

Effect of *Tarhonianthus camphoratus* (Asteraceae) Leaves Extracts on *Culex tritaeniorhynchus* (Culicidae) Life cycle.

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Salama: Effects of *Tarhonianthus camphoratus* on *Culex tritaeniorhynchus*

The *Culex tritaeniorhynchus* mosquito is the primary vector of Japanese encephalitis virus which can cause encephalitis in human and mammals. This mosquito also can transmit other diseases such as filariasis and West Nile virus. It has a wide global distribution. Therefore, it was a necessary to eliminate this insect to reduce the infections. Through this study, we evaluated the effect of methanolic and ethyl acetate extracts of *Tarhonianthus camphoratus* L leaves on different stages of *Culex tritaeniorhynchus* mosquito using five concentrations ranging from 200 ppm to 1000 ppm. Both methanol and ethyl acetate extracts completely eliminated larva by 100 % at the concentration of 1000 ppm. At the concentration of 750 ppm, methanolic extract eliminated larva by 73 %±3.00 %, pupa by 80 %±1.00 % and adult by 80 %±3.00 % whereas ethyl acetate extract eliminated larva by 72 %±3.00 %, pupa by 70 %±5.00 % and adult by 62 %±4.00 %. The LC₅₀ value of methanol and ethyl acetate extracts against larva mortality was 519.95 (ppm) and 454.94 (ppm) respectively. The results showed that both extracts had significant lethal toxic effects on the life cycle of *Culex tritaeniorhynchus*. However, the methanol leaves extract had more effective than ethyl acetate leaves extract. In this study, we suggested that *Tarhonianthus camphoratus* plant could be used as a natural insecticide for combat and control mosquitoes compared to harmful chemical insecticides.

Key words: Methanol, Ethyl acetate, *Tarhonianthus camphoratus* leaves, larvae, pupae, adult emergence, *Culex tritaeniorhynchus* mosquito

The mosquito, *Culex tritaeniorhynchus* (*C. tritaeniorhynchus*) is one genera of the Culicidae family which includes various types of medical and environmental significance. It is considered as the primary vector of Japanese Encephalitis Virus (JEV)^[1-3]. This insect has been spread over the world and is the source of many diseases that affect human and animal. *C. tritaeniorhynchus* is detected in at least 41 countries, across 5 different continents^[4]. It can transmit many diseases such as West Nile virus which cause dangerous neurological disease, Japanese encephalitis disease which affects the central nervous system and lymphatic filariasis disease^[5,6] which cause tissue enlargement due to *Wuchereria bancrofti* parasite^[7]. Thus, there is a critical necessity to combat and control this mosquito to prevent diseases outbreak. The use of insecticides and the nonstop use this harmful materials for mosquitoes control can affect the environment and

human health. These chemicals can disrupts natural adversaries and outbreaks of some species of insect^[8]. Also the high cost and development of resistance of mosquito species to chemical insecticides led to exploiting a biological control using plants and their derivatives against insects^[9]. More than 2000 species of plants have toxic effects to several insect species without harmful to environment^[10]. A number of plant derivatives are found to be effective against mosquitoes with safe manner. *Tarhonianthus camphoratus* (*T. camphoratus*) is a rare shrub has more than 6 meters in height with grey color and distributes in a number of habitats including Saudi

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Arabia, East and South Africa^[11]. The scented tree, *T. camphoratus* has various medicinal applications in traditional healing by smoking of burned leaves and drinking infusion or decoctions. The infusions and tinctures of its leaves used for many diseases such as asthma, inflammation, rheumatism, bronchitis, heartburn, stomach trouble, toothache, headache, cough, indigestion, cerebral hemorrhage and paralysis^[12-15]. This plant has powerful insect repellent action^[16]. The rub of *T. camphoratus* leaves by wild animal used to rid them from mosquitoes and flies. The study of the environmental interactions between insects and plants is a vital area for understanding the ecosystems and developing sustainable strategies to address environmental and health challenges. The current study aimed to explore the relationship between the *C. tritaeniorhynchus* mosquito and *T. Camphoratus* L tree with an emphasis on the chemical properties of plant and its impact on the behaviour of mosquito. Our study investigated the effect of methanolic and ethyl acetate extracts of *T. camphoratus* leaves against various stages of *C. tritaeniorhynchus*. We found that these extracts had inhibition effects on larva, pupa, adult emergence and adult of *C. tritaeniorhynchus* mosquito. This manuscript provided a scientific insight into the possibility to using the *T. camphoratus* plant extract as effective and safe way to control and combat the breeding of mosquitoes which can contribute to reducing transmission of diseases and achieving environmental sustainability.

MATERIAL AND METHODS

C. tritaeniorhynchus Larvae were obtained from the Vector-Borne Diseases Centre in Jazan. They were bred in the lab. For six generations in Department of Biology, Faculty of Sciences, Jazan University under

controlled conditions of ($27^{\circ}\pm 2^{\circ}$), RH (70 %-80 %) and (12-12) light-dark. Adult mosquitoes were kept in (30×30×30 cm) wooden cages. After emergence, the adult was daily provided with cotton pieces soaked in 10.0 % sucrose solution for 3 d. Then, the female mosquitoes were allowed to take a blood meal from a pigeon host, which is necessary for laying the eggs. To facilitate eggs laying, a plastic cup oviposition (15×15 cm) containing dechlorinated tap water was placed in the cage. After that, the egg clusters were picked up from the plastic cup and transferred into plastic pans (25×30×15 cm) has 3 l of tap water and leftover for 24 h. Daily the emerged larvae were supplied.

Plant collection and preparation of plant extract:

In the blooming season (April 2024), leaves of *T. Camphoratus* L (Family: Asteraceae) (fig. 1) were collected at Al-Hashr Mountain in the Jazan region of Saudi Arabia ($17^{\circ}26'34.80''$ N, $43^{\circ}2'34.79''$ E). The plant was recognized at Biology Department, faculty of Sciences, Jazan University, Saudi Arabia, and added in the herbarium collection according to the guidelines of Tackholm (1974) and Boulos (1995)^[17,18]. The collected leaves were washed and dried in the shade at room temperature (27° - 31°) for 10 d up to become a brittle texture. Then they were grounded to a powder using a hammer mill. For extraction, two solvents used, methanol and ethyl acetate. A 100 g of leaves powder were extracted with methanol and ethyl acetate separately three times with 300 ml at room temperature. After 24 h of extraction, the supernatant was aspirated and filtered through Whatman filter paper No. 5. Then the filtered supernatant was concentrated in a rotary evaporator at 40° . Finally we got 12.9 g and 9.1 g of semi-solid crude leaves extracts. Finally, the prepared extracts were stored in a deep freezer



Fig. 1: Morphology of *T. camphoratus* tree

at - 4° for using.

Larval toxicity test:

To investigate extracts toxicity, two drops of Tween 80 was added to the extracted crude to make an emulsifier. Different concentrations of methanol and ethyl acetate leaves extracts were prepared (1000 ppm, 750 ppm, 500 ppm, 400 ppm, and 200 ppm). A 30 late 3rd instar larvae were used and put in 300 ml plastic cups containing 200 ml of dechlorinated tap water and they maintained until the emergence of adults optimized conditions of $27^{\circ}\pm 2^{\circ}$, 70 % \pm 10 % RH, and 12-12 LD. To control larvae, two drops of Tween80 were added in 100 ml water. Every day, mortality was recorded, and the dead larvae and pupae were collected. All experiments were done in triplicates.

Experimental bioassay:

Larval mortality was indicated by a failure to respond to mechanical stimulation^[19]. Larval mortality percent was estimated by using the following equation^[20]: Larval mortality % = $(A - B) / A \times 100$ (Where: A=number of tested larvae. B=number of resulted pupa). Percentage of pupation was estimated by using the following equation: Pupation % = $A / B \times 100$ (Where: A=number of resultant pupae. B=number of tested larvae). Pupal mortality was observed by a failure to developing to adult stage. The pupal mortality percent was determined using the following equation: Pupal mortality % = $A / B \times 100$ (Where: A=number of dead pupae. B=number of resulted pupae. For adult emergence: The males and females of adults emergence had counted and the adult emergence percent was calculated using this equation: Adult emergence % = $A / B \times 100$ (Where: A=number of emerged adults. B=number of resulted pupae).

LC₅₀ and LC₉₀ determination:

The LC₅₀ value which represents the lethal concentration for 50 % larval mortality and the LC₉₀ value which exhibits the lethal concentration for 90 % larval mortality were calculated through linear regression analysis using Microsoft Excel.

Statistical analysis:

Data are represented as mean \pm Standard Deviation (SD). Statistical analysis of the data was carried out using One-way analysis of variance Analysis of Variance (ANOVA) was applied to find the differences between the activity of tested extracts using Tucky's

test at 0.005 probability level, where means with $p > 0.05$ are not statistically significant. Statistical analysis was carried out using SPSS software.

RESULTS AND DISCUSSION

The biological activity of methanolic and ethyl acetate leaves extracts of *Trachnoanthus Camphoratus* at various concentrations (1000 ppm, 750 ppm, 500 ppm, 400 ppm, 200 ppm) had toxic effects against different stages of *C. tritaeniorhynchus* mosquito.

From Table 1 showed that the methanolic extract had a maximum larvicidal effect on larva stage at a concentration of 1000 ppm where 100 % of mortality was observed (Table 1 and fig. 2). At 750 ppm, 500 ppm, and 400 ppm, the percent of larval mortality reached 73 ± 3.00 %, 53 ± 1.00 %, 37 ± 2.00 % respectively (Table1 and fig. 2).

While at low concentration (200 ppm) the larva mortality was 10 ± 1.00 % compared to control (Table1 and fig. 2). The LC₅₀ value against larva mortality was 519.95 (ppm) while LC₉₀ was 904.19 (ppm) (Table1 and fig. 2-fig. 12) this represented a significant toxicity of methanol extract. Duration of larva was 3.33 ± 1.00 d at concentration of 1000 ppm while at concentration 750 ppm was 4.33 ± 1.00 d compared to control (5.00 ± 1.00 d) (Table 1). At the concentration of 750 ppm, the pupation percent was 27 ± 2.00 %.

The higher percentage of pupation showed at low concentration of 200 ppm with 90 ± 3.00 % compared to the control (100 ± 0.00 %) (Table1 and fig. 3). Methanol extract had a toxic effect on pupae life where the pupal mortality was 80 ± 0.00 % at concentration of 750 ppm compared to untreated pupae (Table1 and fig. 4). At the concentrations of 500 ppm, the pupal mortality percent was exceed 50 % by 57 ± 2.00 % this indicated to a significant toxicity of extract (Table1 and fig. 4).

The Effect of methanolic extract on adult emergency and adult mortality was recorded, where at 750 ppm the percent of adult emergency was 20 ± 1.00 % whereas at low concentration 200 ppm, the percentage of adult emergency increased to 88 ± 2.00 % (Table1 and fig. 5). The highest mortality percent of adult mosquito was showed at concentration of 750 ppm by 80 ± 0.00 % (Table1 and fig. 5). A 50 % of adult mortality was exceed at concentration of 500 ppm by 67% this appeared a significant toxicity of methanol extract (Table1 and fig. 5).

TABLE 1: THE BIOLOGICAL EFFECT OF METHANOLIC AND ETHYL ACETATE LEAVES EXTRACT OF *T. Camphoratus* LEAVES ON DIFFERENT STAGES OF *CULEX TRITAENIORHYNCHUS* MOSQUITO

Concs. ppm	Larval mortality %	Larval duration/ days	Pupation %	Pupal mortality %	Adult emergency %	Ault mortality %
Methanol leaves extract						
1000	100±0.00	3.33±1.00	-----	-----	-----	-----
750	73±3.00	4.33±1.00	27±2.00	80±1.00	20±1.00	80±3.00
500	53±1.00	5.33±1.00	47±2.00	57±2.00	43±3.00	67±2.00
400	37±1.00	5.67±1.00	63±4.00	19±1.00	81±1.00	21±1.00
200	10±1.00	5.67±1.00	90±3.00	12±2.00	88±2.00	18±1.00
Control	0	5.00±1.00	100±0.00	0	100±0.00	0
Ethyl acetate leaves Extract						
1000	100±0.00	4.33±1.00	-----	-----	-----	-----
750	72±3.00	5.33±1.00	28±3.00	70±5.00	30±5.00	62±4.00
500	58±3.00	5.33±1.00	42±3.00	48±3.00	52±5.00	40±5.00
400	49±2.00	5.67±1.00	51±3.00	28±4.00	72±7.00	27±6.00
200	32±3.00	5.67±1.00	68±3.00	16±1.00	84±5.00	11±2.00
Control	0	5.00±0.00	100±0.00	0	100±0.00	0

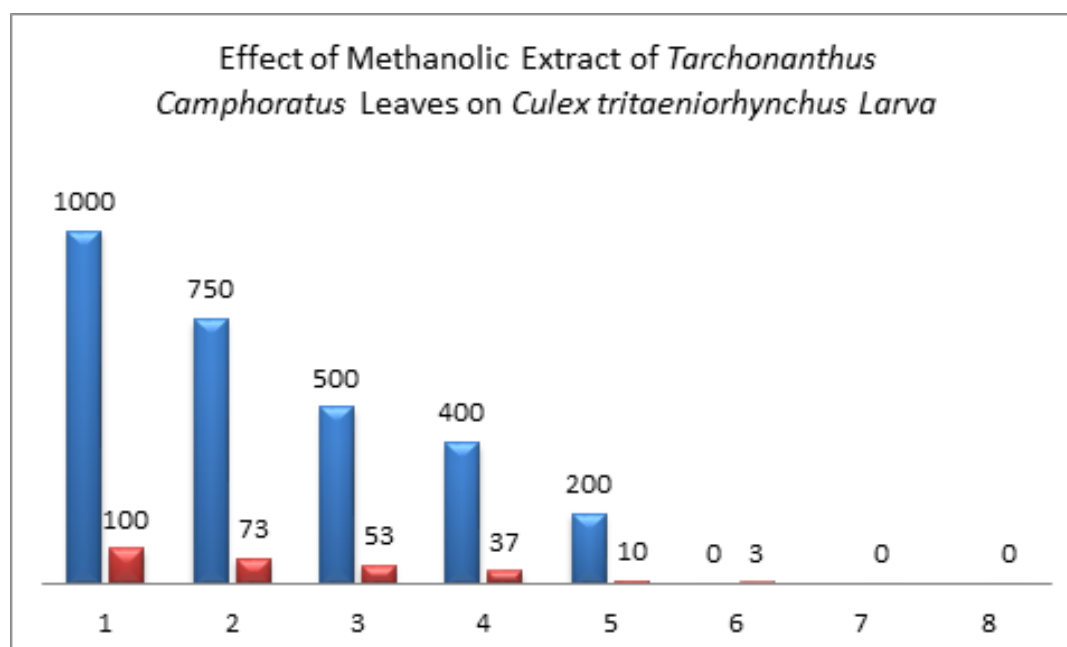


Figure 2: Biological activity of methanolic extract of *Tarchonanthus Camphoratus* Leaves L on *Culex tritaeniorhynchus* larva
 Note: (■): Concentration of extract (ppm) and (■): % of larva mortality

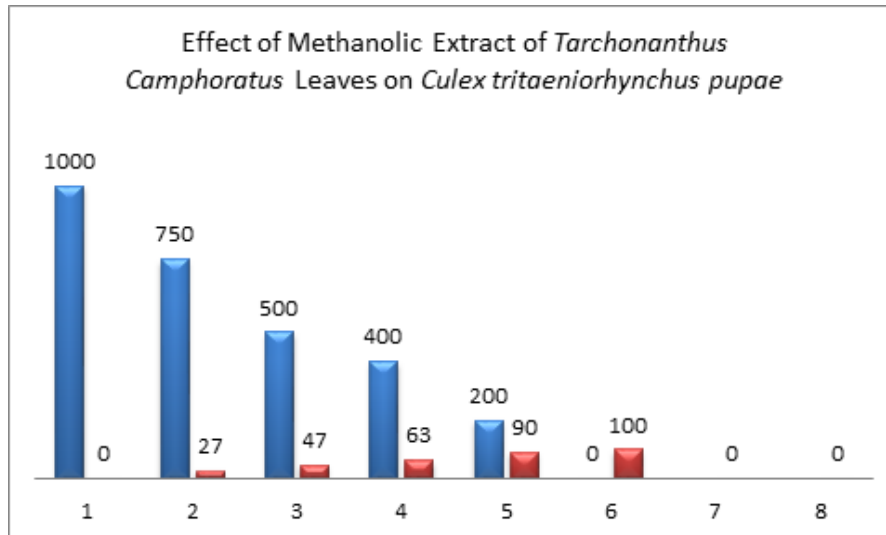


Fig. 3: Biological activity of methanolic extract of *T. Camphoratus* Leaves L on *C. tritaeniorhynchus* pupation
 Note: (■): Concentration of extract (ppm) and (■): % of pupation

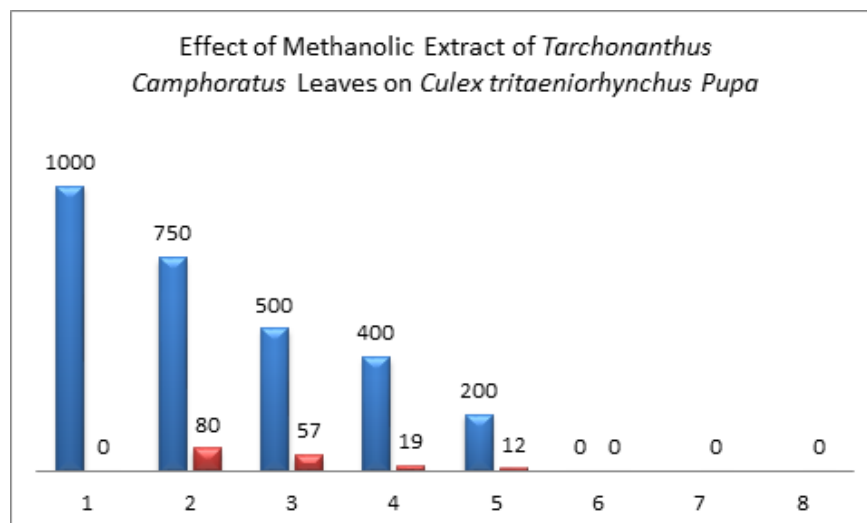


Fig. 4: Biological activity of methanolic extract of *T. camphoratus* Leaves L on *C. tritaeniorhynchus* pupa mortality
 Note: (■): Concentration of extract (ppm) and (■): % of pupa mortality

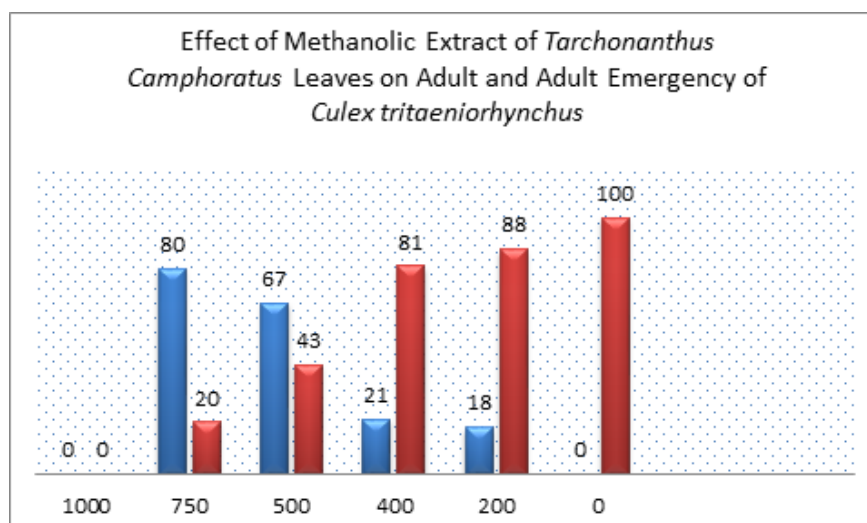


Fig. 5: Biological activity of methanolic extract of *T. Camphoratus* Leaves L on adult and adult emergency *C. tritaeniorhynchus*
 Note: (■): Adult mortality and (■): % of adult emergency

The prepared concentrations showed variations toxic activity against different stages of *C. tritaeniorhynchus*. The maximum larva mortality was found at the concentration of 1000 ppm with 100 ± 0.00 % with duration of 4.33 ± 1.00 d (Table 1 and fig. 6). At 200 ppm the larva mortality reached to 32 ± 3.00 % with duration of 5.67 ± 1.00 d. At 750 ppm the percent of larva mortality was 72 ± 2.00 %. At the concentration of 500 ppm a 50% of larva mortality was exceeded by 58 ± 3.00 % this appeared a significant toxicity of extract (Table 1, Figure 6). The value of LC_{50} against larva mortality was 454.94 (ppm) whereas the value of LC_{90} was 890.19 (ppm) (Table 1 and fig. 11-fig. 12B). The higher percent of pupation was observed at low concentration with 68 ± 3.00 % compared to control (100 ± 0.00 %) (Table 1 and fig. 7). The ethyl acetate extract also represented a lethal toxicity on pupae life, where the highest percent of pupal mortality was showed at 750 ppm with 70 ± 3.00 % (Table 1 and fig. 8). At 500 ppm the pupal mortality had 48 ± 3.00 % did not exceed the 50 % this indicated that there is not significant toxicity found at this concentration against pupae life (Table 1 and fig. 8). The Effect activity of ethyl acetate extract on adult emergency and adult mortality also was reported. A 84 ± 5.00 % of adult emergency was showed at low concentration of 200 ppm compared to control (100 ± 0.00 %) but at high concentration, 750 ppm the adult emergency percent reduced to 30 ± 3.00 % (Table 1 and fig. 9). A 62 ± 4.00 % of adult mortality was observed at high concentration of 750 ppm and at 500 ppm, the adult mortality was 40 ± 5.00 % this value did not exceed the 50 %, this indicated that ethyl extract at this concentration had not significant toxicity against adult mosquito (Table 1 and fig. 9).

Many diseases that infect humans and animals can be transmitted by mosquitoes. *C. tritaeniorhynchus* mosquito is one type of mosquitoes which can transmit several diseases such as West Nile fever, Japanese encephalitis, and Filariasis^[5-7]. These diseases can affect humans health and cause morbidity and mortality. To prevent transmission of such diseases, many chemical insecticides used to control breeding of mosquitoes around the world. But these insecticides can affect environment, animal and humans and create undesirable side effects by continuous using. In recent years, the researchers tend to find a new alternatives of insecticides are harmless and safe to humans and environment. During the past three decades, the manners for controlling insects have been directed to use insecticides from plant origin because

they are safe and friendly to environment, human, and animals. A number of plants had been effected against mosquitoes with safe manner. *T. camphoratus* is one type of medicinal plant has various medicinal applications in traditional healing. It used for many diseases such as asthma, inflammation, rheumatism, bronchitis, heartburn, stomach trouble, toothache, headache, cough, indigestion, cerebral hemorrhage and paralysis^[12-15]. Roark (1947) described around 1200 plant species listed and discussed 344 plant species that exhibited mosquitocidal activity^[21]. *T. camphoratus* exhibits powerful insect repellent action. The life cycle of mosquito starts with laying of its eggs in the water, the eggs hatch and develop to larvae, then to pupae and adult emergence and finally become adult mosquito after that fly out to air. In this study, both the methanol and ethyl acetate extracts of *T. camphoratus* leaves against multiple stages of *C. tritaeniorhynchus* had lethal toxic efficacy. Both the extracts had highest mortality rate against larvae at concentration of 1000 ppm with 100 % (Table 1 and fig. 2 and fig. 6). At 1000 ppm concentration, the pupae, adult emergency and adult were disappeared as a result of complete death of larva. At the concentration of 750 ppm, the methanol extract was more effective against larvae, pupae and adult of mosquito than ethyl acetate extract by percentages of 73 ± 3.00 , 80 ± 1.00 , and 80 ± 3.00 respectively (Table 1 and fig. 2-fig. 5). At 750 ppm of methanol extract, the adult emergency was reduced to 20 ± 1.00 % (Table 1 and fig. 5) compared to ethyl acetate extract effect (Table 1 and fig. 9). The time duration of larva life at concentration of 1000 ppm and 750 ppm of methanol extract had short time compared to ethyl extract (Table 1). At 500 ppm of methanol extract effect, percent of mortality against larva, pupa and adult was exceed 50% (Table 1 and fig. 2 and fig. 4, 5) than ethyl acetate extract effect (Table 1 and fig. 6 and fig. 8, 9). The rate of pupation was increased gradually at the concentrations, 750 ppm, 500 ppm, 400 ppm, and 200 ppm due to decreasing in mortality percent of larvae in both effects of methanol and ethyl acetate extracts (Table 1 and fig. 3 and fig. 7). The values of LC_{50} and LC_{90} for both extracts against larva mortality were approximately similar in effect (Table 2 and fig. 10-fig. 12B). Lethal concentration (LC) 50 % of methanolic extract for kill 50 % of larva is needed at concentration of 519.95 (ppm) and Lethal concentration (LC) 90% for kill 90% of larva is needed at 904.19 (ppm) (Table 2 and fig. 1B2 and fig. 10).

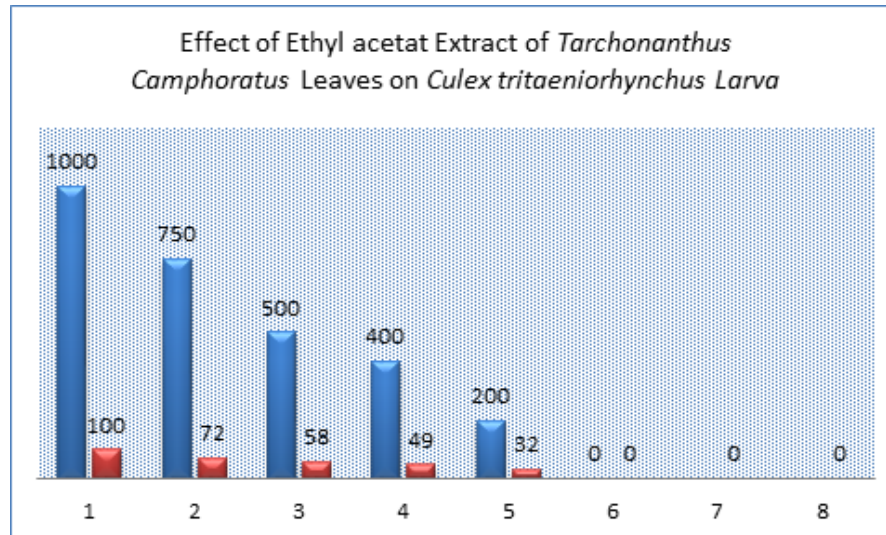


Fig. 6: Biological activity of ethyl acetate extract of *T. camphoratus* Leaves L on *C. tritaeniorhynchus* larva
 Note: (■): Concentration of extract (ppm) and (■): % of larva mortality

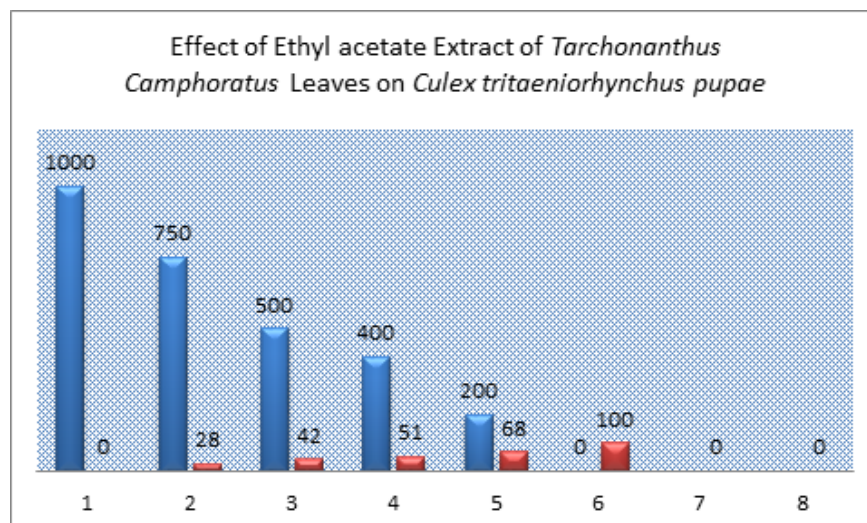


Fig. 7: Biological activity of ethyl acetate extract of *T. Camphoratus* leaves L on *C. tritaeniorhynchus* pupation
 Note: (■): Concentration of extract (ppm) and (■): % of pupation

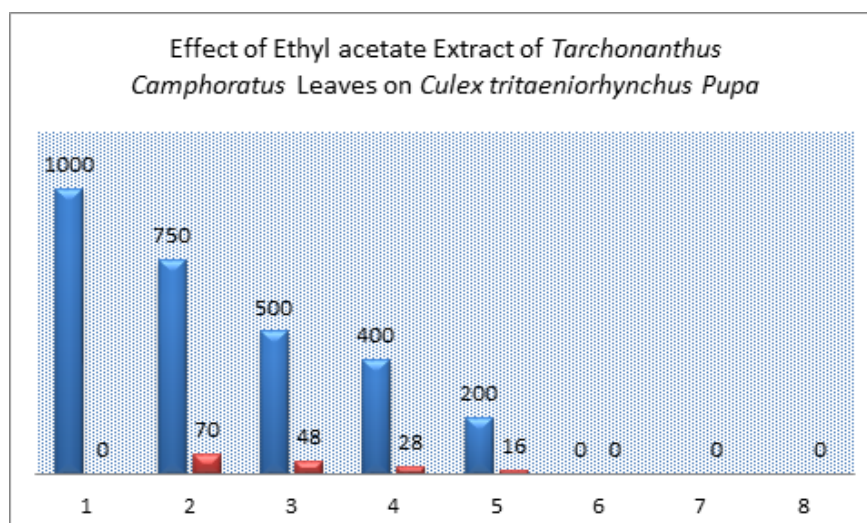


Fig. 8: Biological activity of ethyl acetate extract of *T. camphoratus* Leaves L on *C. tritaeniorhynchus* pupal mortality
 Note: (■): Concentration of extract (ppm) and (■): % of pupal mortality

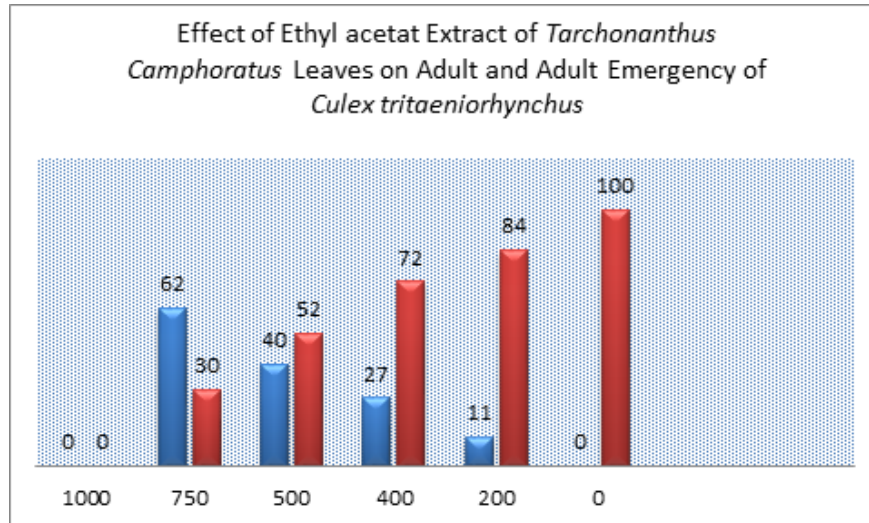


Fig. 9: Biological activity of ethyl acetate extract of *T. Camphoratus* Leaves L on Adult and Adult emergency *C. tritaeniorhynchus*
 Note: (■): % of adult mortality (ppm) and (■): % of adult emergency

TABLE2: LC₅₀ AND LC₉₀ VALUES (PPM) OF METHANOLIC AND ETHYL ACETATE EXTRACTS OF *T. camphoratus* LEAVES AGAINST LARVA MORTALITY OF *C. tritaeniorhynchus* MOSQUITO

Extract	LC ₅₀	LC ₉₀
Methanol	519.95 (ppm)	904.19 (ppm)
Ethyl acetate	454.94 (ppm)	890.19 (ppm)

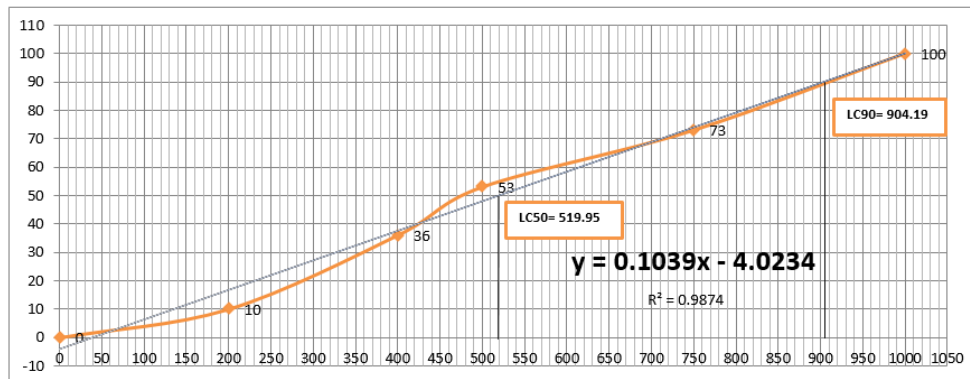


Fig. 10: LC₅₀ and LC₉₀ values (ppm) of methanolic leaves extract of *T. camphoratus* plant against larva mortality of *C. tritaeniorhynchus* mosquito

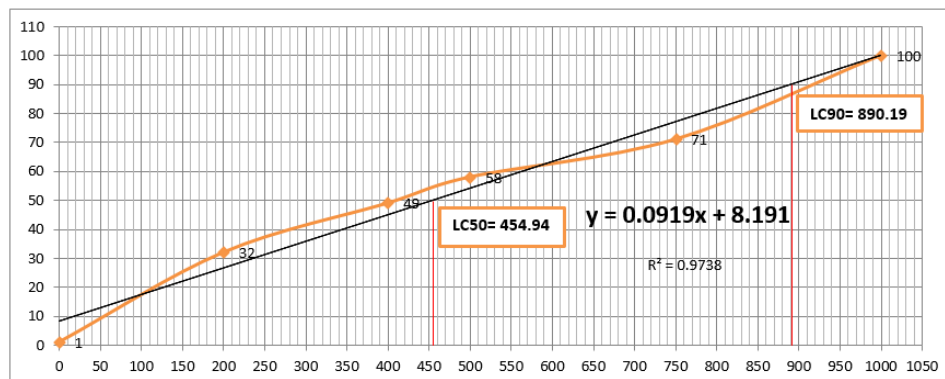


Fig. 11: LC₅₀ and LC₉₀ values (ppm) of ethyl acetate leaves extract of *T. camphoratus* plant against larva mortality of *C. tritaeniorhynchus* mosquito

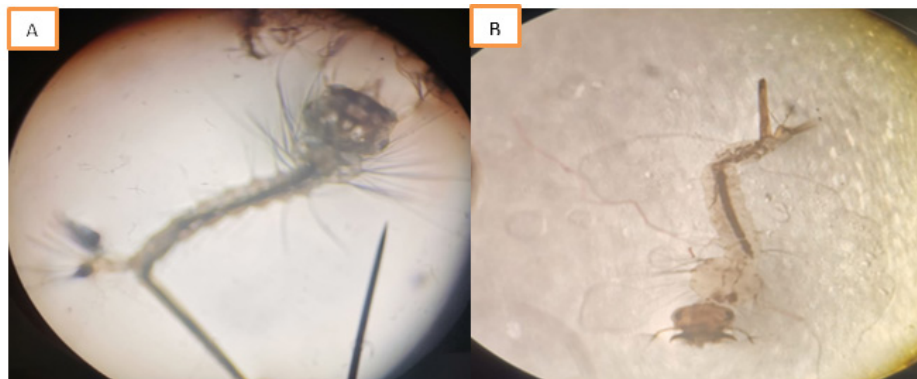


Fig. 12: The effect of methanol and ethyl extracts of *T. camphoratus* leaves on *C. tritaeniorhynchus* larva, (a): Untreated larva and (b): Larva after treatment

However the values of LC_{50} and LC_{90} of ethyl acetate extract against larva were 454.94 (ppm) and 890.19 (ppm) respectively (Table 2 and fig. 12B and fig. 11). In current study, the obtained results indicated that methanolic extract had best efficacy compared to ethyl acetate extract. Additionally, many studies of leaves extract by methanol against mosquitoes life cycle had better effect than ethyl acetate extract^[21-24] this is may be due to the nature and ability of methanol solvent for extraction. The results in this manuscript are proved that the biological activity of methanolic and ethyl acetate leaves extracts of *T. camphoratus* tree exhibited a significant lethal toxicity effect against different stages of *C. tritaeniorhynchus* mosquito. It is suggested that *T. camphoratus* tree could be used as an alternative natural insecticide instead of harmful chemical insecticides for combating and controlling of mosquitoes. Further studies can be conducted such as phytochemical analysis of *T. camphoratus* plant to find the effective derivate against mosquitoes.

The mosquitoes can transmit many diseases to humans and animals which make critical healthy problems around the world. The combating and fighting of mosquitoes became an urgent necessity to reduce prevalence of diseases. The resistant of mosquitoes to chemical insecticides and the harmful effects of these chemical martials make us to tend for create a natural insecticides for fighting mosquitoes. Here in this study, it concluded that the methanolic and ethyl acetate leaf extracts of *T. camphoratus* tree are proved their efficacy against the different stages of *C. tritaeniorhynchus* mosquito which transmits many diseases. These extracts killed the larvae, pupae and adult at various concentrations. The study demonstrates with increasingly likelihood to use this *T. camphoratus* plant as alternative natural insecticides instead of harmful chemical insecticides.

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Conflict of interests:

The authors declared no conflict of interests.

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