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Chemical content of *Ficus carica* L. leaves and the effect of terpenes on the biological performance of *Culex pipienes* mosquitoes (Dipterta: Culicidae Culicinia)

Ashwaq Talib Hameed*[1], Fatima Abdullah Kamil[2], Imtithal I. J.[1]

1. College of Education for Women, University of Anbar, Iraq
2. General Directorate of Education, Anbar, Iraq

** Corresponding author's Emails: ashwaq.talib@uoanbar.edu.iq, Edw.Kalimer2004@uoanbar.edu.iq*

ABSTRACT

In this study, a qualitative and quantitative estimation of the most important active constituents of *Ficus carica* L. leaves was made, and the results showed that they contained good amounts of them. Terpenes were extracted from the leaves and their potential impact on the life performance of *Culex pipienes* mosquitoe, the percentage of cumulative and non-cumulative deaths in immature roles, growth period, and female productivity were selected as criteria for live performance, where concentrations (2.5, 5, 7.5 and 10 mg mL^{-1}) were used for evaluation of its effect on the life of mosquitoes. The results showed that the leaves of the plant had a significant effect on some aspects of the biological performance of *C. pipienes*. The mortality of the larval stages of the insect was significantly increased compared to the control group, and its adult productivity was significantly reduced. The results of the statistical analysis indicated that there were statistically significant differences concerning the concentrations and number of eggs.

Keywords: Terpenes, *Ficus carica*, *Culex pipienes*, Mosquitoes.
Article type: Research Article.

INTRODUCTION

The genus *Ficus* belongs to the family Moraceae, one of the oldest types of fruits appeared on Earth (Mudhafar *et al.* 2021), and also one of the most important sources of chemically-active substances that are characterized by being antioxidants (Suriyakalaa *et al.* 2021). It was found that the leaves of *F. elastica* contain chemical compounds such as ficus elastic acid and glucopyranosyl abscisate sodium, which showed significant activity as antioxidant (Salehi *et al.* 2021). Antioxidants such as xanthotoxol, triterpenoids inhibit oxidizing organic molecules, very important as food preservatives as well as in defence systems against oxidative stress. In addition, the general chemical analysis of the plant shows the presence of arabinose sugar, fatty acids, campesterol, stigmasterol and glycosides. The leaves of *Ficus* are also rich in phenols, essential oils, flavonoids, and other bioactive compounds such as arabinose, β-amyrins, β-carotenes, glycosides, xanthotoxol and β-setosterols, among the secondary metabolites, as they are found in vesicles or freely bound to carbohydrates (Dasqupta *et al.* 2021 & Elhawary *et al.* 2018). Terpenes have vital defensive importance as they work to expel herbivores, especially insects (Gundesli *et al.* 2021). Given the medical importance of *F. carica* and the possibility of the presence of effective compounds against insects in this plant, it was chosen to investigate its biological effects on the live performance of *Culex* mosquitoes in an attempt to find some alternatives from pesticides of plant origin, in a desire to preserve the safety of the environment from pollution resulting from the use of chemical Insecticides.

Collection and diagnosis of plant samples. The leaves of *F. carica* were collected at the beginning of April from a home garden in Ramadi, Iraq in 2020. The plant was classified by Dr. Ashwaq Talib, The leaf samples were dried in laboratory conditions and crushed to obtain a fine vegetable powder, then stored in a tightly-closed bottle, and refrigerated until use.

Insect breeding, collection and diagnosis. The immature roosters (larvae and eggs) were collected from of the drainage sites of Anbar Governorate - the puncture area in Al-Khalidiyah by means of a long-handled ladle. it was then placed in a plastic container with a lid and transported to laboratory, followed by placing it in plastic basins filled with tap water to which a mice diet containing ground flour consisting of yellow corn, wheat, rice and protein at a ratio of 0.25:1:1:1 was added (Saady *et al.* 2021) by 2 g per tank to feed the larvae. For the purpose of obtaining a permanent pure farm, the modern virgins were transported by means of a train with a wide nozzle into plastic containers, which were deposited in a wooden cage, ribs (1 m × 60 cm × 1.20 m) for adult feeding. Petri dishes containing cotton saturated with a 10% sugar solution were placed inside the cage to feed the newly hatched chicks. To get the egg boats, we followed Arora *et al.* (2022), method where the female mosquitoes were fed three days after their emergence on the blood of a pigeon that plucked its feathers from the chest and abdomen area. A water bowl was also placed inside the cage to serve as a place to lay eggs. The egg boats laid in a small brush were transferred to new water bowls containing food for the larvae until the emergence of the adult insects. In order to prevent rotting, water was added every three days or the surface layer of the cloth was removed. This method was repeated until the appearance of the third generation of adults. Samples were taken from the fourth instar larvae and adults of this generation, and slides were prepared for them to diagnose and according to the taxonomic characteristics contained in the taxonomic keys (Dehghan *et al.* 2016). Samples of larvae, pupae and adult instars of the third generation were taken for the purpose of diagnosis in the Natural History Museum / University of Baghdad and confirmed that they are *Cx. pipienes* from the family Culicidae. It should be noted that the insect was reared and its culture was prepared in laboratory conditions of 28 °C, relative humidity of 50% and at a rate of illumination of 12 capacity.

Phytochemical test. The plant was determined some phytochemical compound such as Glycoside, resin, phenolic compounds, flavonoids saponin, alkaloids and tannins using standard methods (Omoregie *et al.* 2010; Evans 2002).

The antioxidant activity. The *F. carica* leaf has been evaluated for the antioxidants activity according to Beta- Carotene method after accommodated method. This report was conducted in relation to the procedure done by Kulisic *et al.* (2004). So, 1 mg β -Carotene and 2 mL chloroform with 20 mg linoleic acid were mixed together, then the chloroform was evaporated under low pressure in 30 °C by rotary evaporator, and added Tween 40 (200 mg). Tocopherol and water were prepared as control sample. The retention of all samples was documented spectrophotometrically at 470 nm after 2 h.

Preparation of the crude terpene extract

To extract the crude terpene compounds, 20 g dry matter powder of *F. carica* leaves was weighed, and extracted with 400 mL chloroform in a succinate extraction device for 24 h at a temperature of 40 °C. The extracted sample was concentrated by the rotary evaporator, and the sample containing the crude turbinate compounds of the plant was dried in an electric oven at 45-40 °C, then dried and placed in a closed glass container in the refrigerator until use after recording its weight when it was empty (Essa *et al.* 2020; Hameed *et al.* 2021). For the purpose of estimating the biological activity of turbines by dissolving 1 g of them in 3 mL 96% ethyl alcohol and completing the volume to 100 mL with distilled water, the concentration of the basic solution became 1% or equivalent to 10 mg mL^{-1}. Then the concentrations of 2.5, 5, 7.5 and 10 mg mL^{-1} were prepared. In the case of control treatment, it was 3 mL of ethyl alcohol, and the volume was completed to 100 mL with distilled water. For the purpose of estimating the effectiveness of turbines for *F. carica* leaves in the cumulative destruction of insect eggs, the newly laid egg boats at 1-2 days old were obtained from the colony bred in the rearing cage. These boats were transferred to plastic containers containing 50 mL of the concentration of crude terpenoid extract at 5 replication for each concentration and each replicate was one boat. The mortality rate was calculated in the eggs after hatching and the mortality rate was adjusted according to the Abbotte equation (Abbotte, 1925). For the purpose of knowing the effect of the extract on the destruction of different larval instar: 10 larvae were taken from fresh first instar larvae with 5 replicates for each concentration. They were transferred to plastic containers containing 50 mL of extract and 0.1 g nutritional mint was added to each replicate. Mortality was recorded in the first larval age after 24 h of trial. The same process was repeated for the second, third, and fourth larval ages, each, separately, and the mortality rate was adjusted according to the Abbotte's equation.

Effect on destroying pupae: 10 pupae were transferred to each replicate with 5 replicates for each concentration. They were transferred to plastic containers containing 50 mL of the extract. Mortality was recorded 24 h after trial. The death rate was adjusted according to Abbotte's equation (Abbotte, 1925). The effect of the extract of terpenes in *F. carica* leaves on the cumulative death, growth period of immature roles and adult productivity of *Culex* mosquitoes: For the purpose of studying the cumulative effect of extracts on immature roles of the insect and after hatching from first-stage larvae, the larvae were distributed on 5 replicates of one concentration and at a rate of 20 larvae per replicate containing 50 mL of the extract was followed up to the complete stage, where the total percentage of total deaths was calculated for the immature roles represented by the different larval stages and pupae. The volume of each daily repeater was completed with distilled water due to evaporation. Dead insects were removed daily from the treatments and examined microscopically to determine phenotypic abnormalities, if any. The growth period of the immature roles was calculated from the egg to the full ones. These complete insects were taken for the purpose of knowing the productivity of female mosquitoes resulting from the previously-treated eggs. The adults emerging from the replicates were isolated for each concentration in wooden cages of cube-shaped with a length of 50 cm sides and covered with a thin cloth. Afterward, it was fed on the blood of pigeons to feed the females on its own blood. Inside each cage, a petri dish covered with cotton was placed saturated with a 10% sugar solution to feed the males. A plastic container containing tap water was also placed to receive the egg boats that the females would later lay. Then the number of eggs laid for each concentration and hatching percentage was calculated.

Statistical analysis

The experiments were designed according to the factorial experiment model and with a completely randomized design (Factorial experiments with completely randomized design), and the percentages of fatalities were corrected according to the Abbott formula (1925).

Corrected perishment (%) = Perishment in the treatment (%) - Perishment in control \ 100 – Loss in control

The Least significant differences (L.S.D) test under the $P \leq 0.05$ level was used to show the significance of the results. Corrected mortality percentages were converted into angular values for inclusion in the statistical analysis.

RESULTS AND DISCUSSION

The phytochemical composition of green portion extracted of *Ficus carica* has characterized seven components. The major constituents were glycoside 0.65 %, phenolic compound 0.88 %, resins 29.6%, flavonoids 43%, saponin 4.7%, alkaloids 0.90 % and tannins 3.8 % (Table 1).

Table 1. phytochemical analysis of *Ficus carica* leaf.

Glycoside	**Brown**	**0.65**
Phenolic compound	Green	0.88
Alkaloids	Brown	0.9
Saponin	Foam	4.7
Tannins	Green	13.8
Resins	Not turbidity	29.6
Flavonoids	Yellow	43

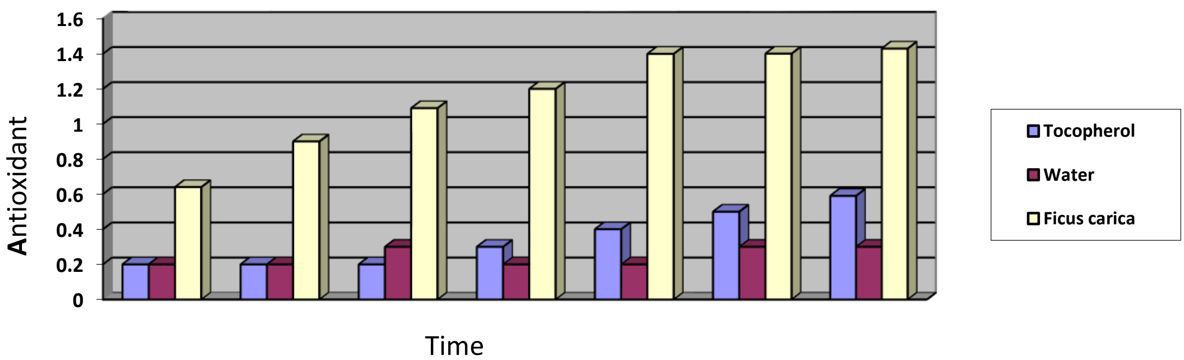

Fig. 1. Antioxidant test of *Ficus carica* by beta Carotene methods over 90 min (0-90 min); LSD 5% = 0.021.

Beta- carotene method was one of important ways to test the oxidative efficacy of plant in cubing and scavenging harmful free radicals in living bodies (Sharma *et al.* 2021). Elevated difference between tocopherol and water were showed in Fig. 1 compared to *F. carica* leaf sample. The capability of various solution of *F. carica* was assessed by lowering in its 517 nm absorption. The efficacy of *F. carica* was appeared in inhibiting free radicals in comparison with vitamin E and BHT which was a good antioxidant (Mudhafer 2021; dos Anjos *et al.* 2021), and used universally. This material was used in this study as coefficient compared with sample (Fig. 1).

Table 2. Effect of factor concentrations of terpenoid extracts of *F. carica* leaves on cumulative mortality of immature roles of *Culex pipienes* mosquitoes.

Terpenoid concentration (mg mL^{-1})	Eggs destruction (%)	First larval instars (%)	Second larval instars (%)	Third larval instars (%)	Fourth larval instars (%)	Destruction of virgins (%)
0	17.54	0	0	0	0	0
2.5	17.80	0	0	0	0	0
5	20.11	10.44	0	0	0	0
7.5	41.21	40.11	41.54	33.15	0	0
10	45.29	46.00	44.11	33.50	10.12	0
LSD at p < 0.05	1.10			2.9		-

Table 2 shows the effect of the concentration factor of the extract on the cumulative destruction of the larval instars of the insect, where the highest mortality rates (45.29, 46.00, 44.11 and 10.12) were recorded in the first, second, third and fourth larval instars, respectively, at 10 mg mL^{-1} compared to the control. These results confirm that the extract of *F. carica* leaves exhibit a significant effect on the different larval stages of the insect, where the L.S.D value of 2.90 was recorded under the 0.05 level of significance, while the death rate has fallen to its lowest level and no significant effect was recorded in the four larval instars at 2.5 mg mL^{-1}. This indicates a direct relationship that mortality rates increase by elevating the leaf extract concentrations. When comparing the average mortality rate of the four larval instars among themselves, it was found that the first instar is the most sensitive compared to the rest of the other larval instars, where the mortality rates were 46.00, 40.11, 10.44 and 0% for 10, 7.5, 5 and 2.5 mg mL^{-1} respectively. While the second instar recorded mortality rates of 44.11, 41.45, 0, and 0%, and in the third larval instar 33.50, 33.15, 0 and 0, the fourth larval instar was the least sensitive, reaching 10.12, 0, 0 and 0% with the same concentrations aforementioned. The results of the statistical analysis showed that there were significant differences between the phases under the level of 0.05 for the extract concentration factor, and it was between (Olikibo *et al.* 2018). The terpenes have a physiological effect on the larval tissues, represented by a direct toxic effect on the target tissues and an indirect effect by causing an imbalance in the neurosecretory system. Guermah *et al.* (2021) reported that the crude extract of phenolic compounds from the leaves of the licorice plant *Glycyrrhiza glabra* L. had a remarkable effect on the cumulative destruction of the different larval stages of *Culex pipienes* mosquitoes, where the highest mortality rate was as a result of treatment with this crude extract at a concentration of 10 mg mL^{-1}, where the rate of ratios phase 1 was 57%. The second phase was 50.60%, the third 60.40%, and the fourth 41.56%. This is consistent with the results of the current study in terms of the effect of the same concentration on different larval instars. In another study, it was indicated that treatment of the different larval stages of *Culex quinqufasciatus* to extract the terpene compounds of some leaves had a significant effect on their mortality (Shehata 2018). By studying the effect of the factor concentrations of crude phenolic compounds extract of *F. carica* leaves on the cumulative destruction of *Culex* mosquito eggs, it was found that the extract had a clear effect on the destruction of insect eggs, which rose to 43.08 at a concentration of 1 mg mL^{-1}. The reason may be attributed to the prevention of gas exchange through the effect of the extract on the egg shell, or it works to harden the egg shell (Anosike *et al.* 2021).

Table 3. Effect of terpentine extract factor concentrations of terpenoid extracts in *F. carica* leaves on immature growth period, adult productivity and egg hatching rate of *Culex pipienes*.

Concentration (mg mL^{-1})	Growth period (day)	Yield of eggs	Hatching of eggs (%)
0	18	238	94.40
2.5	17	191	90.20
5	16	109	84
7.5	13	0	0
10	13	0	0
LSD at p < 0.05	1.6	13.420	4.881

Table 3 shows the effect of the concentrations of the turbinate extract of the leaves on the growth period, as the highest growth period that was taken by the immature roles as a result of treatment with concentrations of 10, 7.5, 5 and 2.5 mg mL^{-1} of the leaf extract were 13, 13, 16 and 17 days, respectively, compared the control, which lasted 18 days. However, no significant differences were observed between the concentrations of the same extract and the growth period. In the case of the productivity of the adults, it decreased significantly by an elevation in the concentrations of the extract of phenolic compounds compared to the control treatment. The average productivity was 109 and 191 eggs/female at 5 and 2.5 mg mL^{-1}, respectively, while no productivity was recorded at the two concentrations, i.e., 10 and 7.5 mg mL^{-1}. In addition, the eggs hatching rate (%) decreased to a great extent by upraise in the extract concentrations, reached to 84 and 90.20 at 5 and 2.5 mg mL^{-1}, respectively, while no percentage was recorded at 10 and 7.5 mg mL^{-1}. The results of the statistical analysis indicated significant differences in the results. The effect of the extract concentrations of turbinate compounds in the *F. carica* leaves on the cumulative death of immature stages of *Culex* mosquitoes (Table 3) and that there was an increase in the mortality rates of the immature stages by elevation in the extract concentration. These results were in agreement with other studies. The crude extract of phenolic compounds from the leaves of the *F. carica* led to a cumulative death of the *Culex* mosquito reported by Jabbar *et al.* (2021) and Suriyakalaa *et al*. (2022) that the destruction of the immature stages of the mosquito may be due to the sensitivity of the larvae to the toxic substances present in the plant or the low efficiency of food conversion.

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