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Research Article
Evidence Based Efficacy of Three Medicinal Plant Extracts Against *Culex quinquefasciatus* (Say) Larvae

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Abstract

Background and Objective: Mosquitoes transmit serious human diseases, which cause millions of deaths every year. Their increasing resistance to chemical insecticides has reinforced their roles as vectors. Plants may be alternative sources of mosquito control agents. It aimed to examine the larvicidal effects of *Tamarix aphylla* (Caryophyllales: Tamaricaceae), *Jacaranda mimosifolia* (Lamiales: Bignoniaceae) and *Lavandula angustifolia* (Lamiales: Lamiaceae) extracts on *Culex quinquefasciatus*. **Materials and Methods:** The plants were screened and evaluated for their phytochemical composition and identified larvicidal effects on *C. quinquefasciatus* larvae. **Results:** Ethanol leaf extracts of *T. aphylla* caused the highest mortality of *C. quinquefasciatus* larvae. It found that exposure to the three plant extracts for 72 h induced 100% larval mortality. Phytochemical analysis showed the presence of flavonoids, alkaloids, tannins and polyphenols in all plant extracts, all of which were demonstrated as potential larvicides. **Conclusion:** These results revealed that the ethanolic leaf extracts of *T. aphylla*, *J. mimosifolia* and *L. angustifolia* can be used effectively as an ecofriendly, biodegradable and economical larvicide in integrated mosquito control programs.

Key words: Mosquito control, phytochemicals, *Tamarix aphylla*, *Jacaranda mimosifolia*, *Lavandula angustifolia*, *Culex quinquefasciatus*

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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

Mosquitoes of the genus *Culex* (Diptera: Culicidae) are known to either develop or find shelter in storm drain systems[1]. These species are notorious biting nuisances and important vectors of diseases, including lymphatic filariasis, malaria, Japanese encephalitis, dengue hemorrhagic fever and yellow fever, which cause millions of deaths every year[2]. *Culex quinquefasciatus* (Diptera: Culicidae) is the principal vector in the outbreak of West Nile Fever that occurred in North America[1].

Globally, farmers largely rely on chemical substances to protect their agricultural products against pests, insects and diseases[3]. Although pesticides are currently the most effective pest management tools, they have been criticized for affecting non-target organisms, leading to resistance development in pests[4]. Repeated use of synthetic insecticides for mosquito control has also disrupted natural biological control systems and led to resurgence in insect populations, therefore, phytochemicals or botanical insecticides have attracted attention as an alternative approach to insect control[3].

Phytochemicals are naturally occurring insecticides obtained from plants. They are biodegradable, have no ill-effects on non-target organisms and do not have the disadvantages associated with injudicious application of synthetic insecticides. Research on the insecticidal effects of phytochemicals has yielded positive results[5,6].

Several groups of phytochemicals, such as alkaloids, flavonoids, tannins, essential oils and phenols, have been reported to show insecticidal effects[7]. Insecticidal effects of plant extracts vary according to the plant species, mosquito species, geographical varieties and parts used and also the extraction methodology used and the polarity of the solvents used during extraction[8].

Jacaranda mimosifolia D. Don (blue trumpet tree, Bignoniaceae) is a common sub-tropical ornamental tree found worldwide. It is native to South America. It is also investigated by Sidjui *et al.*[9] that *J. mimosifolia* tree bark as a bio-monitor of atmospheric trace metals. The leaves. This also exhibits cytotoxic, antimicrobial and larvicidal[10] properties.

Tamarisk aphylla (Caryophyllales: Tamaricaceae) is a Saharan shrub that grows in saline soils. *Tamarisk aphylla* has traditionally been used in folk medicine to cure various ailments, including hepatitis, eczema and skin diseases, such a *Tinea capitis* and *syphilis*[11]. Chemical valorization of *T. aphylla* has shown the presence of strong polyphenols, especially flavonoids and tannins[12] and leaves contain more polyphenols than stems[13]. Tamarisk is also known by its insecticidal effect[14].

Lavandula angustifolia Miller (Lamiaceae), or true lavender, is an evergreen perennial shrub. The essential oil of Lavandula angustifolia was investigated for its biocide activity against *Acyrthosiphon pisum* by fumigation[13].

There is a lack of information regarding the application of plant extract to the control of mosquitoes in the Kingdom of Saudi Arabia (KSA). Therefore, the objective of this study was to investigate the phytochemical properties and larvicidal effects of *J. mimosifolia*, *L. angustifolia* and *T. aphylla.*

MATERIALS AND METHODS

Study area: The study was carried out at Biology Department, Research Lab of King Faisal University between September, 2017-December, 2018

Plant material: Healthy leaves of *T. aphylla*, *J. mimosifolia* and *L. angustifolia* were collected from botanical gardens in Al-Asha, Makkah and Al Medinah in the KSA. For each species, 50 leaves were randomly taken from 20 selected plants. Fresh leaves were washed, air-dried under room temperature (25-27°C) for about 15 days and ground into powder before three cycles of maceration in 95% ethanol, each cycle involved soaking for 3 days at room temperature (4°C). The extracts were filtered and concentrated using a rotary evaporator (R-100, Buchi, Switzerland) under reduced pressure at 40°C to yield a concentrated ethanol extract. The aqueous extract was prepared by further soaking the concentrated ethanol extract in ultra-pure water for 24 h and refiltered. The plant extracts were freeze-dried (Model; Labconco, City, State, USA) and stored dry in a refrigerator at 4°C until used for experiments.

Phytochemical screening: The total phenolic content (TPC) was measured in six samples using the method of Folin and Ciocalteu[15]. TPC was expressed as gallic acid equivalent (GAE, mg g^{-1}) of extract[16].

The total flavonoid content was determined using the method of Chang *et al.*[17]. The total flavonoid content of the extracts was calculated using the standard graph of quercetin and the results were expressed as quercetin equivalent (QE mg g^{-1}) of extract.

The total tannin and alkaloids contents were determined using the method of Schanderl[18]. The total tannin content was calculated using the standard graph for gallic acid (0-100 μg) and the results were expressed as gallic acid equivalent (GAE mg g^{-1}) of extract.

The total alkaloid content was expressed as atropine equivalent (AE mg g^{-1}) of extract[19,20].

Insect rearing: *Culex quinquefasciatus* were collected from Wadi Hanifah, Riyadh, KSA. The insects were reared in the laboratory at King Faisal University, Al-Asha, KSA, at 30±2°C and 70±5% RH. The adult insects were kept in conical flasks containing untreated beans and kept in the dark. The mouth of the flask was covered with a muslin cloth to allow air circulation and prevent the insects from escaping. Larvicidal bioassays of the extracts were performed on late second, third and early fourth instars.

Treatments and data collection: The method of Dua *et al.*[21] was used for the larvicidal bioassays. The larvicidal bioassays were performed at 27°C, 70-80% RH, photoperiod of 12:12 h (L:D) and distilled water at pH 7.0. Second, third and fourth instar larvae of *C. quinquefasciatus* were exposed to different concentrations of *J. mimosifolia*, *L. angustifolia* and *T. aphylla extracts*.

Five concentrations (1, 5, 10, 15 and 20 mg mL^{-1}) of each extract were prepared using dechlorinated water. Solution of each concentration (100 mL) was placed in glass beakers. Ten healthy larvae of each instar of *C. quinquefasciatus* were then introduced into each beaker. Five replicates of each concentration were tested, along with a control (0 mg mL^{-1}) at room temperature. The larvae were fed dried yeast powder on the water surface. The number of dead larvae in each beaker was recorded at 24, 48 and 72 h after introduction. Dead larvae were removed as soon as possible to prevent decomposition, which may affect the survival of the remaining larvae. Three repetitions were conducted with five replicates in each one.

Statistical analysis: The Log-probit analysis was used to determine the lethal concentrations (LC_{50} and LC_{90}) of each extract. Statistical analysis 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: Extracts of *T. aphylla*, *J. mimosifolia* and *L. angustifolia* contained alkaloids, flavonoids, tannins and phenols (Table 1). The quantity of the phytochemicals varied within each species. TPC was 3.75, 16.17 and 23.09 mg GAE g^{-1} for *J. mimosifolia*, *L. angustifolia* and *T. aphylla*, respectively. The highest concentration of tannins was 55.04 mg GA g^{-1} in *T. aphylla* extracts, followed by *L. angustifolia* at 48.05 mg of GA g^{-1} of extract and *J. mimosifolia* at 1.61 mg of GA g^{-1}. The highest concentration of alkaloid was 41.00 in *T. aphylla* extract, followed by *L. angustifolia* with 32 mg of AE g^{-1} of extract and *J. mimosifolia* with 16.25 mg of AE g^{-1} of extract. The concentration of flavonoids was 66.06, 10.74 and 8.84 mg of QE g^{-1} in *T. aphylla*, *L. angustifolia* and *J. mimosifolia* extracts, respectively.

Larvicidal effect of plant extracts: All the plant extracts screened display some effects on the mosquito larvae tested, with varying degrees of toxicity. No mosquito larvae died in beakers containing only water (Table 2). All second instars

Table 1: Concentrations (Mean±SEM) of phytochemicals in leaf extracts of 3 plant species studied

Phytochemicals	*Tamarix aphylla*	*Jacaranda mimosifolia*	*Lavandula angustifolia*
Alkaloids (AE, mg g^{-1} of extract)	41.00±0.031	16.25±0.02	32.00±0.05
Flavonoids (QE, mg g^{-1} of extract)	66.06±0.03	8.84±0.08	10.74±0.04
Tannins (GAE, mg g^{-1} of extract)	55.04±0.02	1.61±0.09	48.05±1.83
Phenols (GAE, mg g^{-1} of extract)	23.09±0.09	3.75±0.05	16.17±0.22

Each value is the Mean±standard deviation for 3 replicates, AE: Atropine equivalent, QE: Quercetin equivalent, GAE: Gallic acid equivalent

Table 2: Mortality (%) (Mean±SEM) of *Culex quinquefasciatus* larvae at different concentrations of plant extracts

Mosquito instar	Plant species	Concentration (mg mL^{-1}) 0	1	5	10	15	20
	Lavandula angustifolia	0^{a}	3.33±3.33^{a}	3.33±3.33^{c}	26.67±3.33a^{bc}	30.00^{b}	56.67±6.67^{b}
	Jacaranda mimosifolia	0^{a}	3.33±3.33^{a}	6.67±3.33^{bc}	10.00^{d}	26.67±3.33^{b}	46.67±8.81^{b}
	Tamarix aphylla	0^{a}	3.33±3.33^{a}	6.67±3.33^{bc}	16.67±3.33^{cd}	43.33±6.67^{b}	100^{a}
Third	*Lavandula angustifolia*	0^{a}	0^{a}	6.67±6.67^{bc}	16.67±3.33^{bcd}	43.33±8.81^{b}	83.33±3.33^{a}
	Jacaranda mimosifolia	0^{a}	0^{a}	23.33±8.81^{a}	33.33±8.81^{ab}	56.67±3.33^{b}	100^{a}
	Tamarix aphylla	0^{a}	6.67±333^{a}	26.67±8.81^{a}	46.67±6.7^{a}	93.33±3.33^{a}	100^{a}
Fourth	*Lavandula angustifolia*	0^{a}	0^{a}	16.67±8.8^{bc}	50.00±5.77^{cd}	73.33±6.67^{b}	100^{a}
	Jacaranda mimosifolia	0^{a}	0^{a}	13.33±6.67^{bc}	60.00±5.77^{bc}	100^{a}	100^{a}
	Tamarix aphylla	0^{a}	23.33±5.77^{b}	66.67±8.8^{a}	100^{a}	100^{a}	100^{a}

Means within a column followed by the same letter are not significantly different ($p>0.05$)

Table 3: LC_{50} and LC_{90} (mg mL^{-1}) of *Culex quinquefasciatus* larvae after 24 h of exposure to ethanolic extracts of different plant species

Mosquito instar	Plant species	$LC_{50}\pm SE$ (mg mL^{-1})	$LC_{90}\pm SE$ (mg mL^{-1})
Second	*Tamarix aphylla*	16.35±0.09	18.98±0.11
Third	*Jacaranda mimosifolia*	13.61±0.65	18.94±0.60
	Tamarix aphylla	11.08±0.18	16.19±0.10
Fourth	*Lavandula angustifolia*	9.06±0.10	18.08±0.14
	Jacaranda mimosifolia	8.35±0.14	13.08±0.14
	Tamarix aphylla	3.58±0.11	8.69±0.21

died when exposed at 20 mg mL^{-1} of *T. aphylla*. Exposure to 20 mg mL^{-1} of *T. aphylla* and *J. mimosifolia* also cause 100% mortality of third instars, except after exposure to *L. angustifolia*, which caused only 83.3% mortality. All fourth instars died at 20 mg mL^{-1} of all plant extracts.

Most of the plant extracts killed 50% of third and fourth instar larvae at 10 mg mL^{-1} (Table 3). The leaf extract of *T. aphylla* was the most toxic, followed by *J. mimosifolia* with LC_{50}. For fourth instar larvae, the LC_{90} was higher for larvae exposed to *L. angustifolia* followed by *J. mimosifolia* and *T. aphylla*.

DISCUSSION

Botanical phytochemicals are now recognized as potential alternatives to synthetic insecticides in mosquito control programs due to their excellent larvicidal and insecticidal properties[20]. In our study, all the tested plant extracts showed different larvicidal action compared to the controls. The larvicidal activity could have been due to any one of the bioactive compounds in the extracts. *T. aphylla*, which had the highest levels of phenols, tannins, alkaloids and flavonoids, had the highest mortality rate of larvae exposed to 20 mg mL^{-1} of the plant extract. This finding concurs with a similar study by Abdul Rahuman *et al.*[22], in which the presence of phenolic compounds in seeds of *Carica papaya* (Brassicales, Caricaceae) showed larvicidal potential. According to Pavela[23], majority of plants with phenolic compounds present strong larvicidal potential.

The high mortality of *C. quinquefasciatus* could have been a result of highly toxic effects of the plant leaves. Phytochemical screening revealed that the three plants had large quantities of phenols.

Flavonoids have been reported to possess potent mosquito larvicidal effects[24]. Figueroa-Brito[25] confirmed that secondary metabolite content in papaya leaves inhibit metabolic processes in larvae. Previous and current results by Hollingworth *et al.*[26] confirm that the larvicidal activity of whole plant extracts is due to the presence of secondary metabolites, especially alkaloids, phenols and flavonoids. Alkaloids have a long history as insecticides. Thus, the presence of alkaloids and flavonoids could have contributed to the larvicidal effects of the plants studied to date. Other secondary metabolites that have been found to have larvicidal effects include alkaloids[27] and tannins[28], which could have contributed to the larvicidal effects observed in this study.

The difference between observed LC_{50} in previous studies could be explained by factors such as plant extract concentrations and larvae instar stage[24]. Different concentrations of plant extracts possess different larvicidal effects against different mosquito species[28]. Additionally, some plant-derived materials are known to be highly effective against insecticide-resistant insect pests[28]. Ahn *et al.*[29] reported that the leaf extracts of five species of cucurbitaceous plants showed larvicidal effects at 465.85 ppm against the fourth-instar larvae of *C. quinquefasciatus*. Similarly, *Ricinus communis* seed extract exhibited larvicidal effects with 100% mortality at the concentration of 32-64 µg mL^{-1} and showed LC_{50} values of 7.10 µg mL^{-1} in *C. quinquefasciatus* larvae[30].

The results of this study supported earlier findings of Govindarajan[31], who showed that ethanol extracts of *Tagetes patula* was less active as only 50% *C. quinquefasciatus* larvae were killed at 100 ppm. The petroleum ether fraction of *Acacia nilotica* and *Citrullus colocynthis* showed 100% mortality at 125 and 62.5 ppm, respectively, against *C. quinquefasciatus*[32]. They also reported that ethyl acetate fractions of *Solanum trilobatum* and *Letiota aspera* showed LC_{50} values of 23.5 and 138.6 ppm against second and third instar larvae of *C. quinquefasciatus*, respectively.

This study suggests that extracts from *T. aphylla*, *L. angustifolia* and *J. mimosifolia* leaves caused mortality of *C. quinquefasciatus* larvae. However, the effectiveness of the extracts differed with dosage and the instar. Nevertheless, the considerable mean larval mortality indicated that these plants could be exploited as alternative control agents against *C. quinquefasciatus*.

The leaves of *T. aphylla* and *J. mimosifolia* showed 100% mortality of fourth instar larvae exposed to 15 mg mL^{-1} and mortalities increased with concentration of all the plant extracts tested. This concurs with Khanna and Kannabiran[27], who reported a positive correlation between concentration and the percentage of larval mortality.

Phytochemicals may serve as suitable alternatives to synthetic insecticides in the future as they are relatively safe and inexpensive and are readily available in many parts of the world. According to Abdul Rahuman *et al.*[22], the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

In this study, plant extracts showed promising larvicidal effects. Plant extracts offer an advantage over synthetic pesticides as they are easily biodegradable and less prone to development of resistance in the target organisms. We recommend application of these potential botanical insecticides against mosquito larvae. Additionally, more studies are needed to elucidate these effects against a wider range of mosquito species and identify the active ingredient(s) in the extracts that produce the larvicidal effect.

CONCLUSION

The ethanol extracts of *Tamarix aphylla*, *Jacaranda mimosifolia* and *Lavandula angustifolia* 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. However, further studies on identifying the active compounds, toxicity and field trials are needed to recommend the active components of these plant extracts for development as eco-friendly controls of insect vectors.

SIGNIFICANCE STATEMENT

This study investigates the mode of action of three natural plant 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.

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