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Research Article
Evidence Based Efficacy of Selected Herbal Extracts Against *Culex quinquefasciatus* (Say) Larvae

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Abstract

Background and Objective: Chemical insecticides have some limitations in vectors control such as the emergence of resistance in vector mosquitoes to conventional synthetic insecticides. It aimed to evaluate the larvicidal efficacy and phytochemical potential of *Azadirachta indica*, *Cymbopogon citratus* and *Allium sativum* L. against *Culex quinquefasciatus* fourth instar larvae. **Materials and Methods:** The plants were screened and evaluated for their phytochemical composition and larvicidal effects on *C. quinquefasciatus* larvae. **Results:** The bioassay results showed that the effects were dependent on time and concentration of the extract used. Results showed the least lethal dose value for ethanolic extract of *C. citrates* (72 h exposure) and the highest value for aqueous extract of *A. indica* (24 h exposure). Chi-square values were significant at $p<0.05$. Phytochemical analysis showed phytochemicals such as alkaloids, flavonoids, cardiac glycosides and resins in all the three plants. However, tannins were absent in *A. indica* and *A. sativum,* while balsam was only present in *A. indica.* Saponins, balsam and phenols were not found in *A. sativum*. Phenols were also absent in *C. citratus.* Terpenes, steroids and resins were absent *A. indica.* **Conclusion:** Findings of this study revealed that these herbal extracts constitute an effective eco-friendly approach for the control of *C. quinquefasciatus* larvae.

Key words: Ethanolic and aqueous extracts, phytochemicals, mosquitoes, *Azadirachta indica*, *Cymbopogon citrates*, *Allium sativum* L.

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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

Culex quinquefasciatus (Say) (Diptera: Culicidae) is a mosquito species found worldwide. It is one of the most successful mosquito species because it can tolerate pollution and breed in almost all type of water habitats, such as ponds, stagnant water, roadside ditches, freshwater ponds, banks of rivers and natural streams[1].

Mosquitoes are competent vectors of pathogens cause serious diseases such as dengue fever, yellow fever and zika viruses[2].

Mosquito control programs have been facing important and timely challenges, including the recent outbreaks of novel arbovirus, development of resistance in several Culicidae species and rapid spreading of highly invasive mosquitoes worldwide[3]. For most vector-borne viral diseases such as Rift Valley Fever Virus (RVFV) there is no available vaccines except some trails still under development and evaluation[4].

The use of traditional insect repellents/killers is widespread among different cultures and communities of Africa and beyond.

Herbal extracts have been used for a long time as repellents/killers for blood-sucking insects[5]. Herbal products with proven potential as repellants can play an important role in the interruption of transmission of mosquito-borne diseases at both individual and community levels[6-9].

The aim of this study was to evaluate the larvicidal efficacy and phytochemical potential of three selected indigenous plant species, namely *Azadirachta indica*, *Cymbopogon citratus* and *Allium sativum* L. against *C. quinquefasciatus* fourth instar larvae.

This study determined the efficacy of these plant extracts to alternate the chemical insecticides in the control *C. quinquefasciatus* larvae. This study, contrary to most relevant studies, evaluated the efficacy of these herbal extracts in the field. This insured the competence of the extracts to tolerate climatic conditions and control *C. quinquefasciatus* larvae in their natural habitats.

MATERIALS AND METHODS

Study duration: The study has been conducted between May, 2017-April, 2018.

Sample collection and identification: The leaves of *A. indica*, *Cymbopogon citrates* and *Allium sativum* L. were collected from different locations in Saudi Arabia within the premises of Princess Nourah Bint Abdurrhman University. The plants were identified and confirmed at the research lab of the Department of Biology, Faculty of Science, Princess Nourah Bint Abdurrahman University.

Sample preparation and extraction: The method of Odey *et al.*[10] has been adopted for the preparation of the herbal extracts as follows: Fresh leaves of each sample were washed, air-dried at 37°C and then ground into a powder form before maceration with 95% ethanol for three cycles. Each cycle involved soaking for 3 days at 37°C. The extracts were filtered using an Agitated Nutsche Filter (Evapodry Manufacturing Company, Uttarakhand, India) and concentrated using a Wiped Film Evaporator (Evapodry Manufacturing Company, Uttarakhand, India) under reduced pressure at 40°C to yield a concentrated ethanol extract. The aqueous extract was prepared by further soaking of the residue from the previous filtration step in ultra-pure water for 24 h followed by filtration. The plant extracts were freeze-dried (Labconco, USA) and stored in dry conditions in a refrigerator at 4°C until use for further experiments.

Rearing of mosquito species: The eggs of species of *C. quinquefasiciatus* were maintained in the mosquito rearing laboratory of Faculty of Science, Princess Nourah Bint Abdurrahman University and reared in white basins containing tap water and maintained between 27 and 29°C. When the eggs hatched into the first instar larvae, they were fed yeast powder and biscuit powder in the ratio of 1:3. The larvae were reared until the fourth instar larvae emerged on the 6th day.

Phytochemical analysis/screening of plant extracts: The phytochemical screening of plant extracts obtained using organic solvents was carried out using standard qualitative procedures: Dragendorff test for alkaloids, sodium hydroxide test for flavonoids, ferric chloride test for tannins, Salkowski test for cardiac glycosides, Liebermann-Burchard test for terpenes and general test for saponins, phenols and balsam.

Cholesterol determination: Cholesterol levels were estimated colorimetrically. The absorbance of standard and samples was measured at 546 nm after prior mixing and incubation in an oven at 37°C for 5 min.

Triglyceride determination: Triglycerides were determined colorimetrically at 546 nm after prior mixing and incubation in an oven at 37°C for 5 min.

High-density lipoprotein determination: Low-density lipoproteins (LDLs) were determined colorimetrically at 510 nm after prior mixing and incubation in an oven at 37°C for 5 min. The LDL was estimated as a calculated value from the other fractions using the Friedewald equation.

Very low-density lipoprotein (VLDL) determination: The VLDL concentration was estimated as follows:

$$VLDL = 0.45 \times TG \ (mmol\ L^{-1})$$

where, TG is triglycerides.

Larvicidal bioassay: The bioassay was performed at a temperature of 27°C, relative humidity of 70-80%, photoperiod of 12:12 (light: dark) and pH 7.0 of distilled water. The test concentrations used for larvicidal bioassay were 5, 10, 20, 30 and 40 mg mL^{-1} of each extract. Each plant extract was weighed to obtain the required concentration and dissolved in 2 mL of ethanol. Distilled water was measured (95 mL) and poured into each container to be used. The extracts dissolved with ethanol were introduced into the containers (WHO standard containers) containing 95 mL of distilled water. Ten fourth instar larvae of mosquitoes were selected, counted using a micro-pipette, placed in small bottles, made up to the 3 mL mark with distilled water and then introduced into the containers.

A control was also maintained by adding 2 mL of ethanol to 95 mL of distilled water and ten fourth instar larvae. Distilled water (3 mL) was introduced later. The larvae were fed yeast powder and biscuit powder in the ratio of 1:3 daily (sprinkled on the surface of water). The larval mortality was measured and recorded in percentage at 24, 48 and 72 h intervals. Dead larvae were removed to avoid decomposition.

Field evaluation of the herbal extracts: The experiments were repeated in the field to insure the quality of the results. Same breeding sites from where mosquitos' larvae have been collected for the first time were selected. Herbal extracts were added (only doses showed significant mortality in the laboratory) to the breeding sites.

Statistical analysis: Statistical analyses were conducted to determine the significance of differences using (χ^2) test. Statistical analysis were performed using SPSS, version 21 software (IBMCorp., Armonk, NY, USA). A $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical analysis: The results obtained showed the presence of phytochemicals such as alkaloids, flavonoids, cardiac glycosides and resins in all the three plants (Table 1). However, tannins were absent in *A. indica* and *A. sativum*, while balsam was only present in *A. indica*. Saponins, balsam and phenols were not found *in A. sativum*. Phenols were absent in *C. citratus.* Terpenes, steroids and resins were also absent in *A. indica*.

Larvicidal effect of plant extracts: The toxicity of *A. indica*, *C. citratus* and *A. sativum* extracts to the fourth instar larvae of *C. quinquefasciatus* mosquito was noted. Mean mortality of larvae was 65, 90 and 95% when exposed for 24 h to *A. indica*, *C. citratus* and *A. sativum* aqueous extracts, respectively. On the other hand mean mortality of larvae was 100% when exposed to the ethanolic extracts of each one of the three plants for 24 h.

This indicated that ethanolic extracts of the three plants induced significant mortality for larvae $p<0.05$ (Table 2). Besides, results showed the least lethal dose (LD) value for ethanolic extract of *C. citrates* (72 h exposure) and the

Table 1: Phytochemical components of *Azadirachta indica*, *Cymbopogon citratus* and *Allium sativum*

Components	*Azadirachta indica*	*Cymbopogon citratus*	*Allium sativum*
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	-	+	-
Saponins	+	+	-
Balsam	+	-	-
Cardiac glycosides	+	+	+
Terpenes and Steroids	-	+	+
Resin	-	+	+
Phenols	+	+	-

+: Denotes present, -: Denotes absent

Table 2: Mean larval mortality (%) of ethanol and aqueous extracts of *Azadirachta indica, Cymbopogon citratus* and *Allium sativum* against *Culex quinquefasciatus* fourth instar larvae

	Exposure intervals (h)			
	Concentration (mg mL^{-1})	Mean larval mortality (%)		
Plant aqueous extract	0	24	48	72
Control (without extract)	0	0.00	0.00	3.33
Azadirachta indica	5	3.33	15.00	35.00
	10	20.00	45.00	60.00
	20	40.00	65.00	80.00
	30	45.00	48.00	69.00
	40	65.00	90.00	100.00
	LD 50	2.40±0.09	1.90±0.06	1.90±0.02
Cymbopogon citratus	5	3.33	15.00	35.00
	10	30.00	50.00	70.00
	20	55.00	70.00	90.00
	30	70.00	90.00	100.00
	40	90.00	100.00	100.00
	LD 50	0.20±0.071	1.20±0.05	0.90±0.03
Allium sativum	5	9.00	24.00	46.00
	10	22.00	41.00	55.00
	20	39.00	46.00	53.00
	30	57.00	66.00	75.00
	40	95.00	100.00	100.00
	LD 50	2.10±0.08	1.33±0.044	1±0.032
Control (without extract)	0	0.00	0.00	3.33
Azadirachta indica	5	13.33	25.00	40.00
	10	23.33	63.33	83.33
	20	33.33	43.33	63.33
	30	62.00	79.00	88.00
	40	100.00	100.00	100.00
	LD 50	1.80±0.3	1.6±0.09	0.80±0.05
Cymbopogon citratus	5	15.00	28.00	59.00
	10	33.33	43.33	78.00
	20	63.33	85.00	97.00
	30	92.00	99.00	100.00
	40	100.00	100.00	100.00
	LD 50	0.50±0.07	0.32±0.04	0.10±0.022
Allium sativum	5	20.67	40.00	60.00
	10	35.33	50.00	61.00
	20	50.00	63.00	69.00
	30	85.00	95.00	98.00
	40	100.00	100.00	100.00
	LD 50	0.79±0.022	0.65±0.053	0.60±0.031

highest value for *A. indica* aqueous extract (24 h exposure). Chi-square values were significant at $p<0.05$ (Table 2). The field results were typical to results achieved in the laboratory.

DISCUSSION

In this study, it aimed to evaluate the larvicidal efficacy and phytochemical potential of *A. indica, C. citratus* and *A. sativum* L. against *C. quinquefasciatus* fourth instar larvae. It found that exposure to the three plant extracts for 72 h induced 100% larval mortality.

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides[11].

Botanical insecticides provide an alternative to synthetic insecticides, because they are generally considered safe, biodegradable and can often be obtained from local sources[12].

The larvicidal activity of extracts of *A. indica*, *C. citratus* and *A. sativum* was determined against *C. quinquefasciatus* fourth instar larvae. Phytochemical analysis of the three plants was conducted to investigate the presence of important components such as essential oils and alkaloids. Alkaloids are involved in the relaxation of muscles and in relieving nasal congestion. They are also present in quinine and aspirin. Cardiac glycosides are antidotes for heart failure and irregular

heartbeats. Terpenes are psychoactive chemicals found in cannabis[13]. Flavonoids have been reported to possess both bacteriostatic and bactericidal effects on some strains of bacteria. In addition, they inhibit the activity of reverse transcriptase and proteases. As vegetables contain flavonoids, their consumption in moderation is beneficial to the body.

The results of this study showed that ethanol extracts of *C. citrates* and *A. sativum* were very effective against *C. quinquefasciatus* mosquito larvae, especially at concentrations of 30, 40 and 50 mg mL^{-1}. A previous study revealed the ovicidal activity of *Moschosmapoly stachyum* leaf extract against *C. Quinquefasciatus*, with 100% egg mortality at a concentration[14] of 100 mL L^{-1}. Another study reported the larvicidal efficacy of leaf extracts of *C. cucumispubescen* with four different solvents against late third instar larvae of *Anopheles stephensi*, *C. quinquefasciatus* and *Aedes aegypti*. Similarly, other researchers investigated the use of garlic and lemon peel extracts as larvicides of *Culex pipiens* and the interaction and persistence of the extracts via organophosphate resistance mechanisms were observed[15].

The results of this study revealed that 72 h of exposure to the three plant extracts induced 100% larval mortality (Table 2). This finding is in agreement with the result of a previous study that reported that *Cleome viscosa* (Leaf), *Clerodendrum viscosum* (Leaf), *Murraya koenigii* (fruit) and *Vitex negundo* (leaf) showed 100% mortality against *C. quinquefasciatus* larvae at a concentration of 0.5% (v/v) within 72 h of exposure. Similarly, the extracts of leaves of *A. indica*, pericarp of fruits of *Alangium salviifolium*, seeds of *Polyalthia longifolia* and the fruits of *Derris indica* and *Solanum sisymbriifolium* showed 100% mortality against the third instar larvae of *C. quinquefasciatus* at 1% concentration of crude extract, within 72 h of exposure[10]. Statistical analysis of the results revealed that the ethanolic extract of *C. citratus* was more effective as a natural pesticide, followed by *A. sativum* and both extracts caused above 50% mortality after 24 h of exposure when applied at a concentration of 20 mg mL^{-1}.

These results are consistent with the findings of another study that evaluated the repellent activity of ethyl anthranilate (EA), a non-toxic, FDA-approved, volatile food additive, against three known mosquito vectors namely, *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. The results revealed that EA exhibited significant mortality rates for the three species of mosquitoes at a concentration[16] of 10% w/v. The ethanolic extract of *A. indica* at 30 mg mL^{-1} concentration induced more than 50% mortality. This finding is in accordance with the results of many studies that reported the significant effect of *A. indica* on the mortality of various species of mosquitoes, such as *A. aegypti* and *C. pipiens*[17-19].

A previous study reported that the chemical composition and broad spectrum of biological activity of the plant extract can vary with plant age, plant tissues, geographical origin of plant and the species and age of the targeted pest organism[20].

A previous study reported that crude extracts or isolated bioactive phytochemicals from *A. sativum* could be used in stagnant water bodies, which are known to be the breeding grounds for mosquitoes[21,22].

Finally, it can be concluded that this study discovered the significant effect of herbal extracts of *A. indica*, *C. citratus* and *A. sativum* L. on mortality of *C. quinquefasciatus* larvae particularly the ethanolic extracts. This can be beneficial for the control of mosquitoes in their natural breeding habitats depending on the findings of this research which approved that the three herbal extracts were sustainable and tolerant to climatic conditions when tested outdoors in the natural breeding sites of mosquitoes.

Despite further studies are needed to investigate the mode of action of these herbal extracts, their efficacy in inducing mortality to other species of mosquitoes and their efficacy as mosquito-repellant products, this study will help the researchers to uncover the critical areas of finding solutions to the environment pollution caused by chemical insecticides that many researchers were not able to explore. Thus a new theory on total dependence of herbal environment friendly insecticides may be arrived at the near future.

CONCLUSION

The ethanol extracts of *C. citratus*, *A. sativum* and *A. indica* demonstrated effective larvicidal properties against fourth instar larvae of *C. quinquefasciatus*. It has been found that exposure to the three plant extracts for 72 h induced 100% larval mortality. These findings open a window for investigating more herbal extracts to be used as natural insecticides. This will reduce the environment pollution and toxicity to humans and animal vertebrates.

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