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Evaluation on the Efficacy of *Tribulus terrestris* (Zygophyllaceae) Leaf Extracts on the Different Stages of the Lymphatic Filariasis Vector, *Culex quinquefasciatus* (Diptera: Culicidae)

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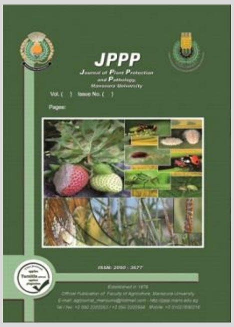

ABSTRACT

The current study aims to estimate the biological impact of methanol, ethyl acetate, and hexane extracts of *Tribulus terrestris* leaves on different stages of *Culex quinquefasciatus*. A range of concentrations of methanolic (150-250-500-600-700 ppm), ethyl acetate (62.5-150-250-500-700 ppm) and hexane extracts (5-15-30-62.5-125-250 ppm) were evaluated. The results showed that methanol extract had the strongest effect on all stages of the mosquito compared to the ethyl acetate and hexane extracts. The methanol extract eliminated the larvae at a concentration of 700 ppm by 100%, with high values of LC_{50}-LC_{90}., while in ethyl acetate extract the highest mean larval mortality was at the highest concentration (700 ppm) but at a mean percent of 69.63 ±4.18. Also, the highest larval mortality rate was at the highest concentration of hexane extract at 94.467±1.86%, The findings suggest that the *T. terrestris* plant extract had strong effect on the different developmental stages of *C. quinquefasciatus*. In fact, more specialized future research is required to identify the active compounds in the plant that affect the vital processes in mosquitoes and use them as environmentally friendly alternatives.

Keywords*: Methanolic extract, mosquito, larval mortality, *Tribulus terrestris*, *Culex quinquefasciatus

INTRODUCTION

Lymphatic filariasis and malaria are the commonest mosquito-borne infectious diseases, that affect human health and other vertebrates (Bhuvaneswari et al., 2023; Ragavendran et al., 2024). Filariasis is among the most prevalent infectious diseases affecting tropical and subtropical regions, caused by a species of nematode called *Wuchereria bancrofti* (Amaechi et al., 2024). Through preventive chemotherapy and vector control measures, two mosquito-borne diseases—malaria and lymphatic filariasis are expected to be eradicated by 2030 (WHO, 2007; WHO, 2018; Bhuvaneswari et al., 2023). *Culex quinquefasciatus* mosquito is the main vector for lymphatic filariasis and other zoonotic diseases (Khan et al., 2021) that found in many countries around the world, including the Middle East, especially Saudi Arabia (Bosly,2021)ᵡ. As a result of the development of resistance against chemical insecticides, which leads to the rebounding vectorial capability, controlling the mosquito population is extremely challenging (Shahi et al., 2010). Eliminating mosquito breeding grounds with environmentally safe larvicides is the most cost-effective way to control mosquitoes (Cetin et al., 2004). Because of its poor movement and localization, larval therapy is more effective in controlling this terrible insect (Howard et al., 2007; Ullah et al., 2018). In order to battle tropical diseases, the WHO aims to eliminate the intermediary hosts or vectors of these diseases. The only effective way to reduce the occurrence of filarial fever is to eliminate and control mosquito vectors, mostly by applying insecticides to larval habitats, as there is currently no viable vaccination for the disease. Since mosquitoes are immobile during their immature stages, controlling them during this time is essential and effective in integrated mosquito management (Elimam et al., 2009; Tennyson et al., 2012; Marc et al., 2021). The use of pesticides has many effects on the environment and human health, so we need a safer and more effective method to eliminate mosquitoes at different stages of their growth without harming the environment. Lately, people have been thinking about finding environmentally friendly solutions to eliminate mosquitoes. Numerous researchers have, over the years, identified traditional medicinal plants as protective agents to enhance integrated pest management (IPM) programs against various insect species (Abdel Kareim et al., 2017 and 2023) including mosquitoes (Tisgratog et al., 2016; Bekele, 2018; Sivakumar and Sivakumar ,2022). Saudi Arabia has many traditional plants that can be used to eliminate mosquitoes, especially in Jazan region. Because *Tribulus terrestris* is prevalent here, we decided to use it for this study. *Tribulus terrestris* has also been used by El-Sheikh et al (2012) to eliminate Anopheles mosquitoes that cause malaria. Belonging to the Zygophyllaceae family, *Tribulus terrestris* L. is a significant traditional medicinal plant that has been used for a variety of reasons since the Vedic era. It is used to treat vesical calculi, piles, sexual dysfunction, and eye issues. Steroids, antioxidants, proteins, amino acids, alkaloids, flavonoids, phenolics, and saponins are all present (Chaudhary et al., 2023). Every part of the *Tribulus terrestris* has unique pharmacological properties, such as immunomodulatory, antibacterial, mitigating,

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sexual enhancer, and antibiotic. The purpose of the present study was to observe the biological effect of methanol, ethyl acetate, and hexane extracts of *T. terrestris* leaves on different stages of *C. quinquefasciatus*. These findings could present a chance to create less costly and environmentally damaging organic insecticide substitutes.

MATERIALS AND METHODS

Mosquito culture:

The larvae of *Culex quinquefasciatus* were sourced from the Center for Vector-Borne Diseases in Jazan and were reared for six generations in the Department of Biology, Faculty of Science, Jazan University, under controlled conditions of 27±2^{oC}, 70-80% RH, and 12-12 LD. Adult mosquitoes were maintained in wooden cages measuring 30×30×30 cm and were provisioned daily with cotton pads soaked in a 10% sucrose solution for three days after emergence. Following this period, female mosquitoes were permitted to take a blood meal from a pigeon host, which is essential for laying eggs. A plastic oviposition cup (15×15 cm) filled with dechlorinated tap water was introduced into the cage to facilitate egg laying. The resultant egg clusters were subsequently collected from the plastic cup and moved into plastic pans (25×30×15 cm) containing three liters of tap water leftover for 24 hours. The newly emerged larvae were supplied on a daily basis.

Preparation of tested extracts:

Mature specimens of *T. terrestris* (Family: Zygophyllaceae) (Fig. 1) were collected in April 2022 coinciding with the blooming season at Al-Hashr Mountain in the Jazan region of Saudi Arabia (17°26′34.80″ N, 43°2′34.79″ E). The identification of the plant was conducted following the guidelines of Tackholm (1974) and Boulos (1995). A voucher specimen (Jaz. 00301010005) was deposited in the Biology Department, Faculty of Science, Jazan University, Saudi Arabia, and incorporated into the herbarium collection. The leaves were thoroughly washed and air-dried in the shade at ambient temperature (27–31 °C) for a period of 10 days until they reached a brittle texture, after which they were ground into a powder using a hammer mill. A total of 100 g of leaf powder was subjected to extraction three times, each with 300 ml of methanol at room temperature. After a 24-hour extraction period, the supernatants were carefully removed and filtered through Whatman filter paper No. 5 before being concentrated in a rotary evaporator at 40 °C, yielding 12.9 g and 9.1 g of semi-solid crude leaf extract, respectively. The dried extracts were stored in a deep freezer at -4 °C until needed for further experimentation.

Fig. 1. morphological characteristics of the leaves of *T. terrestris*.

Larval toxicity test:

The extracted material was diluted in two drops of Tween 80 as an emulsifier to facilitate its dissolution in water to investigate the toxicity of the extracts. To find mortalities, several concentrations of each extract were made. Three replications were conducted for each experimental treatment. For each replicate, 30 late 3rd instar larvae were utilized and introduced into 300 ml plastic cups containing 200 ml of dechlorinated tap water, where they were maintained until the emergence of adults under lab conditions of 27 ± 2 °C, 70 ±10% RH, and 12–12 LD. Only two drops of Tween80 were given to control larvae in 100 ml water. Every day, mortality was recorded, and the dead larvae and pupae were collected and removed prior to the emergence of the adults.

Experimental bioassay:

Larval mortality was determined based on the absence of response to mechanical stimulus (Williams et al., 1986). The percentage of mortality during the larval stage was calculated applying the equation depicted as follows (Briggs, 1960): larval mortality % = A – B / A × 100 (where: A = number of tested larvae, B = number of resulted pupa). The pupation occurrence rate was derived through the application of the formula: Pupation % = A / B × 100 (where: A = number of resultant pupae, B = Quantity of larvae subjected to testing). Pupal mortality was evidenced by a lack of response to mechanical stimulus or the failure to develop to the adult stage. The proportion of pupal mortality was determined using: Pupal mortality % = A / B × 100 (Where: A = number of dead pupae. B = number of resulted pupae. The emergence of male and female adults was quantified and computed using the following equation: Adult emergence % = A / B × 100 (where: A = Quantity of adults that have emerged, B = number of resulted pupae).

Statistical Analysis:

The data are expressed as the Mean ± Standard Deviation (SD). Statistical analyses were performed using One-way Analysis of Variance (ANOVA) to assess the differences in the activities of the tested extracts, with Tukey's test at a significant level of 0.005 probability level; means with $p > 0.05$ were considered not statistically significant. All statistical analyses were performed using SPSS software. The LC50 and LC90 values were determined through linear regression analysis in Microsoft Excel.

RESULTS AND DISCUSSION

Results

The biological effect of different concentrations methanolic, ethyl acetate and hexane leaf extracts of *Tribulus terrestris* on several stages of *Culex quinquefasciatus* is included in the present data. This includes Larval mortality, duration of the larval stage, pupation rate, percentage of pupal mortality, adult emergence rate, and percentage of adult mortality

1- Effect of methanol extract of *T. terrestris* on different stages of *C. quinquefasciatus*

Table 1 showed the effects of methanol extracts of *T. terrestris* leaves on different stages of *C. quinquefasciatus* at concentrations ranging from 150-250-500-600 and 700 ppm. The methanol extract exhibited the maximum larvicidal activity at a concentration of 700 ppm. At this concentration, the complete mortality for larvae was 100.0%. At the lowest concentrations (150 ppm), the larval mortality percentage dropped to 12.70±3.1% (from 3.43±5.95% for the untreated larvae); this effect was statistically significant. While the highest effect of methanol extract on larval duration was at a concentration of 600 ppm with an average of 6.33±0.57 days compared to the control, which set a period of 5 days. The effect on pupation appeared at concentration of 150 ppm with a mean of 86.63±2.57 with statistical significance with the control. The highest pupal mortality rate was at a concentration of 500 ppm, higher than the control (3.33±5.77) with a mean of 25.00±25.00. While the mean percentage of adult emergency (96.67±7.070) approached the control percentage at a concentration of 150 ppm, which is the highest effect compared to the rest of the concentrations. Adult mosquitoes (adult mortality) died at the highest concentration (600 ppm), which has a mean rate of 26.67±0.58.

Table 1. Effect of methanol extract of *Tribulus terrestris* on different stages of *Culex quinquefasciatus*

Conc.ppm	Larval mortality %	Larval duration (day)	pupation	Pupal Mortality %	Adult Emergency %	Adult Mortality %
control	3.43±5.95	5.00±0.00	96.56±5.94	3.33±5.77	96.66±5.77	0
150	12.70±3.17*	5.00±0.00	86.63±2.57*	3.33±5.77	96.67±7.070	14.33±0.58*
250	44.43±3.65*	5.33±0.57	55.56±3.67*	8.33±14.43	91.67±14.43	19.33±1.15*
500	61.63±1.16*	5.67±0.67	38.36±1.16*	8.33±25.00	91.67±14.43	23.33±0.58*
600	86.10 ±2.60 *	6.33±0.57*	13.90±2.61*	16.67±28.86	83.33±28.86	26.67±0.58*
700	100.00±0.00*	-	-	-	-	-

2- Effect of ethyl acetate extract of *T. terrestris* on different stages of *C. quinquefasciatus*.

Table 2 presents the effects of ethyl acetate extract of *T. terrestris* leaves against multiple stages of *C. quinquefasciatus* at concentrations ranging from 62.5, 150, 250, 500 and 700 ppm. The results were almost similar to the effect of methanol extract. The highest mean larval mortality was at the highest concentration (700 ppm) but at a mean percent of 69.63 ±4.18, with statistical significance compared with control (2.20±3.8). The larval duration rate was equal to the control (5 days) at the lower concentration extract, and there was no significance between concentrations. The higher concentration of ethanol extract (700) had a high effect on pupal mortality% (33.33±28.86) compared to control, which had no effect. The lowest concentration listed no effect on adult emergency while the concentration of 700 ppm listed 66.67±28.86%. As for the adult mortality of mosquitoes, they had a significant effect on the higher concentration of ethyl acetatel extract, which reached an average of 66.67±57.73.

Table 2. Effect of ethyl acetate extract of *Tribulus terrestris* on different stages of *Culex quinquefasciatus.*

Conc. ppm	Larval mortality %	Larval duration (day)	pupation	Pupal Mortality %	Adult Emergency %	Adult Mortality %
control	2.20±3.8	5.00±0.00	98.13±3.233*	0	100.00±0.00	0
62.5	35.17±1.89*	5.00±0.00	64.83±1.89*	0	100.00±0.00	0
150	42.33±1.15*	5.33±0.57	57.67±1.16*	6.67±11.54	93.33±11.54	0
250	52.77±1.57*	5.67±0.67	47.23±1.16*	20.00±20.00	80.00±20.00	4.73±8.19
500	61.200±1.82 *	6.67±0.58	38.80±1.82*	10.00±10.00	90.00±10.00	49.43±9.16
700	69.63 ±4.18*	5.44±0.70	30.37±4.17*	33.33±28.86	66.67±28.86	66.67±57.73*

3- Effect of Hexane extract of *T. terrestris* on different stages of *C. quinquefasciatus*

The effects of Hexane extract of *T. terrestris* leaves at concentrations ranging from 5, 15, 30, 62.5, 125 and 250ppm were shown in table 3. The effects of hexane extract were evident at the lowest concentration (5 ppm) on larval duration, pupation, and adult emergency% listed 5 days, 82.10%, and 97.33%, respectively. While the highest mortality rate in larvae was at the highest concentration of hexane extract at 94.467±1.86%, the lethal effect of hexane extract extended to pupal mortality% at 100% with a significant effect at the same concentration.

Table 3. Effect of hexane extract of *Tribulus terrestris* on different stages of *Culex quinquefasciatus*

Conc. ppm	Larval mortality %	Larval duration (day)	pupation	Pupal Mortality %	Adult Emergency %	Adult Mortality %
control	11.00±19.05	5.00±0.00	89±1.91	0	100.00±0.00	0
5	17.90±1.82	5.00±0.00	82.10±1.82*	2.67±4.61	97.33±4.62	0
15	31.27±1.86	5.00±0.00	68.80±1.82*	11.10±8.26	88.90±19.22	0
30	44.57±3.67*	5.67±0.58	55.43±3.69*	14.57±10.79	85.43±25.14	29.40±6.23*
62.5	63.43±3.20*	5.67±0.58	36.57±3.20*	50±5.78	50.00±17.32	33.10±0.17*
125	70.10 ±3.15*	5.00±0.00	29.90±3.15*	38.33±10.10*	61.67±31.75	41.00±8.54*
250	94.467±1.86*	6.33±0.58*	5.53±1.86*	100.00±0.00*	-	-

Table 4 displays the methanol extract's LC_{50} and LC_{90} values (ppm) of methanolic, ethyl acetate and hexane extracts of *T. terrestris* against larval mortality of *C. quinquefasciatus* (Fig. 2b). The methanol extract's LC_{50} and LC_{90} values were 349.23 and 739.25 mg/L, outperformed those of the ethyl acetate, 247.17, 590.22 mg/L and hexane extracts, 68.19 and 212.23 mg/L, respectively.

Table 4. LC50 and LC90 values (ppm) of methanolic, ethyl acetate and hexane extracts of *Tribulus terrestris* against larval mortality of *Culex quinquefasciatus.*

Extracting material	LC50	LC90
Methanol extract	349.23 mg\L	739.25 mg\L
Ethyl acetate	247.17mg\L	590.22 mg\L
Hexane extract	68.19 mg\L	212.23 mg\L

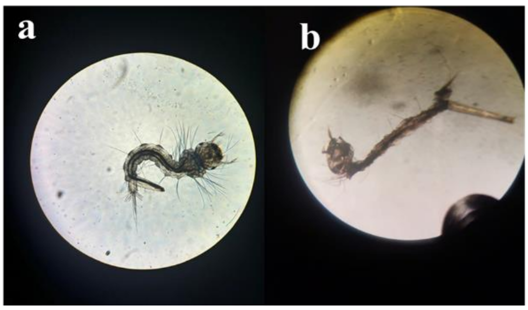

Fig. 2. The effect of extracts of *Tribulus terrestris* on larval mortality of *Culex quinquefasciatus*: a) untreated larva (as control and b) larva after treatment.

Discussion

In the present study, methanolic, ethyl acetate, and hexane extracts from the leaves of *Tribulus terrestris* were utilized to treat various developmental stages of *Culex quinquefasciatus*, yielding significant mortality rates. The methanol extract of *T. terrestris* leaves exhibits a superior efficacy in eliminating the various stages of the mosquito life cycle when compared to the ethyl acetate and hexane extracts, the findings of the current study align with those reported by Tennyson et al. (2012) and El-Sheikh et al. (2012). Additionally, El-Sheikh et al. (2012) reported that extracts from the leaves and stems of the plant *Tephrosia purpurea* (Fabaceae) exhibit activity against the mosquito *C. quinquefasciatus*. Notably, the stem extract demonstrated a lower efficacy against 3rd instar larvae, with an LC_{50} value of 2348 ppm, in contrast to the leaf extract, which had an LC_{50} value of 58.3 ppm. The leaf extract also significantly reduced adult emergence, fecundity, and fertility to a greater extent than the stem extract. These findings are consistent with the current study, which indicates that the leaves of *T. terrestris* leaves with different concentrations of methanol, had the strongest effect in eliminating the stages of the *C. quinquefasciatus* mosquito compared to the concentrations of ethyl acetate and hexane extracts. Ilham et al. (2019) demonstrated that the variations in the active ingredient composition affect larval mortality. Secondary metabolites effectively contribute to mosquito mortality and growth disruption in their immature stages (Pavela et al., 2019). Because plant components of juvinoids and phytoecdysosteroids could operate as agonist and/or antagonist hormones, their interaction with insects lowers development fitness (Mekhlif, & Kafi, 2020). Several studies indicated that the use of plant leaves in larvicidal activity of *C. quinquefasciatus*, such as: Ullah et al. (2018), who used five distinct plant extracts as an effective larvicide against *C. quinquefasciatus*. Using acetone as a reference, the plant extracts were applied at various concentrations (400, 200, 100, 50, and 25 ppm), among the five plant extracts, some were deemed the best in terms of LC_{50} and LC_{90} values as well as % mortalities when compared to other extracts. Moreover, this study cleared that the highest values of $LC_{50,}$ LC_{90} were in the methanol extract compared to the other extracts. Because LC_{50} and LC_{90} are powerful tools for evaluating the toxicity of plant extracts and determining their effectiveness in insect control. A high LC_{50} value indicates that the extract is less toxic, as a high concentration is required to kill half of the sample. The methanol extract eliminated the larvae at a concentration of 700 ppm by 100%. Furthermore, Salama *et al* (2022) in the Jazan region assessed the methanol extract of *Rumex vesicarius* leaves' impact on *Aedes aegypti* mosquito larvae in their third instar. The methanol extract was found to have the highest pupal and adult mortality rates, at 100% and 66.67%, respectively, at concentrations 35 and 15. Moreover, choosing the appropriate solvent to extract active compounds from plants has a significant impact on the effectiveness of the resulting extract in controlling mosquitoes. The fact that methanol is a polar solvent and can dissolve a variety of substances, including physiologically active substances present in plants, may be the reason why the methanol extract worked better than the other extracts. Numerous active substances, including terpenoids, flavonoids, and volatile oils, are extracted by methanol and may combine to boost the extract's potency (Huie, 2002; Sasidharan et al., 2011; Chaudhary et al., 2023).

CONCLUSION

Overall, it can be said that the methanolic, ethyle acetat, and hexane extracts of *T. terrestris* leaf act as antifeedants or repellents on larval mortality, larval duration per day, pupation, pupal mortality%, adult emergency percentage, and adult mortality percentage of *Culex quinquefasciatus*. As an environmentally friendly measure, the current study's findings may help reduce the use of synthetic insecticides, increasing the likelihood that botanical pesticides will naturally control a variety of medically significant pests.

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تقييم تاثير مستخلص اوراق نبات ***Tribulus terrestris*** **(zygophyllaceae)** علي الاطوار المختلفة للبعوض الناقل لحمي الفيلاريا ***Culex quinquefasciatus*** **(Diptera : culicidae)** تحت الظروف المعملية

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الملخص

تهدف الدراسة الحالية الى تقييم التأثير الحيوى لمستخلص أوراق نبات *Tribulus terrestris (Zygophyllaceae)* باستخدام تركيزات مختلفة من مذيبات الميثانول والايثيل اسيتات والهكسان على الأطوار المختلفة لبعوض *Culex quinquefasciatus (Diptera: Culicidae)*. بالنسبة لمذيب الميثانول تم استخدام تركيزات 150, 250, 500, 600, 700 جزء فى المليون ، أما مذيب الايثيل أسيتات فتم استخدام تركيزات 62.5, 150, 250, 500, 700 جزء فى المليون بينما فى مذيب الهكسان فتم استخدام تركيزات 5, 15, 30, 62.5, 125, 250 جزء فى المليون. أظهرت النتائج أن مذيب الميثانول أظهر تأثير قوى ضد الأطوار المختلفة من البعوض مقارنة بمذيبات الايثيل آسيتات والهكسان حيث عند تركيز 700 جزء فى المليون لمذيب الميثانول أدى الى موت 100% من يرقات البعوض ، بينما فى مذيب الإيثيل أسيتات سجلت أعلى نسبة موت لليرقات 69.63% عند تركيز 700 جزء فى المليون ، أما أعلى نسبة موت لليرقات عند استخدام مذيب الهكسان فكانت 94.46 عند أعلى تركيز. من خلال النتائج اتضح أن مستخلص أوراق نبات *T. terrestris* ذات كفاءة عالية لمكافحة الأطوار المختلفة للبعوض الناقل لمرض الفيلاريا ، وبالتالى فى الدراسة القادمة سيتم تحليل مكونات استخلاص أوراق نبات *T. terrestris* باستخدام مذيب الميثانول لمعرفة المواد الفعالة.

الكلمات الدالة: مستخلص الميثانول- يرقات البعوض- نبات *Tribulus terrestris*- بعوض *Culex quinquefasciatus*