on *A. arabiensis* and *C. quinquefasciatus* larvae.

3. Results

The larvicidal activities of *C. colocynthis* seeds and fruit pulp extracts against the 3rd and 4th instars larvae of *A. arabiensis* and *C. quinquefasciatus* after 24 hour were presented in (Table, 1 and Figure 1) and (Table, 2 and Figure, 2), respectively. The seed extract at concentrations of 87.13, 69.74, 52.26, 34.87 and 17.39 ppm showed a range of mean mortality of 35% - 85% on *A. arabiensis* and 45% - 90% on *C. quinquefasciatus* larvae. The probit analysis showed that, the LC_{50}'s were 30.90 ppm and 39.81 ppm for *C. quinquefasciatus* and *A. arabiensis* larvae, respectively, while the LC95's were 97.72 ppm and 158.48 ppm, followed the same order of larvae. According to the obtained LC's values, it was clear that, the ethanol extract of *C. colocynthis* seeds showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae more than *A. arabiensis* larvae.

The fruit pulp extract at concentrations of 87.13, 69.74, 52.26, 34.87 and 17.39 ppm (the same concentrations used for seed extract) showed a range of mean mortality of 45% - 90% on *A. arabiensis* and 30% - 90% on *C. quinquefasciatus* larvae. The probit analysis showed that, the LC_{50}'s were 25.12 ppm and 50.11 ppm for *A. arabiensis* and *C. quinquefasciatus* larvae, respectively, while the LC95's were 89.13 ppm and 83.17 ppm, followed the same order of larvae.

According to the obtained LC_{50} values, it was clear that, the ethanol extract of *C. colocynthis* fruit pulp showed relatively higher larvicidal potentiality against *A. arabiensis* larvae more than *C. quinquefasciatus* larvae (not like the seed extract), also, the seed extract was more potent than the fruit pulp extract by more than two folds against both larvae.

Table 1: The larvicidal effect of ethanol extract of *Citrullus colocynthis* seed on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

C. quinquefasciatus		*A. arabiensis*		Log- Conc	Conc. (ppm)
Probit	**Mortality %**	**Probit**	**Mortality %**		
6.28	90	6.04	85	1.94	87.13
5.84	80	5.74	75	1.84	69.74
5.30	65	5.33	60	1.71	52.26
5.25	60	4.87	45	1.54	34.87
4.87	45	4.61	35	1.24	17.39
0.96		0.93			R^2
2.43		1.89			slope
1.90		2.07			x-coefficient
30.90 ppm		39.81 ppm			LC_{50}
97.72 ppm		158.48 ppm			LC95

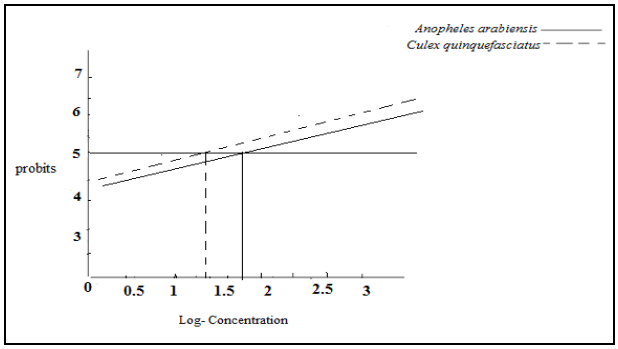

Fig 1: Log- Probity curve of seeds extract of *Citrullus colocynthis* on *Anopheles arabiensis* and *Culex quinquefasciatus* larvae after 24 hour

Table 2: The larvicidal effect of ethanol extract of *Citrullus colocynthis* fruit pulp on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

C. quinquefasciatus		*A. arabiensis*		Log- Conc	Conc. (ppm)
Probit	**Mortality %**	**Probit**	**Mortality %**		
6.28	90	6.28	90	1.94	87.13
5.52	70	5.84	80	1.84	69.74
5.13	55	5.67	75	1.71	52.26
4.75	40	5.30	65	1.54	34.87
4.48	30	4.87	45	1.24	17.39
0.68		0.86			R^2
2.23		2.44			slope
1.68		1.86			x-coefficient
50.11 ppm		25.12 ppm			LC_{50}
83.17 ppm		89.13 ppm			LC95

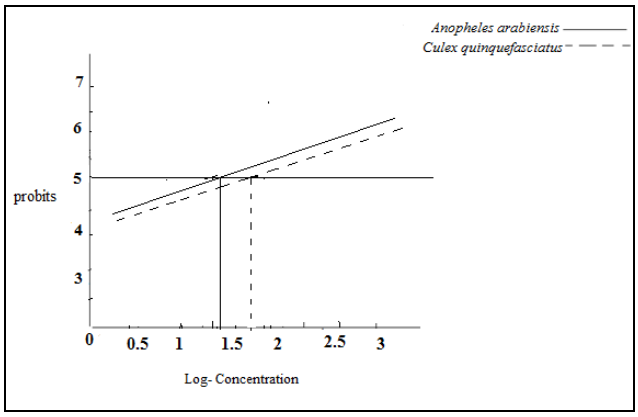

Fig 2: Log- Probity curve of fruit pulp extract of *Citrullus colocynthis* on *Anopheles arabiensis* and *Culex quinquefasciatus* larvae after 24 hour

4. Discussion

The larvicidal activities of *C. colocynthis* due to presence of various alkaloids, terpenoids, cucurbitacin, glycosides and flavonoids (Gurudeeban *et al*., 2010) [6].

This may explain the higher mortality of *A. arabiensis* and *C. quinquefasciatus* larvae in the results of the current study. In similar study, Rahuman and Venkatesan (2008) [10] found that the petroleum ether extract of *C. colocynthis* had higher effect to reduce the larvae of *A. aegypti* (LC_{50}=74.57). Mullai and Jebanesan (2007) [10]. observed 100% mortality of the larval stage of the filarial vector *C. quinquefasciatus* (Say) (Diptera: Culicidae) when this pest was treated with the leaves extract of *C. colocynthis* at 450 ppm, suggesting that the plant *C. colocynthis* exhibits larvicidal, ovicidal and repellent activities against *C. quinquefasciatus* as well and can be used as a natural insecticidal product against mosquitoes. Also, Rahuman and Venkatesan (2008) [14] found out that the leaves extract of *C. colocynthis* had the oleic and linoleic acids, which showed tremendous toxic effects against mosquito larvae.

Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of *C. colocynthis*, was tested against the early fourth instar larvae of *C. quinquefasciatus*. The larval mortality was observed after 24 hour of exposure. All extracts showed moderate larvicidal effects; however, a high larval mortality was found in petroleum ether extract of *C. colocynthis* against the larvae (LC_{50}=74.57 ppm) (Rahuman and Venkatesan, 2008) [14].

Handal is a prostrate plant, containing a very bitter glycosides colocynthin and cucurbitacin, also it contains some resins, pectins and saponins (AOAD, 1988) [3]. Whiting and Yousif (1989) [19] reviewed the characteristics and chemical composition of *C. colocynthis*. Handal was reported to possess a molluscicidal and nematocidal activity (Hmamouchi and Lahlou, 2000) [5], medicinal effect (Nmila and Gross, 2000) [11] and as potential source of edible oil (Schafferman and Beharav, 1999) [16]. The use of *C. colocynthis* in the malarial vector *A. stephensi* control was reported by Kumar and Kumar (1994) [9].

5. Conclusions

In conclusion, this study found that, the ethanol extract of *C. colocynthis* seeds showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae more than *A. arabiensis* larvae. The ethanol extract of *C. colocynthis* fruit pulp showed relatively higher larvicidal potentiality against *A. arabiensis* larvae more than *C. quinquefasciatus* larvae (not like the seed extract), also, the seed extract was more potent than the fruit pulp extract by more than two folds against both larvae. This, in turn, could provide a decision for making a recommendation to use *C. colocynthis* as a safe insecticide alternative for controlling mosquito species.

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