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

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ABSTRACT

Larvicidal activity studies of different concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml)of water extracts of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) were carried out against the 3rd instar larvae of *Culex quinquefasciatus*. Results indicated that, all water extracts of these plants under investigation are larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) against the larvae, with special reference to *Cuminum cyminum* (% of mortality = 84.444±0.001, 64.444±0.000, 48.889±0.001, 28.889±0.001 and 22.222±0.001 respectively, LC_{50}=1.946±0.001 and LC_{80}=3.114 ±0.001 mg), followed by *Pimpinella anisum* (% of mortality = 53.333±0.001, 40.000±0.000, 26.667±0.001, 22.222±0.001 and 17.778±0.001 respectively, LC_{50}=3.028±0.001 and LC_{80}=4.844±0.001 mg). The least effect was obtained by *Cinnamomum sp.* (% of mortality = 17.777±0.001, 13.333±0.000, 8.889±0.001, 6.667±0.001 and 4.444±0.001 respectively, LC_{50}=9.479±0.001 and LC_{80}=15.166±0.001 mg).This larvicidal effect is dose dependent in case of all studied concentrations of water extracts of investigated plants against the larvae. To conclude: All water extracts of these plants under investigation are larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) against the larvae. LC_{50} and LC_{80} of water extract of these plants against the larvae are ranged between 1.946±0.001 to 9.479±0.001 and 3.114±0.001 to 15.166 ±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: *Cuminum cyminum, Pimpinella anisum*, Egyptian Plants, Water extracts, Larvicidal activity, *Culex quinquefasciatus*.

1. INTRODUCTION

Mosquitoes are vector of many blood-borne pathogens as malaria, filariasis, Dengue fever, Yellow fever, Zika fever, West Nile fever, Rift Valley fever and others. Vector control is a very integral part of the current global strategy for the control of mosquito borne diseases. Although it was highly efficient against the target species, insecticide applications are facing numerous threats due to the development of resistance strains. Other undesirable effects include hazardous effects against non-target animals, environmental problems and human health concerns (Farag *et al*., 2018). Lymphatic filariasis stands next to malaria as the most important vector-borne disease in India. *Culex quinquefasciatus* (*Cx. quinquefasciatus*), a vector of lymphatic filariasis affects 119 million people living in 73 countries. A successive change in the

insecticides result in multiple insecticides resistant was developed for vectors. The phytochemicals derived from plant resources can act as larvicides, adulticides, repellents, and ovipositional attractants, having deterrent activities observed by different researchers and may be alternative sources of mosquito larval control agents (Kamaraj, C. and Abdul AbdulRahuman, 2010). Ethanolic extracts of leaves of different species of *Artemisia* showed toxic effects against *Culex quinquefasciatus* larvae (Masotti*et al.,* 2012). Ethanol and hexane crude extracts of *Cassia fistula* reduce pupation, egg production, hatchability and increased percentage of sterility in the cotton leaf worm, *Spodoptera littoralis*. The efficacy of the fruit pulp extracts of *Cassia fistula* Linn (Caesalpiniodae: Leguminosae) extracted with three solvents (viz. water, acetone and n- hexanes) was studied against the 4th instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae) in the laboratory. Larval mortality was observed after 36 hours (khan *et al.,* 2017). In spite of the antimosquito activities of several studied species of plants, relatively little work has been done on the larvicidal activities of essential oils extracted from spices, such as clove and cinnamon. A recent study carried out in Nigeria assessed the activity of clove essential oils against *Aedes aegypti* and *Culex quinquefasciatus* and achieved over 85% larval mortality within 24-hours post-exposure (Thomas *et al.*, 2017). *Syzygium aromaticum* (Clove) essential oil has also shown larvicidal activity against field collected larva of *Aedes aegypti* with LC_{50} of 92.56 and 62.3 ppm in two different reports (Osanloo *et al.,* 2018). Crude and chloroform: methanol (1:1 v/v) extracts of some common spices (*Cuminum cyminum* and others) can be recommended effectively in mosquito control programs. Crude plant extracts were more cost effective and may be employed in localized situation. Chloroform: methanol (1:1 v/ v) extract of these materials were very effective as mosquito larvicide at very low concentration (Singha, S. and Chandra, G., 2011). As compared with other herbal extracts, *Moringa oleifera* seed extract also act as larvicidal agent and studies have been reported on water extracted *M. oleifera* seeds (WEMOS) against *Aedes aegypti* larvae and methanol-extracted *M. oleifera* roots against *Culex quinfasciatus* and *Aedes albopictus*. The obtained larval mortality may be due to active chemical compounds present in *M. oleifera*. Highest larval mortality was observed at highest dose (100mg/l) concentration i.e. 93.33±0.58% at 48 h of exposure time in leaf extracts of *Moringa oleifera* against *Anopheles stephensi*. Larval mortality was highest in 48 h (86.67±0.50per cent) of exposure time as compared to the 24 h (83.33±0.58 per cent) in 80ml/l concentration and this trend is generally observed in all the concentrations whereas lowest was found in lowest dose (20mg/l) i.e 36.67±1.26 and 53.33±0.96 at 24 h and 48 h of time exposure respectively (Sharma *et al*., 2013). The promising essential oils, with larvicidal activity demonstrating LC_{50} ranging between 1-258.5 ppm, are derived from a large number of plants, including *Pimpinella anisum, Cuminum myrrham, Cinnamomum camphora*, *Syzigium aromaticum* and others. *Syzigium aromaticum* was found to contain 1.5 % essential oils, these oil was found to have larvicidal activity against *Aedes aegypti* mosquitoes. This larvicidal activity demonstrating LC_{50}, LC_{95} and LC_{99} with values 124.690, 179.720 and 220.600 ppm respectively (Sutthanont *et al*., 2010). Black seed oil, *Nigella sativum* induced 3, 10, 23, 33 and 50% larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively against *Culex pipiens*. After 48 h of exposure, this oil elicited 7.22, 14.34, 30.93, 45.36 and 76.92 % larval mortality 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively. While after 72 h of exposure, it induced 7.45, 22.34, 36.17, 64.89 and 100 larval mortality at 0.1, 0.3, 0.6, 0.9 and 1.2%, respectively (Abo El-Mahasen, M. M. and Mahmoud, S. H., 2016). The peel powder of *Punica granatum*, extracted with petroleum ether, was proved to have potential toxicological effects against third instar larvae of *Culex pipiens*. The median lethal dose (LC_{50}) value was found to be 95.6632 ppm. Qualitative phytochemical screening of pomegranate peel extract was assessed by standard methods. The phytochemical constituents present in petroleum ether extract of *Punica granatum* peel were phenols and Saponins. The outcome data proved that petroleum ether extract of pomegranate peels is a promising ecological friend mosquito larvicide. The histopathology showed cytotoxic effects of the extracted (Farag *et al*., 2018). Pomegranate contains high levels of phytochemicals including polyphenols, sugars, fatty acids, aromatic compounds, amino acids, tocopherols, sterols, terpenoids and alkaloids (Taher *et al.,* 2012). Phytochemical screening of the *Trigonella foenum-graceum* leaves extract shows the presence of alkaloids, flavonoids, saponins, tannin, glycosides and steroid. The results showed that *T. foenum-graceum* leaves extract has significant larvicidal activity against *Aedes aegypti* and *Anopheles stephensi*. The results of larvicidal activity of *T. foenum-graceum* leaves extract against *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* for the duration of 24 and 48 hours were showed that, the highest mortality (100%) was detected against *A. aegypti* at both 24 and 48 h. Significant activity was observed against *A. stephensi* (93-97% after 24 and 48 h exposure). *T. foenum graceum* leaves extract exhibits

considerable (59-80%) larvicidal activity against *C. quinquefasciatus* (Prabakaran, K. and Rajalakshmi, S., 2018). Larvicidal activity studies of different concentrations (5.000, 2.500, 1.250, 0.625 and0.3125 mg/ml) of water extracts of ten Egyptian plants (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) were carried out in this work against the 3rd instar larvae of *Culex quinquefasciatus* in order to introduce new botanical larvicidal products.

2. MATERIAL AND METHODS

2.1. Plant materials

Ten Egyptian plants were collected from the Egyptian markets, cleaned, air dried, extracted by hot water, then filtered, these extracts (1 ml of each extract = 50 mg Dry Weight) were studied for their larvicidal activity (Alam, A.E., 2019).

Table - 1: Names of Egyptian plants and used parts of these plants under investigation.

Sample Name	Used Parts
Artemisia monosperma	Shoot systems
Nigella sativum	Seeds
Syzygium aromaticum (Caryphyllus aromaticus)	Fruits
Cassia fistula	Fruits
Cinnamomum sp.	Stem bark
Punica granatum	Peels of Fruits
Moringaoleifera	Seeds
Trigonella foenum graecum	Seeds
Pimpinella anisum	Seeds
Cuminum cyminum	Seeds

2.2. Larvicidal Activity Studies

2.2.1. 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.

2.2.2. 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 200ml 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.

2.2.3. 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 water extracts of ten Egyptian plants were prepared. Fifteen larvae in triplicates (the 3rd 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:

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

Additionally LC_{50} and LC_{80} were calculated also for each treatment.

2.2.4. 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

Data in Figures 1 and 2 represented larvicidal activity studies of different concentrations of water extracts of plants under investigation (*Nigella sativa*, *Pimpinella anisum*, *Trigonella foenum graecum, Artemisia monosperma*, *Cuminum cyminum*, *Cinnamomum sp.*, *Moringa olifera*, *Syzygium aromaticum (Caryphyllus aromaticus)*, *Punica granatum*, *Cassia fistula*) against the 3rd instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). Results of larvicidal activity studies of different concentrations of water extracts of these plants against the larvae indicated that, all water extracts of these plants are larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and 0.3125 mg/ml) against the larvae, with special reference to *Cuminum cyminum* (% of mortality = 84.444±0.001, 64.444±0.000, 48.889±0.001, 28.889±0.001 and 22.222±0.001 respectively, LC_{50}=1.946±0.001 and LC_{80}=3.114 ±0.001 mg), followed by *Pimpinella anisum* (% of mortality = 53.333±0.001, 40.000±0.000, 26.667±0.001, 22.222±0.001 and 17.778±0.001 respectively, LC_{50}=3.028±0.001 and LC_{80}=4.844 ±0.001 mg). The least effect was obtained by *Cinnamomum sp.* (% of mortality = 17.777±0.001, 13.333±0.000, 8.889±0.001, 6.667±0.001 and 4.444±0.001 respectively, LC_{50}=9.479±0.001 and LC_{80}=15.166±0.001 mg). This larvicidal effect is dose dependent in case of all studied concentrations of water extracts of investigated plants against the larvae.

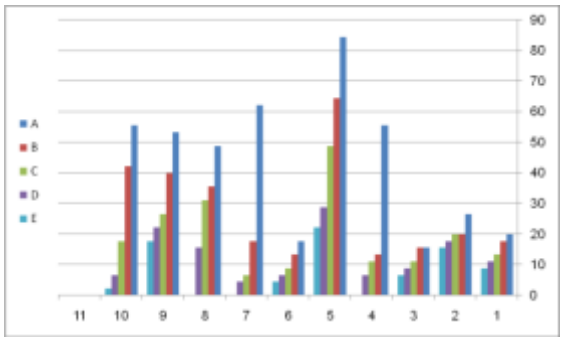

Figure - 1: Larvicidal activity of water extracts of ten Egyptian plants; % of died larvae.

(1= *Nigella sativa*, 2= *Pimpinella anisum*, 3=*Trigonella foenum graecum, 4= Artemisia monosperma*, 5=*Cuminum cyminum*, 6= *Cinnamomum sp.*, 7= *Moringa olifera*, 8=*Syzygium aromaticum (Caryphyllus aromaticus)*, 9= *Punica granatum*, 10= *Cassia fistula*, 11= Control and A= 5.000 mg/ml, B= 2.500 mg/ml, C=1.250 mg/ml, D= 0.625, E=0.3125 mg/ml).

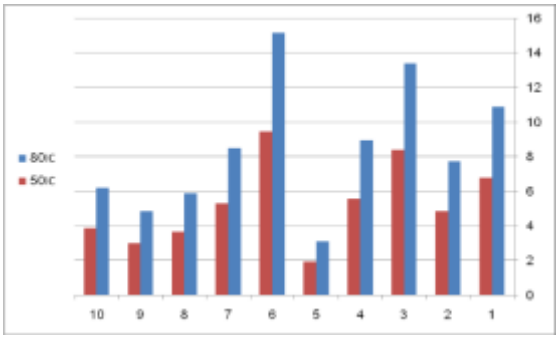

Figure - 2: Larvicidal activity of water extracts of ten Egyptian plants; LC_{50} and LC_{80} in mg/ml.

(1= *Nigella sativa*, 2= *Pimpinella anisum*, 3=*Trigonella foenum graecum, 4= Artemisia monosperma*, 5=*Cuminum cyminum*, 6= *Cinnamomum sp.*, 7= *Moringa olifera*, 8=*Syzygium aromaticum (Caryphyllus aromaticus)*, 9= *Punica granatum*, 10= *Cassia fistula*, 11= Control and A= 5.000 mg/ml, B= 2.500 mg/ml, C=1.250 mg/ml, D= 0.625, E=0.3125 mg/ml).

These results are agreed with findings of others related to the studied larvicidal and insecticidal activity of the studied plants (Prabakaran, K. and Rajalakshmi, S., 2018, Farag *et al*., 2018, Rajan and Dhivya, 2018, Osanloo *et al.,* 2018, khan *et al.,* 2017, Thomas *et al.*, 2017, Abo El-Mahasen, M. M. and Mahmoud, S. H., 2016, Sharma *et al*., 2013, Masotti *et al.,* 2012,Taher *et al.,* 2012, Singha, S. and Chandra, G., 2011 and Sutthanont *et al*., 2010).

4. CONCLUSION

All water extracts of these plants under investigation are larvicidal agents at the studied concentrations (5.000, 2.500, 1.250, 0.625 and0.3125 mg/ml) against the 3rd instar larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae), with special reference to *Cuminum cyminum* (% of mortality = 84.444±0.001, 64.444±0.000, 48.889±0.001, 28.889±0.001 and 22.222±0.001 respectively, LC_{50}=1.946±0.001 and LC_{80}=3.114 ±0.001 mg). This effect is dose dependent in case of all studied concentrations of water extracts of investigated plants against the larvae. LC_{50} and LC_{80} of water extract of these plants under investigation against the larvae is ranged between 1.946±0.001 to 9.479±0.001 and 3.114±0.001 to 15.166 ±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.

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