ISSN 1996-3343

Asian Journal of Applied Sciences

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OPEN ACCESS Asian Journal of Applied Sciences

ISSN 1996-3343
DOI: 10.3923/ajaps.2017.39.44

Research Article
Larvicidal Potency of Arta (*Calligonum comosum* L'Her.) Extract on the Biology of *Culex pipiens* Mosquito

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Abstract
Background and Objective: In recent years, application of synthetic insecticides in mosquito control program has been limited due to the concern about environmental sustainability; therefore, an urgent looking for environmental friendly insecticides against mosquito species was designed. In the current investigation, the green shoot extract of arta (*Calligonum comosum* L'Her.) has been assayed to evaluate its influence as a potential larvicidal on the second instar larvae of *Culex pipiens* mosquito. **Materials and Methods:** The plant samples were collected from the natural vegetation surrounding Riyadh, Saudi Arabia. Different concentrations of ethanolic plant extract (50, 75 and 100%) were applied against *Culex pipiens* mosquito and the effect was monitored every 3 h over a total exposure time period of 24 h. The GC-MS technique was employed to detect the chemical compounds in the ethanolic plant extract. **Results:** It has been found that the shoot extract of arta, *Calligonum comosum* was effective against the second instar larvae of *Culex pipiens* mosquito as indicated by the total mortality rate after 15, 18 and 24 h corresponding to concentrations 100, 75 and 50%, respectively. These results showed that the larvicidal activity of the *Calligonum comosum* extract was concentration-time-dependent. **Conclusion:** The present findings strongly suggest that *Calligonum comosum* extract consists of bioactive compounds which are toxic to *Culex pipiens* mosquito larvae. For gaining betting deeper insights on the mechanism underlying the mode of action behind the efficacy of arta, *Calligonum commosum* extract on controlling mosquito, further detailed studies are required.

Key words: Arta, *Calligonum commosum*, plant extract, *Culex pipiens* mosquito, larvicidal activity

Received: September 25, 2016 Accepted: October 31, 2016 Published: December 15, 2016

Citation: Lamya Ahmed Al-Keridis, 2017. Larvicidal potency of arta (*Calligonum comosum* L'Her.) extract on the biology of *Culex pipiens* mosquito. Asian J. Applied Sci., 10: 39-44.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insect-causing diseases are nowadays regarded one of the most serious environmental and health problem at the global scale. Thus, much concern has been allocated to finding the most adequate and efficient means for combating the harmful insects responsible for the outbreak of several human as well as animal diseases. These insects of which mosquitoes are no exception render general life of the biological community seriously uncomfortable and annoying. It has been well recognized that mosquito insects are the major vectors of some human diseases like malaria, dengue fever and yellow fever, chikungunya fever, filariasis, encephalitis and West Nile virus infection[1-4] resulting in great losses in the lives of human population worldwide. Mosquitoes have been stated by the World Health Organization (WHO) as 'public enemy number one[5]. *Culex pipiens* mosquito is well known as the major vector of the human filarial worm *Wuchereria bancrofti*[6]. Like any other insect, mosquito normally migrates from one region to another mainly in response to changes in climatic conditions basically under elevated levels of temperature as a consequence of the recent phenomenon of global warming. Thus, recently mosquitoes spread dramatically into temperate countries and higher altitude regions[7]. Across borders mosquito movement can be largely linked with the intensive travel and transport of goods from country to another and even inter-continentally.

Recently, the public concern with regard to the spread of diseases caused by mosquitoes has increased. Massive efforts in scientific research have been directed to monitoring the distribution of mosquito. Multi-disciplines including entomologists, virologists and tropical medicine experts have been working in good collaboration to address a set of questions directly or indirectly associated with the risk of disease transmission by mosquitoes with the overall objective of coming out with practically radical solutions to such a problem. In the course of combating mosquito different techniques were tested. The bulk of such techniques is wholly relied on chemical treatment, which though highly effective, however; some drawbacks of this practice made it not fully acceptable and hence the use of synthetic chemical insecticides has become very much reduced[8]. The application of synthetically chemical insecticides is costly, toxic to the environment and under cases of intensive use and long-term exposure to such synthetic chemical insecticides some health complications emerge resulting in critical disturbances to human health such as irritation, allergic dermatitis, nausea, vomiting, headache and central nervous system disorders[9]. Moreover, numerous reports have shown that using chemically synthetic insecticides for the control of different pest including insects has become extremely difficult due to the resistance which the insects have developed to these synthetic insecticides[10]. To address these limitations in using synthetic insecticides for the control of harmful insects, scientists up-to-date have been seeking a technique that is environmentally-friendly, efficient and cost-effective. In the recent past many studies have been performed on the extracts of different plant materials (root, stem, leaf, fruit, etc.) of some plant species to investigate its biological activity as alternatives to the synthetically chemical agents for fighting harmful insects particularly those that have been conventionally used for controlling mosquito vectors[11-15]. Many findings from the previous studies have provided sufficient evidence that such soft insecticides, i.e., insecticides from plant origin for some candidate plant species can successfully be employed as adequate alternative to the synthetic chemical insecticides[16,17]. The insecticidal capability of such plant-derived insecticides was found to be fairly effective against mosquitoes[18-24]. It has been found that the efficacy of insecticides synthesized from plant is attributed to the presence of bioactive chemicals, which inhibit growth development[25] and metamorphosis of insects[26,27] and these bioactive substances may be present singly or in combination. Generally the employment of insecticides synthesized from plant extract has been proven to be a reliably efficient approach in controlling mosquito as this technique is environmentally-safe, readily available, less expensive and the extract used is degradable and target-specific[28,29]. In a recent laboratory investigation, Alouani *et al.*[30] have tested the potency of azadirachtin (Neem extract) against the first and second instar larvae of mosquito *Culex pipiens pipiens*. The researchers have shown that azadirachtin is a promising larvicidal agent against *Culex pipiens pipiens*. Furthermore, the concentration of the extract was found to be positively correlated with mortality of the larvae. Little work has been carried out on using plant extract as a biological treatment to fight mosquito specially the *Culex* mosquitoes which are well known vectors of the pathogens that cause diseases to human. In the present investigation the major goal was to evaluate the effect of *Calligonum commosum* extract on the larvae of *Culex pipiens* mosquito. Arta (*C. comosum*) belongs to the family of the Polygonaceae. It is a plant of tropical and subtropical regions that cultivated around desert plantations to serve as wind breaks and it tolerates saline conditions (http:// www.haad.ae/HAAD/LinkClick.aspx? fileticket=Sr3tj eg5xvo=& tabid = 791). It is a plant that wide spread in Saudi Arabia. The major plant phytochemicals that serves as antioxidants with cytotoxic effect are the anthraquinones and flavonoids[31,32].

MATERIALS AND METHODS

Mosquito rearing: The second instar larvae of laboratory-reared *Culex pipiens* were used in this study. The colony was established in the insectarium of the Department of Biology, Kind Saud University, maintained at 27°C (70±10%) relative humidity, with a photoperiod of 12 h in light and 12 h in the dark.

Plant materials: Fresh leaves from *C. comosum* were dried under shade and grounded to fine powder. About 100 g of dry powder were submitted to extraction with ethanol (80%) during 24 h and this procedure was repeated thrice. The extract was then evaporated in a water path and kept for further usage.

GC-MS analysis of the plant material for active compounds: Ethanol was used to dissolve the plant extract and the mixture was analyzed using GC-MS SHIMADZU QP2010 instrument with Elute DB-5M column. Oven temperature was maintained first at 70°C for 2.0 min and then slowly increased up to 300°C. The carrier gas as well as eluent is Helium (99.99%). About 70 eV and 40000 m/z for duration of 35 min were used. Plant chemical components were identified according to their mass spectra.

Biological study: Moist filter papers were placed on four sets of three petri dishes for the treatment. Twenty mosquito larvae were transferred from the glass jar into each of the 12 petri dishes (20×12) with total number of 240 larvae. For the treatment, from the stock solution of the plant extract, different concentrations (50, 75 and 100%) were prepared and 5 mL from each concentration were added into each set of petri dishes. Distilled water was used as negative control. The number of dead larvae was counted each 3 h for 24 h monitoring the effects of the different extract concentrations. The percentage of larval mortality was corrected according to the bellow equation:

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment-Observed mortality in control}}{\text{100-Control mortality}} \text{x100}$$

$$\text{Percentage mortality} = \frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \text{x100}$$

Statistical analysis: The results in this investigation have been statistically processed and the statistical analysis was performed using JMP software package (version 18.0) with analysis of variance (One-way ANOVA). Statistical differences among the treatments were determined by Dunkan multiple range test ($p \leq 0.05$). The data was computed and expressed as Mean±Standard Deviation (SD) from five replicates per treatment. Data mentioned as means values.

RESULTS AND DISCUSSION

The present investigation was undertaken to evaluate the lethal effect of the bioassay (ethanolic extract) of *C. comosum* to the second instar larvae of *Culex pipiens* mosquito. Plant is a good source of phytochemicals that may widely be used in mosquito control programs[33]. The results obtained from the current investigation showed that application of plant extract to the mosquito larvae has a significantly lethal effect. The mortality rate of second instar larvae of *Culex pipiens* mosquito following plant extract application is presented in Table 1. The mortality rate was differing according to the different extract concentrations.

Our findings have indicated that application of 5 mL of plant extract with the concentration of 100 and 75% killed all the larvae after 15 and 18 h, respectively. Furthermore, application of 50% concentration showed slow capability to kill all larvae since 100% mortality rate was detected after 24 h. Also, it seems that the mortality rate was time-dependent since the death of the larvae was observed from the first 3 h following plant extracts application. It is quite obvious from the current investigations that the mortality rate of the second instar larvae of *Culex pipiens* was increasing as the plant extract concentration increased and similar pattern was also noted with increasing the exposure time. In agreement to our findings, similar trend of observation was also recorded by Alouani *et al.*[30]. The mortality rate of *Culex* larvae that were exposed to ethanolic plant extract was significantly higher than that of the control (Table 1) indicating the ability of *C. comosum* to

Table 1: Mortality rate of different concentrations of *C. comosum* extract on second instar larvae of *Culex pipiens* mosquito

Plant extract concentration	Time (h) 3	6	9	12	15	18	21	24	Total death (%)
50%	1	2	3	5	8	12	17	20	100
75%	1	2	7	9	15	20	20	20	100
100%	2	5	10	17	20	20	20	20	100
Control	0	0	0	0	0	0	1	2	10

Table 2: GC-MS analysis of ethanolic extract of *C. comosum*

Compound name	Retention time	Molecular formula	Molecular weight
2-propanol, 1-(1-methylethoxy)	3.74	$C_6H_{14}O_2$	118
Sec-butyl nitrite	3.74	$C_4H_9NO_2$	103
Acetic acid, diethyl	3.74	$C_6H_{12}O_2$	116
Boric acid	4.09	BH_3O_3	62
2-Butanol	4.09	$C_4H_{10}O$	74
Propylene glycol	4.09	$C_3H_8O_2$	76
Ergoline-8-carboxamide,9, 10-didehydro-6-methyl-, (8.beta)-	44.33	$C_{16}H_{17}N_3O$	267
Bentazone	47.16	$C_{10}H_{12}N_2O_3S$	240
Cyanazine	47.16	$C_9H1_3ClN_6$	240

mosquito larvae control. Different studies confirmed the efficiency of *C. comosum* against disease organisms, bacteria and fungi[34,35]. Some previous investigations tried to isolate and identify the exact components responsible for the insecticidal effect using the application of GC-mass technique. It has been found that the plant extracts possess a wide range of biologically active compounds such as alkaloids, steroids, terpenoids, essential oils and phenolics that has insecticidal ability[36]. In our current investigation and according to GC-MS analysis, the major constituents of the *C. comosum* are shown below (Table 2).

Phytochemicals are secondary metabolites and naturally occurring insecticides obtained from floral resources that help the plant withstand the continuous pressure from different environmental factors[2]. One of the compounds detected in the plant extract by GC-MS is the cyanazine, generally known as Triazine herbicides that has been extensively used in the agricultural parts in the United States[37] and is one of the most frequently detected pesticides in surface and ground waters[38,39]. Furthermore, propylene glycol is used in air sanitization and hard surface disinfection. Pest (fleas, mites, red lice and various bacteria and viruses) control for pets (cats, dogs and birds) is also a major active practice using propylene glycol[40]. Moreover, boric acid is one of the chemical compounds that have been identified by the GC-MS and showed insecticide ability when evaluated on German cockroach, *Blattella germanica* L.[41]. Generally, the plant secondary metabolites are active toxic ingredients that protect the plant against enemies. Phytochemical have been widely used against mosquito larvae instead of synthetic insecticides and the efficacy of these phytochemicals against mosquitoes was well reviewed by Kishore *et al.*[42]. Larvicidal activity of the phytochemicals of the plant *S. campanulata* against dengue vector mosquito *Aedes aegypti* was well documented[43]. The adverse effect of the plant phytochemicals on the insect might be due to its effect on the insect physiology such as the abnormalities in the nervous system besides its interference with the proper functioning of mitochondria[44].

CONCLUSION AND FUTURE RECOMMENDATION

The current investigation provided evidence on the potential of *C. comosum* shoot extract as a larvicidal agent against *Culex pipiens* mosquito. Further experimental study will assist in optimally utilizing *C. comosum* shoot extract for development of preparations that can be employed for controlling mosquito.

SIGNIFICANT STATEMENTS

This study would appropriate in a broader structure of life science knowledge because it is an invented study in Saudi Arabia. In this investigation, the larvicidal ability of arta (*Calligonum comosum* L'Her.) Extract on the Biology of *Culex pipiens* mosquito was evaluated. Furthermore, the chemical compounds that may contribute to the larvicidal ability was detected using GC-MS. Moreover, findings from this research pointed the ability of the plant extract to fight the *Culex pipiens* mosquito. Therefor, the conclusion of this study will be important for scientists and it will help to find out substitutes for the chemical synthetic compounds against mosquito.

ACKNOWLEDGMENTS

The author wish to thank all colleagues in the Department of Biology, Faculty of Science, Princess Nourah Bint Abdul-Rahman University, Riyadh, Saudi Arabia for their constructive comments and suggestion during preparation of this manuscript.

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