

## Larvicidal Activity of Ethanol Extracts of Five Egyptian Plants against *Culex quinquefasciatus* Say (Diptera: Culicidae) Larvae Collected from Zaria in Nigeria

<sup>1</sup>Eman A. Alam and <sup>2</sup>Shawulu SY.

<sup>1</sup> Botany Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.

<sup>2</sup> Department of Biology, Ahmadu Bello University, Zaria, Nigeria.

\* Corresponding Author: E-Mail: aalam.eman@gmail.com

Received: 17<sup>th</sup> September 2019, Revised and Accepted: 24<sup>th</sup> September 2019

### ABSTRACT

Preliminary phytochemical screening of ethanol extracts of five Egyptian plants; *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* (*Caryophyllus aromaticus*) and *Boswellia carterii* revealed that, these plants are rich in flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances, with special reference to *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.*. Total phenolic contents of ethanol extracts of these plants under investigation revealed also that, these plants are rich in phenolics, with special reference to *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* (14.167±0.002, 12.500±0.001 and 10.416±0.001mg/ml respectively). Results indicated that, all ethanol extracts of these plants under investigation are potent larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) against the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus*. All studied concentrations of ethanol extracts of *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* caused 100%±0.000 mortality of the larvae. To conclude: *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* and *Boswellia carterii* are potent larvicidal agents (LC<sub>50</sub> and LC<sub>80</sub> of ethanol extracts of these plants under investigation against the larvae are ranged between 1.218±0.001 to 1.321±0.001 and 1.948±0.001 to 2.114±0.001 mg respectively). These plants could be alternative larvicidal agents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects.

**Keywords:** *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Caryophyllus aromaticus*, *Boswellia carterii*, Phenolics, Larvicidal activity, *Culex quinquefasciatus*, Phytochemical Screening.

### 1. INTRODUCTION

More than 17% of all infectious diseases around the world are vector-borne diseases, such as dengue fever, yellow fever, and malaria. The number of death for such diseases is more than 1 million annually e.g., malaria caused 429000 deaths just in 2015. Mosquito transmit diseases like malaria, dengue, filariasis is accounted for global mortality and morbidity with increased resistance to common insecticides. Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. Mosquitoes being vector for many tropical and subtropical diseases are the

most important single group of insect well known for their public health importance. Mosquito borne diseases are still a major problem in the world particularly in tropical and subtropical regions and WHO has declared the mosquitoes as "Public enemy number one". They are still representing the world's number one vector of human and domestic animals comprising approximately 3500 species. They are distributed globally and most female mosquitoes take blood meals from vertebrates to obtain the necessary nutrition to produce their eggs, injecting saliva

(which may contain pathogens) into host animal. Mosquitoes breed in water, occasionally depositing eggs directly on water, but generally using a variety of moist surfaces, tree holes and containers. Because of the acquired enhanced resistance of mosquito populations to conventional insecticides, the demand for the development of new products has emerged. On the other hand, the recent public perception against synthetic chemicals has shifted the research effort toward the development of environmentally sound and biodegradable agents that consumers conceive as naturals. Synthetic insecticides have created a number of ecological problems such as the development of the resistant insect strains, ecological imbalance and are harmful to mammals. Hence there is a constant need for developing biologically active plant materials such as ovicides, which are expected to reduce the hazards to human and other organisms by minimizing the residue accumulation in the environment. Natural products are generally preferred because of their less harmful nature to non-target organisms and their innate biodegradability. A survey conducted on 344 plant species, revealed that certain phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction, or act on the olfactory receptors, eliciting responses of attractancy or repellency. Botanical extracts with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal and adulticidal properties. Plants could be an alternative source for mosquitocide because they constitute a potential source of bioactive chemicals and typically are free from harmful effects (Rajan and Dhivya, 2018, Osanloo *et al.*, 2018, Deepalakshmi and Jeyabalan, 2017 and Evergetis *et al.*, 2009).

Ethanol extracts of leaves of different species of *Artemisia* showed toxic effects against *Culex quinquefasciatus* larvae with special reference to *A. molinieri* (Masotti *et al.*, 2012). *Boswellia sp.* extracts of acetone, chloroform and ethanol were tested against the eggs of *Culex pipines* at different concentrations. Results revealed that acetone extract of *B. sacra* possessed strong ovicidal activity. Phytochemical profiling of this extracts showed the presence of many secondary metabolites, which might be reason for its high efficacy (Rajan and Dhivya, 2018). Ethanol and hexane crude extracts of *Cassia fistula* reduce pupation, egg production, hatchability and increased per cent sterility in the cotton leaf worm, *Spodoptera littoralis*. The efficacy of the fruit pulp extracts of *Cassia fistula* Linn (Caesalpiniaceae: Leguminosae) extracted with

three solvents (viz. water, acetone and n-hexanes) was studied against the 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae) in the laboratory. Larval mortality was observed after 36 hours (khan *et al.*, 2017). Studies on the effect of some botanical extracts including *Cinnamomum sp.* extract revealed that, all extracts are active against the larvae of *Culex pipines*, induced some morphological abnormalities in pupae and adults. The malformed pupae were not able to develop normally and then died. Also, the present results showed that the percent and degree of malformation were concentrations dependent. In general it could be concluded that, these plant extracts including cinnamon extract act as larvicidal and possess growth and emergence inhibiting against the mosquito vector *Culex pipines* (Deepalakshmi and Jeyabalan, 2017). *Syzygium aromaticum* (Clove) essential oil has also shown larvicidal activity against field collected larva of *Aedes aegypti* with LC<sub>50</sub> of 92.56 and 62.3ppm in two different reports. Use of clove essential oil as a green larvicide against *Anopheles stephensi* preferred compared with its major constituent (Eugenol). Considering the fact that the essential oil is a lot cheaper than eugenol and is composed of several components, thus, has lesser chance of occurring resistance, the whole essential oil may be suggested as a proper larvicide (Osanloo *et al.*, 2018). Ethanol extracts of five Egyptian plants; *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* (*Caryophyllus aromaticus*) and *Boswellia carterii* will be studied in this work regarding their phytochemical constituents and larvicidal activity against the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus* in order to introduce new efficient larvicidal botanical products.

## 2. MATERIAL AND METHODS:

### 2.1. Plant materials:

Five Egyptian plants were collected from the Egyptian markets to be studied in these experiments.

**Table - 1: Names of investigated ten Egyptian plants and used parts of these plants.**

Plant Number	Used Parts
1- <i>Cassia fistula</i>	Fruits
2- <i>Artemisia monosperma</i>	Shoot systems
3- <i>Cinnamomum sp.</i>	Stem bark
4- <i>Syzygium aromaticum</i> ( <i>Caryophyllus aromaticus</i> )	Fruits
5- <i>Boswellia carterii</i>	Gum

## 2. 2. Phytochemical studies:

### 2.2.1. Extraction:

All samples are air dried and extracted by ethanol (80 %), then filtered; each 1 ml of ethanol extract of different plant materials contains 50 mg Dry Weight (Alam, A. E., 2019). These ethanol extracts of these plants were used for both phytochemical screening, estimation of total phenolics and larvicidal activity studies.

### 2.2. 2. Preliminary Phytochemical Screening:

#### 2.2.2.1. Carbohydrates and/ or Glycosides:

The ethanolic extract (5ml) was mixed with 0.5 ml of ethanolic  $\alpha$ - naphthol reagent, then 1ml of sulphuric acid was carefully poured on the walls of the test tube. A violet ring was formed at the interface indicating the presence of carbohydrates and/or glycosides (Stank *et al.*, 1963).

#### 2.2.2.2. Saponins:

Saponins were determined according to the methods adopted by Hungund and Pathak, (1971).

##### *a-Forth test:*

About 3 grams of the dried sample were extracted with boiling water then filtered. After cooling, the aliquot was shaken vigorously until froth was obtained, then allowed to stand for 15-20 minutes and classified according to their saponin contents (No froth means negative, froth less than 1cm height = weakly positive and froth 1-2 cm or higher means positive).

##### *b-Blood hemolysis test:*

About 5 grams of the dried sample were extracted with hot ethanol (95%). One ml aliquot portion was added to 10 ml of 1:4 suspensions of erythrocytes in physiological saline solution and hemolysis was observed indicating the presence of saponins.

#### 2.2.2.3. Tannins:

About 5 grams of the dried sample were extracted with ethanol (50%) and filtered. The addition of ferric chloride reagent to the filtrate gave a green color, then changed to a bluish black color or precipitate indicates the presence of tannins (Trease and Evans, 1978).

#### 2.2.2.4. Unsaturated sterols and/or Triterpenes:

The alcoholic extract (corresponding to 2 grams of the dried sample) was evaporated. The residue was treated with anhydrous chloroform (10 ml) and filtered; the filtrate was divided into two portions and subjected to the following reactions:

##### *a-Liebermann- Burchardt's test:*

To the first portion, 1 ml of acetic anhydride was added, followed by 2 ml of  $H_2SO_4$  down on the wall of the test tube. If a reddish - violet ring was produced at the junction of two layers, then the solution become bluish- green in color in the acetic acid layer it indicates the presence of unsaturated sterols and / or triterpenes (Claus, 1967).

##### *b-Salkowiskit's test:*

To the second portion, an equal volume of sulphuric acid was added, if a red color was produced it indicates the presence of unsaturated sterols and/or triterpenes (Schmidt, 1964).

#### 2.2.2.5. Alkaloids and/or Nitrogenous bases:

About 10 grams of the dried sample were extracted with 100 ml of dilute hydrochloric acid. The acidic extract was filtered, adjusted to be alkaline with ammonium hydroxide solution and extracted with chloroform. The chloroformic extract was evaporated to dryness and the residue was dissolved in about 2 ml of hydrochloric acid. The acidic solution gave faint brown precipitate with Wagner's reagent {1.3 grams of Iodine, 2 grams of Potassium iodide, dissolved in 100 ml dist. water} and very slight yellow precipitate with Mayer's reagent {1.36 grams of Mercuric chloride, 5 grams of Potassium iodide, dissolved in 100 ml dist. water } (Shellard, 1957).

#### 2.2.2.6. Cardiac glycosides:

About 2 grams of the dried sample were boiled with 15 ml of 70 % methyl alcohol for five minutes and filtered. The filtrate was diluted with distilled water and 0.5 ml of concentrated solution of lead acetate was added (to remove chlorophyll and other pigments) and filtered, to remove the excess of lead acetate,  $H_2SO_4$  (10%) was added drop wise until no further precipitate was formed, then filtered. The filtrate was extracted with 10 ml chloroform. The chloroform extract was evaporated to dryness and the following tests were carried out according to Balbaa *et al.*, (1981).

##### *a-Killer -Kiliani test :*

About 1ml of ferric chloride solution (3.5 %) in glacial acetic acid was added to one portion of chloroform residue and left, concentrated sulfuric acid was added carefully down the wall of the test tube. On standing, a brown or red layer appeared at the interface (due to the aglycone) and the upper acetic acid layer becomes blue to green (due to desoxy sugar).

##### *b-Kedde's reaction:*

To another portion of the chloroform residue, 3,5-dinitrobenzoic acid (2%) in 90%

methanol and one drop of NaOH (2%) were added. The solution acquired a violet color on standing.

*c- Libermann's reaction:*

The third portion of the chloroform residue was dissolved in glacial acetic acid, then acetic anhydride (2 ml) was added. Concentrated H<sub>2</sub>SO<sub>4</sub> was added carefully down the wall of the test tube. On standing, two layers were afforded, pink color (upper layer) and green color (lower layer).

#### 2.2.2.7. Flavonoids:

About 5 grams of the dried sample were soaked for one day with 150 ml of 1% HCl and filtered. The filtrate was tested for flavonoid compounds as follows:

About 10 ml of the filtrate were adjusted to be alkaline with sodium hydroxide. The formation of a yellow color indicates the presence of flavonoids. About 5 ml of the filtrate were mixed with 5ml HCl and small pieces of magnesium metal (0.5 g). The formation of red color after 3 minutes, indicates the presence of flavonoids (Mabry *et al.*, 1970).

#### 2.2.2.8. Anthraquinones:

About 2 grams of the dried sample were boiled for few minutes with 0.5 N KOH (10 ml) to which 1ml of diluted H<sub>2</sub>O<sub>2</sub> was added. After cooling, the mixture was filtered and acidified, then extracted with benzene (10 ml). The benzene extract was shaken with NH<sub>4</sub>OH (5ml). The presence of anthraquinones was indicated by the formation of red color in the alkaline layer (Farnsworth *et al.*, 1969).

#### 2.2.2.9. Coumarins:

A small amount (5 g) of the moistened dried sample was placed in a test tube that covered with a filter paper moistened with diluted NaOH (0.1 N) solution. The tube was then removed and examined under U.V. light and any fluorescence is indicated for the presence of coumarins (Feigl, 1960).

#### 2.2.2.10. Irodoids:

About 2 grams of fresh samples were cut into small pieces and placed in a test tube with 5 ml of 1% aqueous HCl. After 3-6 hours, 0.1 ml of the macerate was decanted into another tube containing 1 ml of the Trim and Hill reagent (10 ml acetic acid, 1 ml 0.2 % Cu SO<sub>4</sub> in water and 0.5 ml conc. HCl). When the tube is heated for a short time on a flame, a blue color is produced if a certain irodoid is present (Weiffering, 1966).

#### 2.2.2.11. Chlorides and Sulphates:

Chlorides and Sulphates were determined according to the methods adopted by Islam *et al.*, (1993).

*a-Chlorides:*

Silver nitrate solution gives with a solution containing chlorides a white flocculent precipitate of silver chloride which dissolves in ammonium hydroxide solution and does not dissolve in dilute nitric acid.

*Note:* The color of the precipitate changes gradually in direct sunlight to violet.

*b-Sulphates:*

Barium chloride solution gives a white precipitate of barium sulphate which does not dissolve in mineral acids.

#### 2.2.2.12. Sublimation:

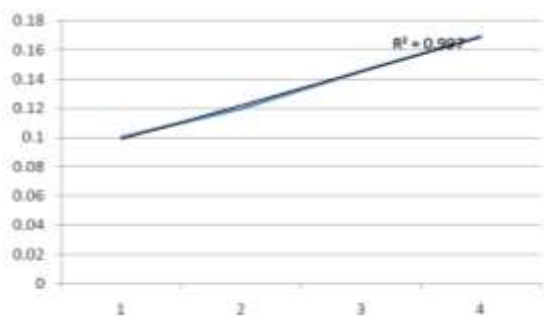
One gram of each sample was carefully subjected to microsublimation in dry crucible, covered with a clean slide. Dark yellowish-brown fumes were evolved and condensed on the lower surface of a slide as a dark brown oily condensate which dissolved in potassium hydroxide solution producing red color indicating the presence of anthraquinones ( Afifi, 1972).

#### 2.2.3. Assay for total phenolics:

Total phenolics were estimated following the method of Gursoy *et al.*, (2009) involving Folin-Ciocalteu reagent and Gallic acid as standard. 1 ml of ethanol extract of different plant materials (contains 50 mg Dry Weight) was added to 1 ml Folin-Ciocalteu reagent in a volumetric flask then 45 ml distilled water was added. The flask was shaken vigorously. After 3 minutes, 3 ml of Na<sub>2</sub> CO<sub>3</sub> (2%) solution were added and the mixture was allowed to stand for 2 hours by intermittent shaking. Each sample was done in triplicate. Absorbance was measured at 760 nm (by using UV 2401 Pc, UV-VIS recording spectrophotometer, Shimazu, Germany). Concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard Gallic acid graph.

The calibration curve of reference standard (Gallic acid) was made using four different concentrations.

Absorbance = 0.0167 Gallic acid (µg) + 0.017 (R<sup>2</sup> = 0.997).



1= GA (25 ppm), 2= GA (50 ppm), 3= GA (100 ppm), 4= GA (200 ppm).

**Figure - 1: Standard curve of Gallic acid (Total phenolic contents).**

### 2.3. Larvicidal Activity Studies:

#### 2.3.1. Study Location:

This study was carried out at Zaria, Nigeria, located at Latitude 11.085541 and Longitude 7.719945. Zaria is an old large city formerly called Zazzau, situated in the central part of Nigeria, in the state of Kaduna. The area is known for its hot climate; however the city is a center of agriculture and cultivating a few local crops important for national economy. The population of Zaria is about 700,000 people, and it is one of the most crowded cities in the country. There is a large university, Ahmadu Bello University, in the city, which is considered to be one of the best higher educational establishments in Nigeria.

#### 2.3.2. Tested mosquito:

Adult *Culex quinquefasciatus* Say (Diptera: Culicidae) mosquitoes were trapped using test tube from class rooms in the main campus of Ahmadu Bello University, Zaria, Nigeria. Collected samples of mosquito species were transported to the Entomology and Parasitology Laboratory of Zoology at Ahmadu Bello University in plastic containers. The adult *Culex quinquefasciatus* Say (Diptera: Culicidae) mosquitoes were released into the Entomological Cages directly containing 200 ml of tap water in a 700 ml of bowl plastic containers for oviposition. Blood fed female mosquitoes laid eggs on water which hatched into larvae and were identified up to species level using keys developed by Hopkins (1952). Cyclic generations of the mosquito species were sustained as described by Raveen *et al.* (2014) using restrained quail birds in the cages.

#### 2.3.3. Larvical Bioassays:

The guidelines for laboratory and field testing of mosquito larvicides recommended by WHO (2005) with little modifications were followed in this study. The larval tests were conducted in plastic bowl (700 ml). A series of

concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) from the stock solutions of ethanol extracts of five Egyptian plants were prepared. Fifteen larvae in triplicates (the 3<sup>rd</sup> instar larvae) were introduced into each plastic bowl containing 100 ml (10 ml of each tested extract were added to 90 ml of tap water). Mortality was observed for 24 hours after treatment. The larvae were considered dead when they showed no sign of movement when probed using a needle (Raveen *et al.*, 2014). Tap water was used as untreated control (C). The percentage of mortality of larvae was calculated using the following equation:

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae in a treatment} - \text{Number of dead larvae in a control}}{\text{Total number of larvae in a treatment}} \times 100$$

Additionally LC<sub>50</sub> and LC<sub>80</sub> were calculated also for each treatment.

### 2.4. Statistical analysis:

Statistical analysis was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5 and 1% probability level to determine the differences among treatment means (Steel and Torrie, 1984). The CO-STAT computerized package program was subjected to the regular statistical analysis of variance (Nissen *et al.*, 1985), using two designs - 1- Anova-1 completely randomized design (CRD) - 2- Factorial implemented in completely randomized design. Each reading = mean of three replicates + SE for all experiments.

## 3. RESULTS AND DISCUSSION

### 3.1. Phytochemical studies:

#### 3.1.1. Preliminary Phytochemical Screening:

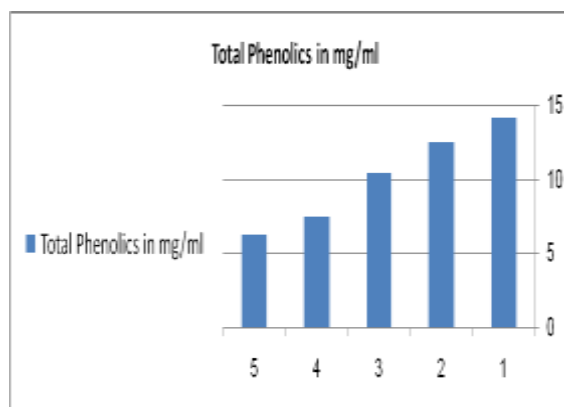
Data in table 2 represented preliminary phytochemical screening of ethanol extracts of five Egyptian plants: *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* (*Caryophyllus aromaticus*) and *Boswellia carterii*. Results revealed that, these plants are rich in flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances, with special reference to *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.*

**Table - 2: Preliminary phytochemical screening of ethanol extracts of five Egyptian plants: (1- *Cassia fistula*, 2-*Artemisia monosperma*, 3- *Cinnamomum sp.*, 4-*Syzygium aromaticum* (*Caryphyllus aromaticus*) and 5-*Boswellia carterii*).**

Experiment	1	2	3	4	5
1-Carbohydrates and/or Glycosides	++	++	++	++	++
2-Tannins	++	++	++	++	+
3-Anthraquinones	++	++	++	++	+
4-Sublimable Substances	++	++	++	++	+
5-Flavonoids	++	++	++	++	+
6-Unsaturated sterols and/or Triterpenoids	++	++	++	++	++
7-Alkaloids	++	++	++	++	++
8- Saponins	++	++	++	+	+
9-Cardiac Glycosides	++	++	++	++	++
10-Iridoids	++	++	++	++	+
11-Chlorides	++	++	++	++	++
12-Sulphates	++	++	++	++	++
13- Coumarins	++	++	++	+	+

**3.1.2. Assay for total phenolics:**

Data in figure 2 represented total phenolic contents of ethanol extracts of plants under investigation. Results revealed that, these plants: *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* (*Caryphyllus aromaticus*) and *Boswellia carterii* are rich in phenolics (14.167±0.002, 12.500±0.001, 10.416±0.001, 7.500±0.001 and 6.250±0.000 mg/ml respectively), with special reference to *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* (14.167±0.002, 12.500±0.001 and 10.416±0.001 mg/ml respectively).



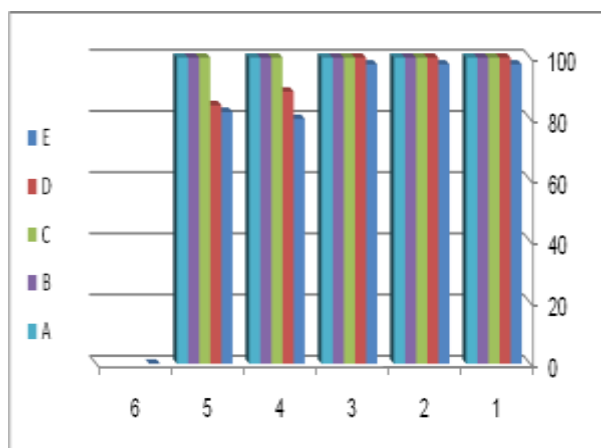
**Figure - 2: Total Phenolics of ethanol extracts of five Egyptian plants;**

**1- *Cassia fistula*, 2-*Artemisia monosperma*, 3-*Cinnamomum sp.*, 4-*Syzygium aromaticum* (*Caryphyllus aromaticus*) and 5-*Boswellia carterii*.**

**3.2. Larvicidal Activity Studies:**

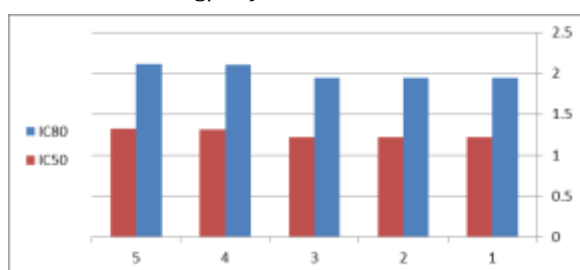
Data in Figure. 3 (a, b) represented larvicidal activity studies of different concentrations of ethanol extracts of plants under investigation (*Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* and *Boswellia carterii*). Results indicated that, all ethanol extracts of these plants under investigation are potent larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) against the 3<sup>rd</sup> instar larvae, with special reference to all examined concentrations of ethanol extracts of *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* (% of mortality = 100±0.000, LC<sub>50</sub>=1.218 ±0.001 and LC<sub>80</sub>=1.948 ±0.001 mg), followed by *Syzygium aromaticum* (*Caryphyllus aromaticus*) (% of mortality = 100±0.000, 100±0.000, 100±0.000, 88.889±0.001 and 84.444±0.001 respectively, LC<sub>50</sub>=1.313±0.001 and LC<sub>80</sub>=2.101 ±0.001 mg). The least effect was obtained by *Boswellia carterii* (% of mortality = 97.778±0.001, 97.778±0.001, 97.778±0.001, 82.222±0.001 and 80.000±0.001 respectively, LC<sub>50</sub>=1.321±0.001 and LC<sub>80</sub>=2.114 ±0.001 mg). This effect is dose dependent in case of ethanol extracts of both *Syzygium aromaticum* and *Boswellia carterii*. Meanwhile, all studied concentrations of ethanol extracts of *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* caused 100%±0.000 mortality of the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus*.





**Figure - 3.a: Larvicidal activity of ethanol extracts of five Egyptian plants; % of died larvae.**

(1- *Cassia fistula*, 2-*Artemisia monosperma*, 3-*Cinnamomum sp.*, 4-*Syzygium aromaticum (Caryophyllus aromaticus)*, 5- *Boswellia carterii* and 6-Control). (A= 5.000, B= 2.500, C=1.250, D= 0.625 and E=0.3125 mg/ml).



**Figure - 3.b: Larvicidal activity of ethanol extracts of five Egyptian plants; LC<sub>50</sub> and LC<sub>80</sub> in mg/ml.**

(1- *Cassia fistula*, 2-*Artemisia monosperma*, 3-*Cinnamomum sp.*, 4-*Syzygium aromaticum (Caryophyllus aromaticus)* and 5- *Boswellia carterii* (A= 5.000, B= 2.500, C=1.250, D= 0.625 and E=0.3125 mg/ml).

Results of larvicidal activity studies of different concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) of ethanol extracts of *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum (Caryophyllus aromaticus)* and *Boswellia carterii* against the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus* agreed with results of other researchers, those found that, these plants are natural potent toxic agents against different insects at different stages of growth (Rajan and Dhivya, 2018, Osanloo *et al.*, 2018, Khan *et al.*, 2017, Deepalakshmi and Jeyabalan, 2017 and Masotti *et al.*, 2012). However, there is a shortage in matching the phytochemical composition of extracts of these plants with the potent larvicidal activity of these extracts. In this regard and in the light of the obtained results, *Cassia fistula*,

*Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum (Caryophyllus aromaticus)* and *Boswellia carterii* are potent larvicidal agents (LC<sub>50</sub> and LC<sub>80</sub> of ethanol extract of these plants under investigation against the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus* are ranged between 1.218±0.001 to 1.321±0.001 and 1.948±0.001 to 2.114±0.001 mg respectively). All studied concentrations of ethanol extracts of *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* Gave 100 % ±0.000 mortality of the larvae. These results can be explained on the basis that these plants are rich in total phenolics and phytochemical screening of these extracts indicating the presence of flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in these extracts in good quantities.

#### 4. CONCLUSION

In the light of the obtained results, *Cassia fistula*, *Artemisia monosperma*, *Cinnamomum sp.*, *Syzygium aromaticum* and *Boswellia carterii* are potent larvicidal agents (LC<sub>50</sub> and LC<sub>80</sub> of ethanol extract of these plants under investigation against the 3<sup>rd</sup> instar larvae of *Culex quinquefasciatus* are ranged between 1.218±0.001 to 1.321±0.001 and 1.948±0.001 to 2.114±0.001 mg respectively). All studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) of ethanol extracts of *Cassia fistula*, *Artemisia monosperma* and *Cinnamomum sp.* gave 100 % ±0.000 mortality of the larvae. These results can be explained on the basis that these plants are rich in total phenolics and phytochemical screening of these extracts indicating the presence of flavonoids, tannins, alkaloids, anthraquinones, carbohydrates and/or glycosides, saponins, coumarins, unsaturated sterols and/or triterpenoids, cardiac glycosides, chlorides, sulphates, iridoids and sublimable substances in these extracts in good quantities. These plants could be alternative larvicidal agents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects.

#### Acknowledgement:

This work is supported by the Centre for Science & Technology of the Non-Aligned and Other Developing Countries (NAM Centre for Science and Technology), India and Ahmadu Bello University (ABU), Zaria, Nigeria and African Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB), ABU, Zaria, Nigeria. Personally great thanks to Prof. Dr. Y.K.E. Ibrahim and Prof. Dr. M. Mamman the Centre Leader and Deputy Centre

Leader of ACENTDFB, ABU, Zaria, Nigeria for their great support to the work continuation during the fellowship and all staff members of this great Centre. Also great thanks to Prof. Dr. Iliya S. Ndams Head of the Department of Zoological Sciences, ABU, Zaria, Nigeria for his great support to the work and also great thanks to all staff members of this great Department, with special reference to Mr. Kamba B.

## 5. REFERENCES

1. Afifi, M.. Pharmacological studies on some genera of Polygonaceae and Cucurbitaceae grown in Egypt. Ph.D. Thesis, Pharmacology Department, Faculty of Pharmacy, Cairo University, Egypt, 1972: 68.
2. Alam, E.A. Natural Phytochemical Products and Our Life (Herbal Medicine from the Land to the Hand), Osiris Publisher, Cairo, Egypt. 2019.
3. Balbaa, S.I.; Sayed, H.H. and Ashgan, Y.Z.: Medicinal plant constituent, General organization for university and school books, 3<sup>rd</sup> ed. 1981:190-255 .
4. Claus, E.P.: Pharmacognosy 1967, Henery Krimpton, London, 5<sup>th</sup> ed.: 168.
5. Deepalakshmi, S. and Jeyabalan, D. Studies on Mosquitocidal and biological activity of endemic plants of Nilgiris Hills against filarial vector, *Culex quinquefasciatus* (Say) (Insecta: Diptera: Culicidae). International Journal of Advanced Research in Biological Sciences, 2017; 4 (3): 137-151.
6. E. Evergetis, A. Michaelakis, E. Kioulos, G. Koliopoulos and S. A. Haroutounian. Chemical composition and larvicidal activity of essential oils from six Apiaceae family taxa against the West Nile virus vector *Culex quinquefasciatus*. Parasitology Research, 2009; 105:117-124.
7. Farnsworth ,N.R.; Fong ,H.H. ;Blomster, R.N. and Draus ,F.G.: Studies on Vinca major (Apocynaceae). Journal of Pharmaceutical Science. 1969; 51(3): 217-224.
8. Feigl, F.: Spot tests in organic analysis. Elsevier Publishing Co, New York, 6<sup>th</sup> ed., 1960:2959.
9. Gursoy, N.; Sarikurikcu, C.; Cengiz, M. and Solak, M.H.. Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. Food and Chemical Toxicology, 2009; 47: 2381- 2388.
10. Hopkins, G.H.E. Mosquitoes of the Ethiopian Region I. Larval Bionomics of Mosquitoes and taxonomy of Culicine Larvae. 2<sup>nd</sup> Edition, British Museum (Natural History), London, 1952: 1-355.
11. Hungund, B.L. and Pathak, C.H.: USDA forest 1971, Service Research Paper, NE: 201.
12. Islam, A.M.; Hassan, E.A. and Hannout, I.B.: Manual of Practical Chemistry, Dar AlMaaref, Egypt, 2<sup>nd</sup> ed. 1993: 19-39.
13. Khan, H. R., Tanjina, T., Rahman, J. and Afia, H. Efficacy of Fruit Pulp Solvent Extracts of *Cassia Fistula* Linn. against The Fourth Instar Larvae of The Mosquito *Culex quinquefasciatus* Say. Journal of Asiat. Society Bangladesh, Scientists, 2017; 43(1): 1-9.
14. Mabry, T.T.; Markhan, K.R. and Thomas, M.B.: The systemic identification of flavonoids. Springer, Verlag, New York, 1970: 46-54 .
15. Masotti, V., De Jong, L., Moreau, X., Rabier, J., Isabelle, L. and Thiery, A. Larvicidal Activity of Extracts from *Artemisia* species against *Culex pipiens* L. mosquito: Comparing Endemic Versus Ubiquist Species for Effectiveness. Comptes Rendus Biologies. 2012; 335 (2012): 19-25.
16. Nissen, O.; Eisensmith, S.P.; Freed, R.; Everson, E.H.; Smail, V.; Weber, M.; Tohme, J.; Anderson, J.; Rorick, K.; Portice, G.; Rittersdorf, D.; Wolberg, P.; Bricker, B. and Heath, T.. A microcomputer program for the design, management and analysis research experiments. Version 4, Michigan State University and Agriculture University of Norway, USA, 1985.
17. Osanloo, M., Sedaghat, M.M., Emaaeili and Amani, A. Larvicidal Activity of Essential Oil of *Syzygium aromaticum* (Clove) in Comparison with its Major Constituent, Eugenol, Against *Anopheles stephensi*. Journal of Arthropod Borne Diseases, 2018;12 (4); 361-369.
18. Rajan, H.P. and Dhivya, R. Phytochemical profiling and Ovicidal efficacy of *Boswellia sacra* Resin extracts against the filarial vector *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Mosquito Research, 2018; 5(4): 01-06.
19. Raveen R, kamakshi JT, Deepa M, Arivoli S, Tennysin S. Larvicidal activity of *Nerium oleander* L. (Apocynaceae) of flower extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). International Journal of Mosquito Research, 2014; 1(1):38-42.
20. Schmidt, J.: Textbook of Organic Chemistry 1964. Olivar and Poyed ed., London: 673.



21. Shellard, E.J.: Practical plant chemistry. Pitman 1957, Medicinal publishing Co., LTD, London: 53-54.
22. Stank, J.; Cerny, M.; Kocoursk, J. and Pacok, J.: The monosaccharides. Publishing House of the Czechoslovak, Academy of Sciences, Prague, 1963: 22-100.
23. Steel, R. G. D. and Torrie, J. H. Principles and Procedures of Statistics, 1984, MC Graw Hill Book Co. Inc, New York, USA, 2<sup>nd</sup> ed.
24. Trease, G.T. and Evans, W.C.: Text book of Pharmacognosy 1978, Bailliere Tindall and Cox, London , 11<sup>th</sup> Ed.:536.
25. Weiffering, J.H.: Aucubinartige glucoside und verwandte heterosideals systematische merkmale. Phytochemistry, 1966; 5: 1053.
26. World Health organization. Guidelines for laboratory and field testing of mosquito larvicides.2005. WHO/SDs/WHOPES/GCDPP/2005.13.