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Research Article

Effect of crude ethanol extract of *Tamarix aucheria* and *Suadae vermiculata* on mortality larvae instar of *Culex pipiens*

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Abstract: *Culex pipiens* mosquitos are thought to be vectors for many arboviruses, including West Nile virus and encephalitis virus, which have a global impact on human health. The natural management of this pest's aquatic stages is critical for sustaining an insecticide-free environment. The current study focused on the biological and biochemical effects of crude ethanol extract of *Tamarix aucheria* and *Suadae vermiculata* on *Culex pipiens* laboratory colony larvae of instar. The results showed the effect of overlapping the concentrations of crude ethanol extract *T. aucheria* and *S. vermiculata* on mortality of the first and second larval instars of *C. pipiens* mosquito which seems the exceed *T. aucheria* more than *T. harizanium* in mortality of first and second larva instars and for all concentrations. The concentration of 150ppm and of 120 hours exposure showed effective concentrations in mortality for all plants.

Keywords: *Culex pipiens*, Crude ethanol, Mortality, Plant.
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Introduction

Because of the female's blood-feeding habits, *Culex pipiens* is closely associated with humans and other animals (Forattini et al. 1993). *Culex pipiens* breeds in clean and polluted ground pools, animal waste lagoons, sewage of treatment plants, and other organic waste-polluted sites (Molaei et al. 2007). It is frequently distributed in sewage in Basrah and Mosul during the year (Mohsen et al. 1995; Abdulkade 2000). *Culex pipiens* is a major vector of periodic filariasis in many parts of the world, infecting approximately 11 million people. It is also a primary vector of West Nile Virus (WNV) in some areas of the world, and Japanese Encephalitis virus has been isolated from these mosquitoes in Taiwan (Weng et al. 1999; Lok et al 2000; Molaei et al. 2007).

There is a need to find an alternative to synthetic pesticides as new control agents derived from natural products such as plant secondary metabolites, botanical insecticides that are generally pest-specific, safe to use, unique in the mode of action, easy to process and apply, less toxic to higher animals and the environment, and can be produced by farmers and small-scale industries. Since they are a mixture of biologically active compounds, insects develop resistance slowly. Since 1950, approximately 2500 plants belonging to 247 families have been reported to have some sort of toxic property against insects (Silva-Aguayo 2004; Talukder 2006). A large number of plants have been tested for insecticidal activity against mosquitos, with some showing promise (Komalamisra et al. 2005; Mullai & Jebanesan 2007; Chowdhury et al. 2008; Kaushik & Saini 2008; Kihampa et al. 2009). The growing need to find more plants with insecticidal properties has led to the current study, which aims to discover the larvicidal activity of

some plant extracts of *Tamarix aucheria* and *Suadae vermiculata* against fourth-instar larvae of *C. pipiens* mosquitoes, which are more resistant than other stages.

Materials and Methods

Culture of *C. pipiens*: Mosquito larvae were collected from a sewage channel in Basrah and identified as *C. pipiens*. The method of Abdulkader (2000) was used to rear the mosquito larvae, using glass pools with dimensions of 30×30×20cm. The mosquito larvae were placed in these pools and a piece of clothing was used as a cover, and when the adults emerged and they were transferred to wooden cages using a suction tube Aspirator. The adults of mosquitoes were fed sugar syrup and birds as a source of blood meal, and a 14-liter glass was placed inside the cage for the females to lay eggs. *Culex pipiens* fourth larvae instar were isolated from culture using a paint brush according to AL-Jebori (1982) that they have large size, the head smaller than the thorax, clearly identify eight body segments, and the head has a pair of antennae (Al-Jebori 1982).

Plant materials: The experimental plants of *T. aucheria* and *S. vermiculata* in this study were washed and dried in the shade at room temperature, and then grounded to a fine powder.

Soxhlet extraction: Soxhlet extraction was performed in a traditional apparatus using standard methods on 20.0g of ground leaves with 200mL n-hexane for 24 hours (within three days, 3x8 h). In two steps, the resulting extract was evaporated. After two replications, the extracted oil was weighed and the extraction efficiency was calculated (Eikani et al. 2012).

Statistical analysis: Mortality percentages were computed and adjusted by Abbott's formula statistical analysis of the experimental data to find the significance between the concentration of plant extract and mortality at different periods with different extracts using SPSS software by applying General Line Model was fitted using F-test, in 0.05 significance level (P<0.05).

Results and discussion

Using the ethanol crude extract of *S. vermiculata* and *T. aucheriana* to control the non-complete and complete stages of *C. pipiens* mosquitoes (Tables 1-4). In the first instars of larvae, the use of crude ethanol extract of *S. vermiculata* and *T. aucheriana* to control the first instar of *C. pipiens* resulted when the concentration of 150ppm used as 53.33 and 60.66%, respectively, while the exposure period 120 hours was the highest mortality percentage that were 56.1 and 61.1%, respectively. In the second instar of larva, the concentration of 150ppm had the highest reaching mortality in the *S. vermiculata* and *T.aucheriana* at 48.66 and 54.66%, respectively. The exposure period of 120 hours had more mortality than the rest of the periods for the plant extracts at 46.66 and 51.1%, respectively. The current study's findings indicate that plant extracts have the potential to contribute to alternative pesticides for disease vector control. The environment is an incomparable reservoir of natural products with structural characteristics not found in terrestrial natural products (Sukumar et al. 1991). Several studies have shown that plants are an excellent source of biologically active components such as antifungal, antiviral, phytotoxic, and larvicidal activity (Thomas et al. 2017). The screening of medicinal plants for mosquito larvicidal activity may eventually lead to their use in mosquito abatement practices based on natural products (LaBeaud et al. 2011). Plants may be a suitable alternative source of insecticides in the future to supplement the toolbox of available synthetic insecticides, because of their low mammalian toxicity, crude extracts contain a large number of active compounds with various mechanisms of action, are relatively inexpensive, and are widely available in many parts of the world (Ramkumar et al. 2015).

The results of a study revealed that the acetone extract of *Cipadessa baccifera* inhibited egg hatching by 98% (2% hatch ability) at 24, 48, and 72 hours at

Table 1. Effect of different concentrations from *T. aucheriana* plant of ethanol extract in mortality first larval instar of *C. ipiens* under effect of different exposure periods.

Exposure period / Concentration	Mortality after the exposure period					Rate
	24h	48h	72h	96h	120h	
25	6.66	16.66	26.66	33.33	36.66	23.994
50	13.33	23.33	30	43.33	46.66	31.33
75	20	30	33.33	43.33	60	37.332
100	26.66	30	40	63.33	66.66	45.33
125	23.33	40	56.66	73.33	76.66	53.996
150	33.33	50	63.33	76.66	80	60.664
Rate	20.55	31.665	41.66	55.55	46.1	

L.S.D. 0.05 (con.) = 12.13, L.S. D.0.05 (ex.p.) = 9.6, L.S.D. 0.05 (interference.)= 10.67

Table 2. Effect of different concentrations from *S. vermiculata* plant of ethanol extract in mortality first larval instar of *C. pipiens* under the effect of different exposure periods.

Exposure period / Concentrations	Mortality after the exposure period					Rate
	24h	48h	72h	96h	120h	
25	10	13.33	20	26.66	30	19.998
50	13.33	16.66	30	33.33	43.33	27.33
75	13.33	20	26.66	36.66	53.33	29.996
100	13.33	20	3.33	50	60	35.332
125	16.66	23.33	43.33	63.33	73.33	73.996
150	26.66	40	50	73.33	76.66	53.33
Rate	15.55	22.22	33.88	47.21	56.1	

L.S.D 0.05 (con.)= 12.56, L.S.D 0.05 (ex.p.)= 8.98, L.S.D 0.05 (interference)= 10.6

Table 3. Effect of different concentrations from *T. aucheriana* plant of ethanol extract in mortality second larval instar of *C. ipiens* under effect of different exposure periods.

Exposure period / Concentration	Mortality after exposure period					Rate
	24h	48h	72h	96h	120h	
25	3.33	13.33	23.33	23.33	30	18.664
50	13.33	16.66	26.66	36.66	40	26.662
75	13.33	16.66	26.66	33.33	46.66	27.328
100	13.33	23.33	36.66	36.66	50	31.966
125	20	33.33	43.33	56.66	63.33	43.33
150	30	43.33	56.66	66.66	76.66	54.662
Rate	15.55	24.44	35.55	42.21	51.1	

L.S.D. 0.05 (con.)= 10.5, L.S.D. 0.05 (ex.p.)= 9.11, L.S.D. 0.05 (interference.)= 9.63

0.1, 0.3, 0.5, 1, and 2mg/mL, respectively (Ramkumar et al. 2019). AL-Dehamee (2022) showed the percentage of hatching eggs that were exposed to mixture extracts were 31, 43, 65, 71, and 83%, respectively which agreed with the results of the current study. The uses was flowering branches *Tamarix jordanis* to control *C. pipiens* that performed by Schlein (2014) reached a death rate of 60.5%, while Ahmad et al. (2022) reported that the use of plant extract *T. baluchistanica T. Androssowii* and *T. mascatensis* with control 3rd instar larvae of *C. quinquefasciatus*, after 24 h of exposure resulted as follow (LC50) for ethyl acetate equal 19.46 and n-hexane fraction (LC50) was 1.26. These results disagreed with the findings of the current study. In the study of Suresh et al. (2018) even at low doses of *S. maritima* leaf extract was moderately toxic to *Aedes aegypti* larval instars (I-IV) and pupae LC50 values range from 135 to 242ppm (Kazim 2012).

Conclusion

Table 4. Effect of different concentrations from *S. vermiculata* plant of ethanol extract in mortality second larval instar of *C. pipiens* under the effect of the different exposure periods.

Exposure period / Concentrations	Mortality after the exposure period					Rate
	24h	48h	72h	96h	120h	
25	3.33	13.33	16.66	26.66	26.66	17.328
50	6.66	13.33	23.33	26.66	33.33	20.662
75	10	13.33	20	26.66	40	21.998
100	6.66	20	30	36.66	46.66	27.966
125	23.33	33.33	40	53.33	60	41.398
150	23.33	36.66	50	60	73.33	48.664
Rate	12.21	21.66	29.99	38.32	46.66	

L.S.D. 0.05 (con.)= 10.46, L.S.D. 0.05 (ex.p.)= 9.15, L.S.D. 0.05 (interference)= 9.63

The present study revealed the potential role of the leaf extracts of *T. aucheriana* and *S. vermiculata* as control agents for *C. pipiens*, either directly through larval kill, or indirectly through its latent effects expressed in the reduction of survival, fecundity, hatchability, and induction of dominant lethality in the subsequent generation.

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