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EFFECTS OF PUNCTURE VINE PLANT (*TRIBULUS TERRESTRIS* L.) EXTRACT ON ALL LARVAL STAGES OF MOSQUITO, *CULEX PIPIENS* (DIPTERA: CULICIDAE)

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ABSTRACT:

The current study was conducted in Duhok city to investigate the biological impact of extracting secondary chemical compounds from punctured vine plants (*Tribulus terrestris* L.) on the mortality of all larval stages of (*Culex pipiens*). Bioassay experiments were carried out by using solvent extraction methods and different concentrations (0.1,0.3,0.5,0.7) mg/ml. The results showed that the ethyl extract of puncture vine plant's leaves demonstrated a promising larvicidal activity, and fatal results were observed where the high rate of mortality was within the 1st larval instar 97.3% and 100% at a concentration(0.5 mg/ml) after 24 and 72 hours of exposure respectively, while the lowest mortality rate was within the 4th larval instar 7.4% and 38% at a concentration(0,1mg/ml) after 24 and 72 hours of exposure, respectively . Moreover, the study found that the leaf extracts modified the morphology of the larval development stage. According to the present study, those plants are possible alternatives to mosquito larvicide which can be used to produce cost-effective, safe chemicals to control mosquitoes.

KEYWORDS: *Tribulus terrestris* L., biological aspects, mosquito, *Culex pipiens*, larvicidal, Phytochemical.

1. INTRODUCTION

Haematophagous insects are nauseating to people and animals
and global disease pathogen transmitters bymosquitovectors have been used to spread the pathogens of various deadly diseases (Mekhlif, and Kafi, 2020). The "public enemy number one" is the mosquito, according to a World Health Organization report (1996), one of the Culicidae family's most widely distributed genera, Culex has roughly 768 species and is further classified into 26 subgenera (Harabach, 2011). Numerous pathogens, including multicellular ones like filariasis (*Wucheriria spp.*), protozoans like malaria (*Plasmodium spp.*), and arboviral ones like dengue fever, are transmitted by most genera of mosquitoes, *Aedes, Anopheles, and Culex*(Severson, 2021). The World Health Organization (WHO) has recommended using phytochemicals instead of synthetic pesticides to avoid the issues associated with these products, as they decompose rapidly in the environment, have inexpensive material costs, and donot harm non-target species (Sharma *et al,*2018).

Plants have been existing as a source of numerous chemical substances, including phenols, alkaloids, and terpenes .Due to their great effectiveness and environmental safety and health, insecticides of plant origin play the primary role in controlling mosquitoes (Muhammad and Mekhlif, 2021). *Tribulus terrestris* is found in tropical climates of the world, and it is commonly known as 'Gokhru' which is a flowering plant belonging to the family Zygophyllaceae (Singh et al., 2022). The plant is widely distributed throughout the world as well as in Iraq. Bioactive compounds like Flavonoids, steroids saponins, flavanol glycosides, lignan amides, N-trans-caffeoyl tyramine, and alkaloids are a few of the active components found in *Tribulus Terrestris* (Bashir and Al-Habib, 2021). As *T. terrestris* is used as a folk medicine for a variety of purposes and treatments, (Pavin *et a*l., 2018), different nations have historically chosen distinct uses for this plant impotence, rheumatism. The plant also provides protection against oxidative stress and exhibits antitumor, cytotoxic, antifungal, anti-helminthic, anthelmintic, and larvicidal (Chhatre et al.,2014) .Numerous survey investigations on the larvicidal effectiveness of mosquitoes against *Culex pipiens* on the native flora of world and Iraq were carried out experimentally as a study done by Al-Chalabi and Taha(2017) and Alhamadanyl and Mekhlif, (2022). The current study's objective is to investigate the insecticidal activity which may be present in the extract of the Puncture vine (*T. terrestris*) plant against the mosquitoes (*Culex pipiense*) as well as the - plant's mosquito repellent characteristics.

2. MATERIALS AND METHODS

2.1. Plant collection and preparation of plant extract

The plant species(*T. terrestris*) was gathered from Miserik in Duhok province, Northern Iraq, Kurdistan Region in August 2021, the species recognition was done at Agriculture College. At the University of Zakho's Physiology Research Lab, part of the Biology Department, the plant extract was prepared according to Bashir and Al-Habib(2021) , the ethanolic extraction of the leaves was prepared. The plant was first dried in the shade, and then it was dried in a 40°C oven until the weight remained stable. Then, after being grounded in a grinder and sieved, the dried leaves were assembled into a fine powder (1000 g). The powdered leaves were steeped in the appropriate volume of methanol (99%) for a full day. For four days, the technique was repeated three times with sporadic shaking. The extract was concentrated using a thin-film filter, the powder was produced using a rotary evaporator (BCHI), and its bottles were maintained at -4°C for additional use.

2.2. Laboratory colony of mosquitoes

A stock culture of culex mosquitoes was generated and maintained for three generations utilizing 500 ml plastic bowls filled with water from the tap and supplied rabbit chows as

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nutrition. After the appearance of the adults, they were given a cotton piece soaked in a solution of 10% of sugar (Abdulmajeed, and AL-chalabi, 2017). The Culex mosquito species applied in this study as *Culex pipienes* L. Diptera: Culicidae was recognized by The Research Centre and Natural History Museum in Baghdad.

2.3. Preparation of stock solution.

Four grams of plant extract were dissolved in 100 ml of distilled water to obtain the stock solution (40 mg/ml), and the following concentrations: 0.1, 0.3, 0.5 and 0.7 mg/ml were used.

2.4. Larvicidal Bioassay

The impact of various plant extract concentrations was evaluated, four groups of 1st, 2nd, 3rd and 4th instar larvae (each of 20 larvae) were maintained in dechlorinated tap water, the larvae were provided with rabbit chow (0.25 g/bowel), and the net fabric was placed over the plastic containers. The experiments were conducted in (27± 2 oC) controlled laboratory conditions. The larval mortality was determined by counting, recording, and removing the dead larvae using disposable droppers after 24, 48, and 72 hours (Mekhlif, 2017). The mortality was then adjusted in accordance with the Abbott equation (Abbott, 1925) to make sure that the results were correct. All the data have been analysed by using the program Graph Pad Prism 5(USA), and one-way analysis (ANOVA).

2.5. 2.5 Ethical approval

Ethical approval was obtained from the Protocol Review Committee of the Biological Sciences Committee (BSCZ) of the University of Zakho (ID: "BSCZ/30/6/2021").

3. RESULTS

3.1. The effect of *Tribulus terrestris* L. extract on the first larval instar

Table(1) is concerned with the mortality rate of larvae in the first instar according to the three variables of time(24h, 48h, 72h) and the estimated concentration rate(0.0, 0.1, 0.3, 0.5, 0.7) mg/ml . The results showed that the lowest mortality rate was 42.8 at a concentration 0.1 after 24 hours of exposure, while the highest mortality rate was 100% at a concentration 0.3 after(72h) 72 hours of exposure. All the mentioned percentages are statistically significant at the mentioned significance levels 0.05,0.01,0.001, while the percentage 42.8 is significant at the level of significance 0.01 only.

Table 1. The effect of Tribulus terrestris L. extract on the first instar larvae of Culex pipiens at different exposure periods

Concentration mg/ml	Mortality%		
	24 h	48 h	7 2h
0.0	1.00	2.00	2.50
0.1	42.8 **	52.00**	78.0***
0.3	82.5***	91.0***	100.0***
0.5	97.3***	100.0***	100.0***
0.7	100.0***	100.0***	100.0***

*=(P-value<0.05) **=(P-value<0.01)***=(P-value<0.001)

3.2. The effect of tribulus terrestris L. extract on the second larval instar

For the second instar, Table (2) shows that the lowest mortality rate starts with 37.0% at a concentration of 0.1 and exposure period of 24 hours, and this value is statistically significant at the significance level of 0.01, while the highest mortality rate was at the concentration of 0.5mg/ml in an exposure period of 72 hours, it reached 100%.

Table 2: The effect of *Tribulus terrestris* L. extract on the second instar larvae of *Culex pipiens* at different exposure periods

Concentration mg/ml	Mortality%		
	24 h	48 h	72 h
0.0	0.00	1.5	2.0
0.1	37.0 **	42.00**	65.5***
0.3	71.8***	80.0***	95.0***
0.5	80.5***	87.0***	100.0***
0.7	98.0***	100.0***	100.0***

*=(P-value<0.05) **=(P-value<0.01)***=(P-value<0.001)

3.3. The effect of Tribulus terrestris L. extract on the third larval instar

According to the data shown in Table (3), the third instar larva had the lowest mortality rate found at a concentration (0.1) with a rate of 23.5% at the exposure period of 24 hours, and this value is statistically significant at the level of 0.05, while the highest mortality rate reached 100% at a concentration of 0.7 after 27 hours of exposure, which is statistically significant at the level of significance 0.001

Table 3: The effect of Tribulus terrestris L. extract on the third instar larvae of Culex pipiens at different exposure periods.

Concentration mg/ml	Mortality%		
	24 h	48 h	7 2h
0.0	0.5	0.5	1.0
0.1	23.5 *	34.00*	45.0**
0.3	65.2**	72.3***	89.2***
0.5	73.5***	80.4***	98.0***
0.7	80.0***	88.0***	100.0***

*=(*P*-value<0.05) **=(*P*-value<0.01)***=(*P*-value<0.001)

Those instars larvae of *C. pipiens* which were killed within the present investigation showed obvious morphological abnormalities and were provided with 0.7 mg/ and 0,5 mg/ethanolic extracts of *Tribulus terrestris* respectively (Fig. 1and 2)

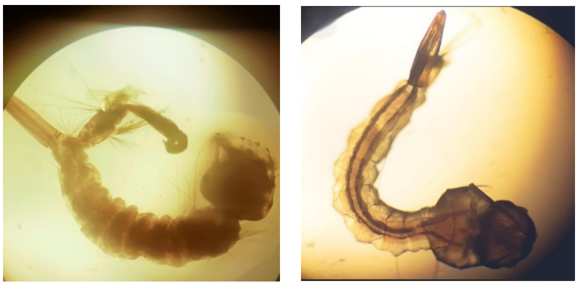

Figure (1): The right side is abnormal third instar larva after 72 hours of exposure to the 0.7 mg/ml of Tribulus terrestris extract compared with left side normal larva (20x).

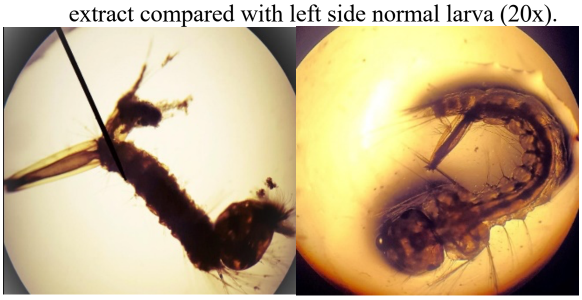

Figure (2): Right side is abnormal 3 rd. instar larva with dark body treated with 0.5 mg/ml of Tribulus terrestris . extract compared with the left-side normal larvae after 72hrs.exposure (20x).

3.4. The effect of Tribulus terrestris L. extract on the fourth larval instar

The lowest mortality rate, which can be seen in Table (4), was 7.4 at the exposure period of 24 hours and concentration (0.1), which is statistically significant at the level of significance 0.05, while the highest mortality rate was at the exposure period of 27 hours and at a concentration (0.7), as the mortality rate decreases with the decreases concentration at all exposure periods.

Table 4: the effect of Tribulus terrestris L. extract on the fourth instar larvae of Culex pipiens at different exposure periods.

Concentration mg/ml	Mortality%		
	24 h	48 h	7 2h
0.0	0.00	0.00	0.00
0.1	7.4 *	11.00*	38.0**
0.3	33*	63.7***	88.0***
0.5	46.3***	78.5***	96.5***
0.7	60.7***	86.0***	100.0***

*=(P-value<0.05) **=(P-value<0.01)***=(P-value<0.001)

Many observations of various morphological abnormalities have been observed in the 4th larval instar dead (Fig. 3) where the treated larvae have a small head and abdominal deformities.

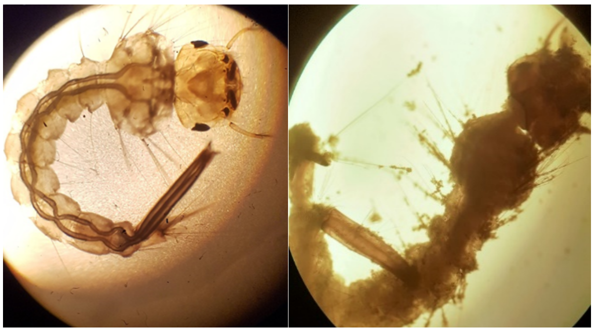

Figure (3): Right side is abnormal dead 4th instar larva with small head treated with 0.5 mg/ml *Tribulus terrestris* extract compared with left side normal larva (28x)

3.5. The effect of the ethanolic extracts of *Tribulus terrestris* L. on all larval stages of Culex pipiens

According to the data of this investigation, *Tribulus terrestris* L. provided strong insecticidal qualities, the larvicidal activity of this extract against all larval stages of *Culex pipiens* was demonstrated in Figures (4, 5 and 6) following exposure for 24, 48, and72 hours. The analysis also indicated that larval mortality was determined by time of exposure and concentration.

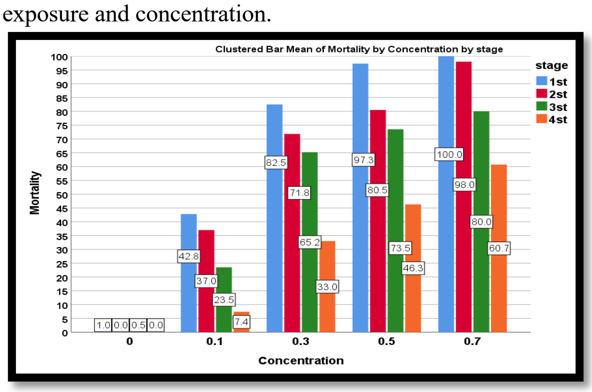

Figure (4) The comparative effect of the plant extract concentration extract on the mortality of all larval stages after 24h exposure.

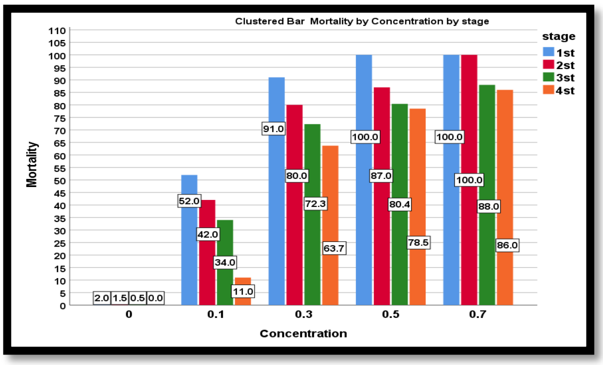

Figure (5) The comparative effect of the plant extract concentrations on the mortality of all larval stages after 48h exposure.

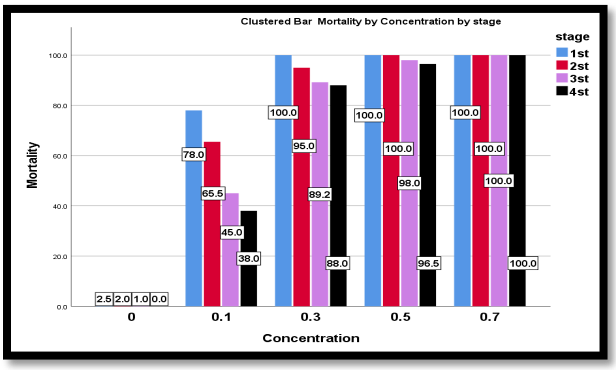

Figure (6) The comparative effect of the plant extract concentrations on the mortality of all larval stages after 72h exposure.

4. DISCUSSION

The present study indicated the larvicidal activity of ethanolic extracts of *Tribulus terrestris* L., which included more active ingredients and were highly harmful to mosquito larvae in their all-larval instars. The results suggested a direct correlation between concentrations and the mortality rate of larvae . According to a study done in Iraq by Alhamadanyl and Mekhlif, (2022) , they recorded different levels of larval mortality, and LC50 and LC90 values were computed. The findings showed that *Geranium savilíticum* was more effective when both LC50 and LC90 were lower (135, 120 ppm) . It was also confirmed that the duration of larval exposure had a positive impact on the fatality rate and that the duration of exposure to concentrations enhanced the probability of larval death. The same pattern was observed on the first instar larvae of Culex quinquefasciatus used, the fresh plants *Clerodendron inerme, and Acanthus ilicifolius* in a study performed by Kovendan and Murugan (2011). Moreoevr these observations supported the results arrived at by Muhammad. and Mekhlif (2021), the medicinal herbs *Physalis angulate*, *Peganum harmala, Teucrium polium, and Thymus vulgaris* had their bio-insecticidal effects tested on the fourth instar *Culex pipiens molestus* larvae . They started after 24 and 48 hours of exposure, the LC50 values at 24 and 48 hours were 1.8, 26.0, 34.0, and 45.0 ppm. Respectively. According to a study done by Jawale (2014), the increase in the percentage mortality of the treated mosquito larvae is supported by the presence of phytochemicals in the plant extracts. Also, the death of the larvae may be due to the presence of saponins, flavonoids, glycosides, alkaloids, and tannins in *Tribulus terrestris* L (Usman, 2007). Compounds saponins' insecticide activities are actually identified by their toxicity to poisonous insects (anti-feeding, disruption of the moult, growth regulation, mortality...) . The insecticidal activity of saponins disrupted the production of ecdysteroids. Furthermore, some insects are cytotoxic to these chemicals, or they act as protease inhibitors (Chaieb, 2010). There is evidence that alkaloids, saponins, and tannins have both therapeutic and pesticide effects (Azmathullah *et a*l., 2011). In many various insect species, flavonoids were antifeeding, and the antifeedant chemical like triterpenes azadirachtin can interact directly with specialized "deterrent" chemoreceptors on the mouth parts of insects . This antifeedant action increased with the prolonging of exposure, as a result, it prevents eating upon touch or may have an impact on the central nervous system, causing the insect to starve , then to death (AL-Dhaher, 2021). Alkaloids influence Acetylcholinesterase (AChE) receptors in the nervous system, control hormonal activity, and interfere with key cellular and physiological activities through toxicity. The mode of action of alkaloids differs depending on the type of alkaloids (*Chowanski et al*.,2016). Singh *et al.* (2008) and El-Sheikh *et al.* (2012) used crude ethanol and acetone extracts, the mosquito carrying the dengue virus, *Aedes aegypti*, was reported to have better larvicidal action against the third instar larvae and adult of *Tribulus terrestris,* with an LC50 of 64.6 ppm. recorded in the latest studies, which all subsequent larval instars had a higher mortality rate than the first instar larvae, and the second instar larvae had a higher mortality rate than the third . The effect on them was close, even though the effect on the second larval instar was slightly greater than on the third larval instar and fourth instar larvae did. Although the larvae of the 4th instar are more resistant than the previous three instars Almost all of the fatalities existed during the four instars of larva displayed a number of phenotypic distortions, where they were in the form of short, shrinking larvae with a black color, which confirms the growth-inhibiting action of this extract. The present study also revealed obvious morphological abnormalities, in addition to morphological abnormalities . The results also demonstrated that larval mortality was time-related, and the results were consistent with those of Dhavan and Jadhav(2020).

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