Larvicidal effects of aqueous extracts of *Citrullus colocynthis* (Handal) Plant as Control agents against the larvae of mosquitoes In Sudan.

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Abstract

The aim of present study is to investigate the potential larvicidal activity of aqueous extract from leaves of *Citrullus colocynthis* (Handal) selected indigenous plants, activity of the mosquitoes A. arabiensis (the main vector of malaria in Sudan) and C. quinquefasciatus (the main vector of Filariasis in Sudan). Laboratory experiments were conducted in the Department of Biology, Faculty of Education, University of Khartoum and Insectary of Health National Laboratory (ISTAC), during the period 2008-2011.

The larvicidal activity of leaves aqueous extract of *Citrullus colocynthis*(Handal) were tested against the 2nd ,3rd and 4th instars larvae of A. arabiensis and C. quinquefasciatus.

Twenty-five laboratory reared each of A.arabiensis and C.quinquefasciatus second, third and fourth instar larvae were exposed to a wide range of test concentration (2500, 5000, 10000, 15000, 20000, and 25000ppm) and a control using de-chlorinated tap water. Four replicates of each of the concentrations of plant water extract separately, were undertaken for each of the two mosquito species to find out the activity range of the extract of leaves of the tested plant. The larval mortality for each stage was determined at 48 hours and by using log

dosage–probit mortality analysis, regression line using computer software programs to determine the values of each of the lethal concentrations, 50% (LC^{50}) and 90%(LC^{90}).

The larvae in the toxic aqueous extracts exhibited behavioral responses and intoxication symptoms that vary in degree according to the type and concentration of the extract.

The current study revealed toxic and lethal effect doses of plant leaf extracts on larvae. The larvae of A.arabiensis were more susceptible than the larvae of C. quinquefasciatus to *Citrullus colocynthis*(Handal). In all cases the, 2nd instar was more susceptible to the effect of the extract than the 3rd instar and the latter was more susceptible than the 4th instar against the two species of mosquitoes.

The current study revealed that plant leaves extracts have high efficacy in the control of A. arabiensis and C. quinquefasciatus. Consequently, these water extract can be induded in programs of biological control of mosquitoes after its effect on other aquatic organisms and its safety to man are tested.

المستخلص :

هدفت هذه الدراسة لبحث فاعليه المستخلص المائي لأوراق نبات الحنظل في مكافحه الطور اليرقي كنوع من المكافحة الحيوية ضد بعوض انوفليس ارابينسيس *Anopheles arabiensis* (الناقل الرئيسي للملاريا في السودان) و الكيولكس كوينكويفاسيتس *Culex quinquefasicatus* (الناقل الرئيسي لمرض الفيلاريا في السودان).

أجريت التجارب المعملية بمعمل الحشرات في كل من كليه التربية جامعه الخرطوم ومشروع الخرطوم خالية من الملاريا و معمل أستاك القومي في الفترة ما بين (2008-2012)م.

تم اختبار تأثير المستخلص المائي لأوراق نبات الحنظل *Citrullus colocynthis* على الطور البرقي الثاني والثالث الرابع لنوعي البعوض.

عُرضت 25 من يرقات البعوض (الطور اليرفي الثاني والثالث والرابع) لفعل خمس تركيزات مختلفة (10000,15000,20000,25000 و 2500 جزء من مليون) من المستخلصين مع وجود تجربة ضابطة فيها ماء الصنبور الخالي من الكلور وتم عمل أربعة مكررات للتجربة.

حسبت نسبة الموت ل 50% و كذلك 90% بعد مرور 48 ساعة باستخدام جداول بروبت ومعادلة الخط المستقيم عن طريق حزم الحاسوب الإحصائية. أظهرت اليرقات في المستخلصات المائية استجابات سلوكية وأعراض سمية تفاوتت درجتها حسب نوع المستخلص وتركيزه.

أظهرت الدراسة الحالية الجرعات السامة والمميتة للمستخلص المائي من أوراق النباتين على اليرقات في حدود الجرعات المستخدمة, أن لأوراق النباتين المستخدمين تأثيرا مميتا لليرقات. كما وجد أن العمر اليرقى الثاني أكثر تأثراُ بالمستخلص, أن العمر اليرقى الثالث هو الأكثر تأثراُ بالمستخلص من العمر اليرقى الرابع. كذلك وجد أن بعوض الانوفلس أكثر حساسية من بعوض الكيولكس للمستخلص المائي .

كان الارتباط بين الجرعات المستخدمة وتأثيرها على الأطوار اليرقية المختلفة مثاليا إذ أنه يقترب من الواحد الصحيح.

أظهرت الدراسة الحالية أن مستخلص النبات المستخدم أظهر فعالية في مكافحة بعوض الانوفلس ارابينسيس *Anopheles arabiensis* والكيولكس كوينكويفاسيتس *Culex quinquefasicatus* بالتالي فانه بالإمكان إدراجهما ضمن برنامج المكافحة الحيوية للبعوض ولكن لا يكون ذلك إلا بعد المزيد من الدراسات علي الإحياء المائية الأخرى وسلامة الإنسان.

Key Word:

Mosquitoes, *Anopheles arabiensis* ,*Culex quinquefasciatus, Citrullus colocynthis*

INTRODUCTION:

Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, such as dengue Japanese encephalitis and yellow fever [(1).] Mosquitoes constitute a major public health menace as vectors of serious human diseases [(2).] About 380 species of *Anopheles* occur around the world. Some 60 species are sufficiently attracted to human to act as vectors of malaria [(3).] Malaria is caused by single –celled protozoan parasite of the genus *Plasmodium*. The parasites are transmitted from person to person by Anophelines mosquitoes [(3).] Mosquito –borne diseases not only cause high levels of morbidity and mortality but also have economic impacts, including loss in commercial labor outputs, and social disruption, particularly in countries with tropical and sub tropical climates. However, no part of the world is free from vector borne disease [(4).]

Greater efforts have been made to control mosquitoes than any other biting insect (1, 2003). Control of *Anopheles* larvae is one of the oldest and most historically successful interventions to prevent malaria particular in urban and pre urban areas [(5).] Biological control is the use of living organisms or their products to control vector and pest insect (1, 2003). Bacterial control of mosquito larvae with produce protein toxins has proved useful, safe and non-polluting [(6).]

Many plant extract have been studied for their efficacy in controlling larvae of different mosquito species the world over, [(7,8).] Some plant extract have been found to show considerable activity against mosquitoes and flies. Exploitation of locally

available plants with insecticide properties will avail us new modes of action and new tools for vectors and pest control in developing countries [9]. Recently in Sudan plant extracts has been tested experimentally to control mosquitoes [10].

The overall objective of the current study is to investigate the effects of a queues extract from the leaves of *Colocynthis* vulgaris on *Anopheles arabiensis* (Malaria vectors) and *Culex quinquefasciatus* (vector of filariasis) mosquitoes in Sudan.

MATERIALS AND METHODS

The study area:-

This study was carried out laboratory of Department of Biology at Khartoum University, faculty of Education at Omdurman, Sudan, during the period October 2008 to September 2011. The experiment was performed under laboratory conditions at 28-30^{o} C.

Collection and rearing of mosquitoes:-

Larvae of mosquito were collected from breeding sites, rain pools, canals, ponds and river within the study area, and reared under laboratory condition at 28-30^{0}c. The larvae were fed on powdered food fish. Pupae were collected daily, and transferred to small bowls containing clean water. The bowls were placed in a net cage 30 ×30 cm for adult emergence. From the day of emergence, Adult mosquitoes were provided with the cotton soaked with a 10% sugar solution as a carbohydrate source, and the female (*Culex quinquefasciatus, Anopheles arabiensis)* mosquitoes were fed on blood of pigeon. Petri dishes provided with moist cotton or

filter paper were fit at the bottom of cage for oviposition. The female will lay eggs within 48 hours. To rear larvae for toxicity assays single egg rafts were placed in plates (30 cm diameter) containing 1 liter of de chlorinated tap water by adding powdered food fish and the water was changed every day.

Collection of plant material and extraction:-

Leaves of *Citrullus colocynthis* (Handal) were collected from EL-Silleit Agricultural Scheme (Khartoum North) and the area of Faculty of Education, University of Khartoum. They were collected during the flowering season of the plant, dried under shade and finely ground to powder. Five grams from the powder leaves was soaked in separate plastic bottle containing 200 ml of distilled water. The solution was allowed to stand for 24 hours with stirring several times a day. The solution was filtered through filter paper (Whatman No.1). The final volume was adjusted to 200ml (25000ppm) by adding distilled water. The stock solution is then serially diluted as follows: To prepare a concentration of 20000 ppm 80 ml from aqueous extract were added to 20 ml of de chlorinated tap water, then 60, 40, 20 ml and 10 ml of each aqueous extracted were separately completed to 100 ml (in 200 ml plastic cup) by adding de chlorinated tap water, the dose of test concentration obtained were 15000, 10000, 5000 ppm and 2500ppm respectively.

Larvicidal effect:-

Larvicidal effects of water extract of plant mentioned above were determined by following the WHO standard procedure [(11).] The mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the water extract of the plant under test. After determining the mortality in 24 and 48

hours is used, to determine the lethal concentration of 50% (LC_{50}) and the lethal concentration of 90% (LC_{90}) values.

Twenty-five laboratory reared second , third and fourth instar larvae of vector mosquito species were transferred by means of droppers to the small test cups (150ml) plastic cups , each containing 100 ml of de chlorinated tap water to which the required concentration were added . Four replicates were set up for each test concentration in each replicate, 25 larvae were used, with four replicate of control. The experiment was performed under laboratory conditions at 28-30^{o} C.

Mortality in larvae was recorded after 24 and 48 hours. Moribund larvae are counted and added to dead larvae for calculating percentage mortality. The mortalities of treated groups were corrected according to Abbott's formula (1925) as follows:

$$\text{Mortality (\%)} = \frac{(X-Y)}{Y} \times 100$$

Where:

X: represents survivals in the untreated control.

Y: represents survivals in the treated control.

Statistical Analysis:-

Data were subjected to probit analysis. The regression equation (Y= a + bx), the lethal concentration that kills 50% (LC_{50}) and the lethal concentration that kills 90% (LC_{90}) of the population, (SE - X), (SE - Y), fiducially limits (F.L) with 95 %(C.

L.) And the regression coefficient (r^2) values were calculated according to Finney (1952 &1963).

RESULTS AND DISCUSSIONS:-

In this study it was observed that, the aqueous extract of the leaves of *Citrullus colocynthis* has been found to possess activities against the mosquitoes *Anopheles arabiensis* and *Culex quinquefasciatus* .

Larvicidal activity of aqueous extract from leaves of *Citrullus colocynthis* against 2^{nd}, 3^{rd} and 4^{th} instars larvae of each mosquito species was recorded after 48 hours.

The aqueous extract of *Citrullus colocynthis* showed a high level of toxicity to the larvae of *Anopheles arabiensis* (Table 1 and Fig 1). The results showed that the 50% mortality (LC_{50} values) was at 3027, 5297 and 5297 ppm for 2^{nd}, 3^{rd} and 4^{th} instar larvae, respectively of *Anopheles arabiensis*. On the other hand, the aqueous extract of *Citrullus colocynthis* showed low levels of toxicity against *Culex quinquefasciatus* larvae (Table 1 and Fig 2). The results showed that the 50% mortality (LC_{50} values) was 11000, 11995 and 15552 ppm for 2^{nd} 3^{rd} and 4^{th} instar larvae respectively, of *Culex quinquefasciatus*. There was a significant difference between LC_{90} values (90% mortality) of *Anopheles arabiensis* and *Culex quinquefasciatus* larvae due to the use of *Citrullus colocynthis* at $P<0.05$. Thus, LC_{90} values were 10593, 12912 and 23933 ppm for 2^{nd}, 3^{rd} and 4^{th} instar larvae respectively, for *Anopheles arabiensis* and 19933, 22500 and 25000ppm for *Culex quinquefasciatus* (Table 1 and Fig 3). From LC_{50} and LC_{90} values it was evident that 2^{nd} instars were more susceptible than

3rd and 4th instar in both mosquito species. This difference may be due to biological reasons such as the small size of the larvae of *A. arabiensis* as compared to that of *Culex quinquefasciatus* larvae (table .1).In addition, the size of the second instar was of course smaller than the third instar, hence it was more sensitive. This agrees with El-Imam (2007) who reported similar finding although he used different plants aqueous extracts *(Calotropis procera* (Ushar) and *Ricinus communis*(Khirwi).(17)

. A high concentration of *Citrullus colocynthis* at 25000 ppm tested on the 2nd instar larvae of both mosquito species produced 100% mortality. The 3rd instar larvae were more susceptible than 4th instar larvae of *Anopheles arabiensis* but, there was no different between 3rd and 4th instar larvae of *Culex quinquefasciatus* (fig 2and 3). All tested larval stage of *Anopheles arabiensis* was more susceptible to *Colocynthis vulgaris* than the same larval stage of *Culex quinquefasciatus*.

(El-Imam,2007) reported that, the aqueous extract of two plants *Calotropis procera* (Ushar) and *Ricinus communis*(Khirwi) showed high level of toxicity against *Anopheles arabiensis and Culex quinquefasciatus* mosquitoes larvae. (12) *Acacia nilotica* (Garad) exo|endo carp aqueous extract was also studied by (Ali, 2010) for control of 3rd instar larvae of *Culex quinquefasciatus* its toxicology. It showed LC_{50} of 0.635% and LC_{95} of 2,713% after 24 hours.(13)

In the present study, the lc_{50} of the aqueous extract of the plant was higher than that obtained by other workers on mosquito larvae using organic or aqueous extract. The ethanol crude extract of *Prosopis julifora* (Arabic and southern U.S: mesquite) and *Senna alexandarina* (Arabic:sana makka) showed larval mortality at LC_{50} of 60.395

mg|L and 249.780 mg|L respectively against the third instar larvae of *Culex quinquefasciatus* after24 hours treatment. However aceton crude extract were more effective as larvicide LC_{50} equal 244.966mg|L and 454.988mg|L respectively [13]

Crude ethanol extract of three plants, *Lawsonia inermis* L (Arabic:Henna) ,*Salvadora* Persica L (Arabic:Araak) and *Zizyph spina*-christi (Arabic:Sider) exhibited larvicidal activity against 3rd instar larvae of *Culex quinquefasciatus* mosquitoes.The LC_{50} values were 945.63, 525.76 and 893.31 mg|L respectively. While crude acetone extract gave best resultLC_{50}were 649.46, 477.00 and 594.43 mg|L respectively [14].These values were higher than those obtained in the present in visitation.

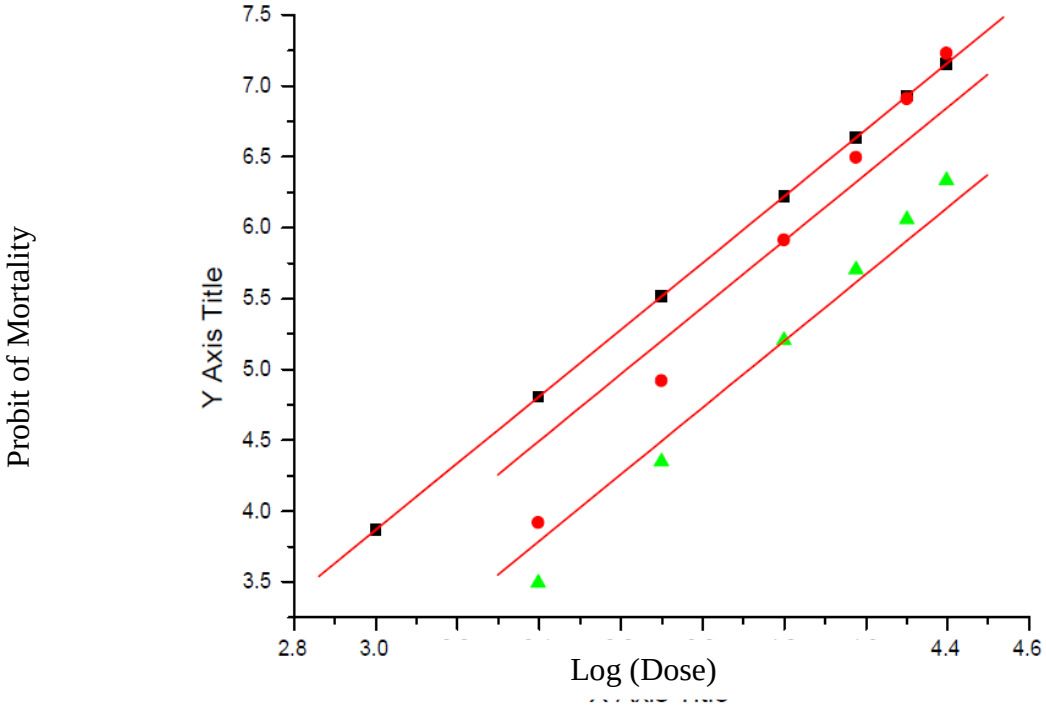
Acacia nilotica (Garad) exo|endo carp aqueous extract was also studied by (Ali, 2010) [16] for control of 3rd instar larvae of *Culex quinquefasciatus* its toxicology. It showed LC_{50} of 0.635% and LC_{95} of 2,713% after 24 hours.

These values were higher than those obtained for *Citrullus colocynthis* used in the present study at concentration of (25000ppm) (equivilant to 2.5%(25gLl). The *Calotropis procera* (Ushar) aqueous extract were tested against *Anopheles arabiensis* and *Culex quinquefascituslarvae* ,the 50% mortality (LC_{50}values) was at 273.53 ,36644 and 454.99 ppm for 2nd ,3rd and 4th instar larvae and 187,93, 218.27 and 264.85 ppm for 2nd ,3rd and 4th instar larvae of both species respectively [15].

Species of mosquito	Larva instar	LC_{50} ppm	LC_{90} ppm	Regression equation
Anopheles arabiensis	2nd	3027	10593	Y= 2.352X - 3.187
	3rd	5297	12912	Y= 3.31X - 7.328
	4th	13492	23933	Y= 2.841X - 6.161
Culex quinquefasciatus	2nd	11000	19933	Y =3.337X - 8.099
	3rd	11995	22500	Y= 4.004X - 11.33
	4th	15552	25000	Y= 3.601X - 9.882

Table 1: Larvicidal activity of leaves extract of *Citrullus colocynthis* against 2nd, 3rd and 4th instar larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* expressed as LC_{50} and LC_{90}.

Fig1. *Larvicidal activity of aqueous extract from leaves of* Citrulluscolocynthis *against 2n, 3rd and 4th instars larvae of* Anopheles arabiensis *expressed as linear regression.*



Second larval instar: Y= 2.352X- 3,187

Third larval instar: Y= 3.31 .X- 7.328.

Fourth larval instar: Y= 2.84 X- 6.161.

Fig2. *Larvicidal activity of aqueous extract from leaves of*Citrullus colocynthis*against 2nd, 3rd and 4th instars larvae of*Culex quinquefasciatus *expressed as linear regression.*

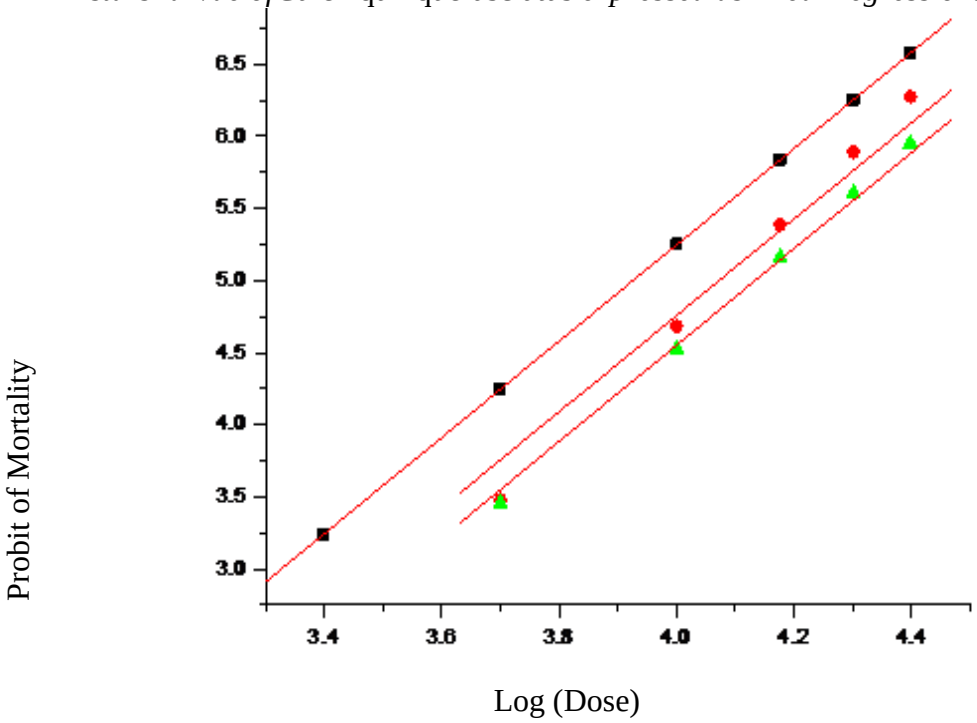
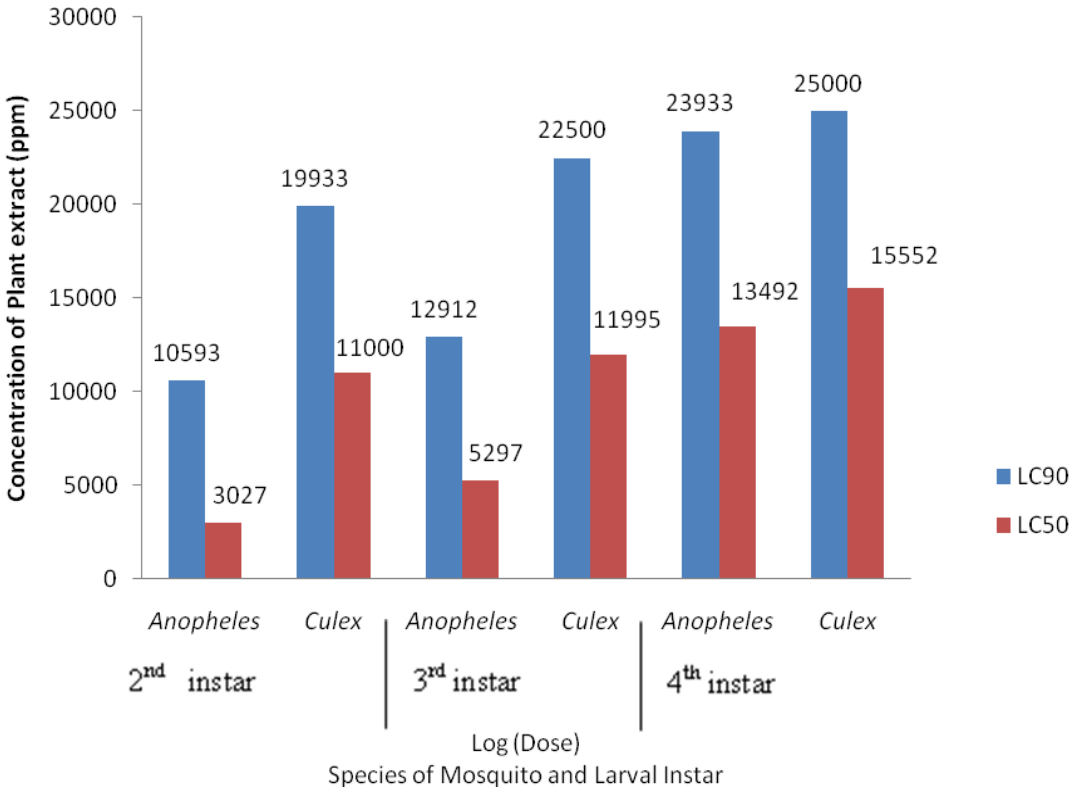


Fig 3. Larvicida lactivity of aqueous extract from leaves of *Citrullus colocynthis*against 2nd, 3rdand 4th instars larvae of *Anopheles arabiensis* and *Culex quinquefasciatus*, expressed as LC_{50} and LC_{90}.



Conclusions and Recommendations:

In Conclusions, this study indicated that :

1. Aqueous extract from leaves of C. colocynthis can be considered as potential larvicidal against A. arabiensis and .C quinquefasciatus mosquitoes.

2. 2nd, 3rdand 4th instar larvae of A. arabiensis was more effective to aqueous extract from leaves of C. colocynthis than 2nd, 3rdand 4th instar larvae of C.quinquefasciatus mosquitoes (48 hour after treatments).

3. The indigenous herbs C. colocynthis and C. co locynthis which are growing around the irrigation areas are readily available and inexpensive .

Recommendations :-

The following recommendations are suggested ;

1. Further studies on the efficacy and toxicity of the water extracts of C. colocynthis is needed to verify their toxicity against mosquitoes .

2. In addition, studies on the effect of this water extracts on non-target aquatic organisms and human to determine their safety .

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