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Comparative Studies on the Larvicidal Activity of *Lantana camara* Linn. (Wide Sage) and *Tithonia diversifolia* (Hemsl) A.Gray (Mexican Sunflower) Leaf Extract on *Anopheles* Mosquito Larvae

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Abstract The larvicidal activity of *Lantana camara* and *Tithonia diversfolia* leaf extract against anopheles mosquito larvae was investigated in this study during October 2019 in the Centre for Disease Surveillance (Malaria Research Laboratory) University of Jos. Anopheles mosquito larvae was obtained from stagnant water pond behind Zoology Undergraduate Research Laboratory. Aqueous and methanolic extraction was carried out on the dried and pulverized leaves of both plants. Three different concentrations were used for the treatments which were 100mg/ml, 50mg/ml, and 25mg/ml. 48 containers(specimen bottles) were used with each containing 10 larvae. Each concentration had three replicates and a control also in triplicate. The treatments were observed for 24 hours after which it was observed that all the extracts were effective against the larvae and the difference being only in the lethal time. The mean mortality was highest for *Tithonia diversifolia* aqueous extract followed by *Lantana camara* aqueous extract, after which is *Lantana camara* methanolic extract and then *Tithonia diversifolia* aqueous extract. Mortality of *Anopheles* mosquito larvae exposed to the plant extracts increased with exposure and concentration of extracts. The higher the concentration, the higher the mortality rate of the larvae. Going by the above deductions, it can be rightly put that the effect of the extract is dose and time dependent as evidenced by increase in the percentage mortality with increase in concentration and time. The extracts from both plants contain bioactive compounds against larvae of anopheles mosquito and could be used in stagnant water bodies and drainages which are known to be breeding sites for mosquitoes and the leaves of both plants could be used in the production of mosquito repellants. The study also recommends that both plants be planted around residential areas to prevent breeding of anopheles mosquitoes.

Keywords: *Lantana camara, Tithonia diversfolia, Anopheles mosquito larvae, larvicidal activity, extraction*

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1. Introduction

Malaria is a public health problem most especially in the tropical countries where majority bear the burden of the disease (Multilaria Initiative for Malaria, 2004). It is one of the six killer diseases in the world to-day and it has been estimated that 40% of the world's population is at risk and 500 million people suffer from the disease annually [18]. About two million children, mostly less than five years and pregnant women die from malaria related illness each year and nine out of ten cases are found in Sub-saharan Africa [39]. In most cases, malaria is transmitted through the bites of female Anopheles mosquito. There are more than 400 different species of Anopheles mosquito; around 30 are malaria vectors of major importance. Mosquitoes are accountable for over 300 million clinical cases and one million deaths yearly worldwide. Ineffective measures and the complexity of illness life cycles need integrated approaches to control, eliminate, and eventually eradicate diseases [19]. Mosquitoes are tiny blood-sucking insects that depend on standing water to reproduce. Female mosquitoes must feed on blood to lay eggs. They feed by sticking their mouthparts into the skin of an animal and sucks blood rapidly. More often, they carry viruses that can be transmitted to a person while they are feeding [20].

The female Anopheles mosquito will lay her eggs in a wide range of locations. Malaria mosquito breeding grounds include fresh water or salt-water, vegetative or non-vegetative, shady or sunlit. Ground pools, small streams, irrigated lands, freshwater marshes, forest pools, and any other place with clean, slow-moving water are all considered prime Malaria mosquito breeding grounds for egg-laying. Females, particularly fertilized females, may survive winter by hibernating in caves, which means the malaria breeding cycle can virtually last year-round in some locations. Eggs are capable of surviving cold temperatures; however, freezing usually kills eggs. [21]. Larval counts and density of anopheles mosquitoes are known to be high during rainy seasons and decline during dry seasons. This is obviously due to loss of some habitats and decline in mosquito populations during the dry season. [24].

Like all mosquitoes, anopheles goes through four stages in their life cycles:

Egg, larva, pupa, and imago. The first three stages are aquatic and together last 5-14 days, depending on the species and the ambient temperature. The adult stage is when the female

Anopheles mosquito acts as malaria vector. The adult females can live up to a month (or more in captivity), but most probably do not live more than two weeks in nature [37]

Copulation:

Mosquitoes copulate while flying during the night. It is believed that the pitch of sound produced during flight is higher in females, and this helps the male mosquitoes to locate the female mosquitoes and copulate [14].

Oviposition:

After copulation the female Anopheles lays about 40 to 100 eggs after midnight in standing water of some pond, ditch, pool, puddle, lake, well, water-storage tanks etc., or even in water containers in our houses. A blood-meal by the female is necessary before oviposition. The Female anopheles mosquito lays its eggs singly [14].

Eggs:

Adults lay 50-200 eggs per oviposition. The eggs of Anopheles are smaller, spindle-shaped and black. On each side of its middle, thicker part, the egg bears an umbrella-like membranous structure filled with air and called "air float". These floats give buoyancy to the egg. Eggs are resistant to drying and hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climates [12].

Larva:

Within one to three days, the embryonic development is completed in an egg and a larva, called wriggler, hatches out in water from it.

The mosquito larva has a well-developed head with mouth brushes used for feeding, a large thorax and a nine-segment abdomen. It has no legs. In contrast to other mosquitoes, the Anopheles larva lacks a respiratory siphon, so it positions itself so that its body is parallel to the surface of the water. Larvae breathe through spiracles located on the eighth abdominal segment, so must come to the surface frequently. The larvae spend most of their time feeding on algae, bacteria , and other microorganisms in the surface micro layer. They dive below the surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes [7].

Larvae develop through four stages, or instars, after which they metamorphose into pupae. At the end of each in star, the larvae molt, shedding their exoskeletons, or skin, to allow for further growth [38].

Pupa:

Soon after the fourth moult, the larva becomes inactive, sinks down to the bottom and metamorphosis into a comma-shaped stage called pupa. Unlike the pupa of housefly, the pupa of mosquitoes is without a tough covering and it is as active as the larva. The pupa of Anopheles is greenish grey. Its body is differentiated into two regions—a cephalothorax in the front region and abdomen in the back region. The pupa stage lasts around 2-3 days in temperate areas [17].

Adults

The duration from egg to adult varies considerably among species, and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as five days, but it can take 10-14 days in tropical conditions. Like all mosquitoes, adult Anopheles species have slender bodies with three sections: head, thorax and abdomen [25].

The head is specialized for acquiring sensory information and for feeding. It contains the eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odors, as well as odors of breeding sites where females lay eggs. The head also has an elongated, forward-projecting proboscis used for feeding, and two maxillary palps. These palps also carry the receptors for carbon dioxide, a major attractant for the location of the mosquito's host. The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax [3].

The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal [25].

Some plants possess certain phytochemicals, which are naturally occurring botanicals that possess insecticidal and larvicidal properties. Applications of phytochemicals in mosquito control were in use since the 1920s, but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effect on non-target organisms was appreciated [27].

In this study, the larvicidal activity of *Tithonia diversifolia* (Mexican sunflower) and *Lantana camara* (wide sage) was determined.

***Tithonia diversifolia* (Mexican sunflower)**

The plant *Tithonia diversifolia* belongs to the Asteraceae family commonly known as the Mexican sunflower, tree marigold and or Japanese sunflower. This species (*Tithonia diversifolia)* is a 2-5m tall perennial shrub, which is a native to Mexico and now widely distributed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa. In Kenya, the green biomass of the plant has been found to be an effective source of nutrients for maize [15]. *Tithonia diversifolia* is considered to be a medicinal plant that is widely used in folk medicine to treat various illnesses, it is commonly known as Mexican Sunflower, tree marigold, shrub sunflower or Japanese sunflower (English), "sepeleba"

(Yoruba), pua renga (Cook Island). Ethno botanical surveys have shown that extracts from the plant exhibited antimalaria, antidiarrhoeic, anti-inflammatory, antibacterial, antiproliferation properties and its effectiveness in the treatment of haematomas and wounds had been demonstrated as well [11]. The leaf is reported to contain sequiterpene lactones taginin C as an active component against Plasmodium [11], diversifolin, diversifolin methyl ether and tirotundin as active components against inflammatory activity. In using the plant for treatment, it can be administered in several forms such as oral decoction of the leaves for treatment of hepatitis, diabetes, malaria and pain [16]. External application and infusion of leaves for treatment is also carried out [1]. Previous phytochemical studies of this genus have shown that the major constituent of this plant include three sub types of lactones, flavones, and chromenes [40]; [2].

***Lantana camara* (wide sage)**

Lantana camara Linn (Verbenaceae) is also known as wide sage or lantana weed. It is a hefty extensive evergreen shrub which can grow up to 3 m in height and has a strong scent. It is a perpetual shrub found growing up to 2000 m altitude in tropical, subtropical and clement parts of the world. All parts of this plant have been used conventionally for numerous illnesses all through the world. The leaves of this plant were used as an antibacterial and antihypertensive agent, roots for the treatment of malaria, rheumatism, and skin rashes [34]. Extract from the leaves of *Lantana camara* possessed larvicidal activity while extract from flowers of the plant showed repellent activity against adult mosquitoes [26]. The plant is a gregarious, upright or half climbing shrub with a strong smell. Leaves are egg shaped, five to nine centimetres long, sharp at the tip and curved at the bottom and saw like in the borders. Flowers are pink, orange, yellow, lilac and other shades, according to the diversity and bear in stalked heads which are 2 to 3.5 centimetres in diameter. Fruit is drupaceous, sweet tasting, purple or black, fleshy ovoid and about 5mm long. Phytochemical screening of leaves yielded saponins, flavonoids and glycosides. The larvicidal activity noted was attributed to the phytochemicals. *Lantana camara* has therapeutic value because of the presence of natural agents and greater part of their activity is due to the phytochemicals present in the plant [10].

2. Materials and Method

2.1. Study Area

The research was carried out in Jos North Local Government area of Plateau State. The experiment was carried out in the centre for disease surveillance (Malaria Research Laboratory) university of Jos, Nigeria.

2.1.1. Larva Collection and Breeding

Anopheles mosquito larvae were collected from unpolluted stagnant water pond behind zoology undergraduate research lab University of Jos, Nigeria. The research utilized 480 larvae throughout the experiment. Ten larvae per container was the set-up, consisting of 48 containers. The larvae were placed in the containers, allowing them to disperse on their own just like in their original habitat.

2.1.2. Collection of Plant Material

Fresh and matured leaves of *Lantana camara* and *Tithonia diversifolia* were collected from full grown plants in different areas in the University of Jos Bauchi road campus and Federal College of Forestry Jos, Plateau state. The plant leaves were taken to the herbarium, university of Jos for proper identification by the curator in charge. The leaves were then cleaned and washed with tap water and rinsed with distilled water [26]. The leaves were shade dried at room temperature for 21 days and then the dried leaves were pulverized to fine powder using mortar and pestle [26].

2.1.3. Containers

Forty eight pieces of 20ml specimen bottles were used.

2.1.4. Extraction Process

Hundred grams each of pulverized leaves was used for the extraction using methanol and water as the extraction solvents. The 50 grams of pulverized leaves was introduced into 250ml of methanol and also into 250ml of water and was allowed to stand for 24hours with periodic shaking. Afterwards, the mixture was filtered using Whatman's filter paper and the final filtrates were concentrated using rotary vacuum evaporator and evaporated to dryness [26].

2.1.5. Phytochemical Analysis

Phytochemical screening was carried out on the extracts using standard procedures to identify the constituents as described by [13,29,36].

2.2. Research design

The experimental design used was complete randomized block design. The anopheles mosquito larvae were randomly assigned to treatments, with different concentrations and each concentration had three replicates [32].

2.2.1. Treatments

Three concentrations of 100mg/ml, 50mg/ml and 25mg/ml were prepared using the double fall serial dilution method. Three samples of each concentration were needed in order to obtain three trials or replicates. This was done for each extract. The treatments were administered to 36 of the bottles containing the larvae. The other 12 bottles containing ten larvae each did not have any treatment diluted and served as the control for each extract.

2.2.2. Larvicidal Assay

The treatments were placed in the 36 specimen bottles prepared and were observed until results were gathered. The treatments was observed only for 24hrs and recorded for each two hour interval [19].

2.2.3. Statistical Tool

Analysis of variance (ANOVA) was carried out on the result and the Kaplan Meier survival statistics using graph pad prism version 8.2 was used to statistically analyse the results. Survival analysis is used to analyse data in which the time until the event is of interest. The response is often referred to as failure time, survival time, or event time [6]. The term survival time specifies the length of time taken for failure to occur. The rime from a specified point to the occurrence of a given event, is called the survival time and hence, the analysis is referred to as survival analysis [33].

3. Result and Analysis

3.1. Phytochemical Screening

There is the presence of active phytochemicals like flavonoids, tannins, terpenoids, alkaloids, saponins and glycosides in both plants, with the absence of coumarin in the aqueous and methanolic extract of *Lantana camara* and, the absence of anthraquinone in the aqueous extract of *Tithonia diversifolia* as shown in Table 1 below.

Table 1. Phytochemical Screening of the Plants showing the phytochemicals and bioactive compounds present in the plant.

Phytochemical	*Tithonia diversifolia* (aqueous extract)	*Tithonia diversifolia* (methanolic extract)	*Lantana camara* (aqueous extract)	*Lantana camara* (methanolic extract)
Flavonoids	++	+	++	+
Saponin	++	+	+	+
Glycosides	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	++
Anthraquinones	-	+	+	+
Terpenoid	++	+	++	+
Coumarin	+	+	-	-

Keys
+ Positive
+ + Strongly positive
- Not detected

3.1.2. Mean Mortality of Mosquito Larva

The mean mortality is highest for *Tithonia diversifolia* aqueous extract followed by *Lantana camara* aqueous extract, after which is *Lantana camara* methanolic extract and then *Tithonia diversifolia* aqueous extract. As such, Table 2 shows that the most effective is *Tithonia diversifolia* methanolic extract.

Table 2. Showing the Mean Mortality of Mosquito Larva Exposed to Different Concentration of Plant Extract.

Plant extract	100mg/ml	50mg/ml	25mg/ml
Lantana camara (aqueous extract)	10 ±0.00	9.67 ±0.56	7.33 ±0.56
Lantanacamara (methanolic extract)	9.67 ±0.50	9.00 ±1.00	7.67 ±0.56
Tithonia diversifolia (aqueous extract)	10 ±0.00	10 ±0.00	9.00 ±0.00
Tithonia diversifolia (methanolic extract)	9.67 ±0.50	9.00 ±0.00	7.00 ±1.00

3.1.3. Percentage survival of *anopheles* mosquito larva exposed to *L. camara* aqueous extract

At $p<0.05$, there was a significant difference in the mortality rate of mosquito larva with different concentration and time.

The survival curve of the mortality rate of anopheles mosquito larva exposed to different concentration of *L. camara* aqueous extract shows that at two hours, 50%, 77% and 84% of the larvae died at 25mg/ml, 50mg/ml and 100mg/ml respectively. At the end of six hours, all the mosquito larvae at 100mg/ml had died, with survival of 3.3% at 50mh/ml and 26.67% at 25mg/ml. For the lethal time (LT_{50}), it took one hour ten minutes, one hour thirty minutes, and two hours to have 50% larva mortality at 100mg/ml, 50mg/ml and 25mg/ml respectively (Figure 2).

The graph shows probit mortality plotted against log concentration and the LC_{50} evaluated from the graph is 17.38 (Figure 1).

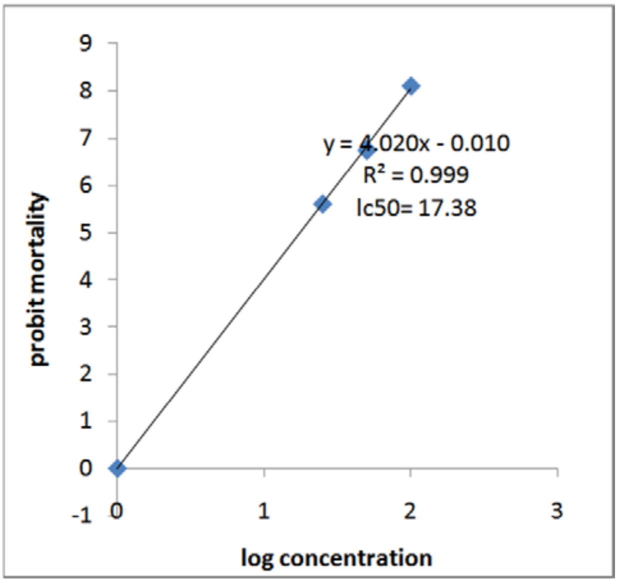

Figure 1. Showing the lethal concentration of *Lantana camara* (aqueous extract) (LC_{50}= 17.38)

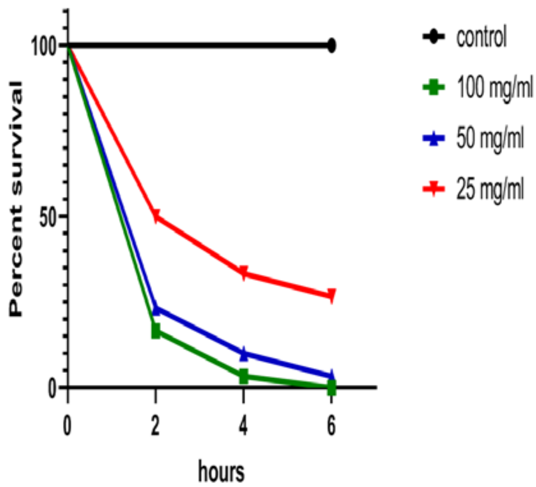

Figure 2. Showing the Percentage Survival of *Anopheles* Mosquito Larva Exposed to Different Concentration of *Lantana camara* Aqueous Extract.

3.1.4. Percentage survival of *anopheles* mosquito larva exposed to *L. camara* methanolic extract

At $p<0.05$, there was a significant difference in the mortality rate of mosquito larva with different concentration and time.

The survival curve of the mortality rate of anopheles mosquito larva exposed to different concentration of *L. camara* methenolic extract shows that at two hours, 44%, 77%, and 85% of the larvae died at 25mg/ml, 50mg/ml and 100mg/ml respectively. At the end of six hours, 3.1%, 10% and 23.3% of the larva survived at 100mg/ml, 50mg/ml, and 25mg/ml respectively. For the lethal time (LT_{50}), it took one hour nine minutes, one hour thirty minutes and two hours forty five minutes to have 50% larva mortality at 100mg/ml, 50mg/ml and 25mg/ml respectively (Figure 4).

The graph shows probit mortality plotted against log concentration and the LC_{50} evaluated from the graph is 22.91 (Figure 3).

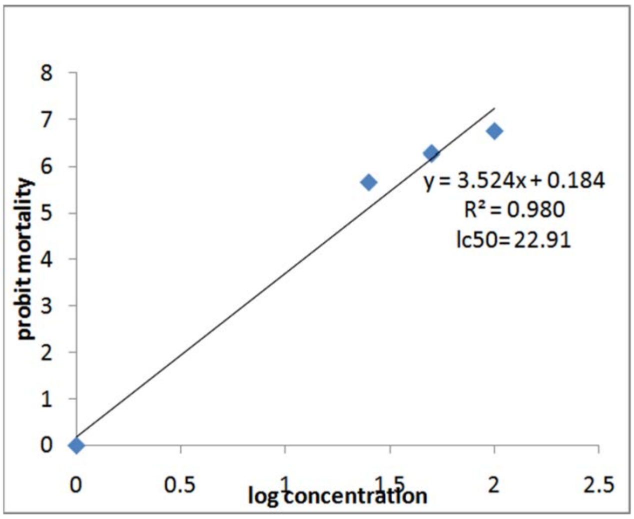

Figure 3. Showing the lethal Concentration of *Lantana camara* (methanolic extract) (LC_{50}= 22.91)

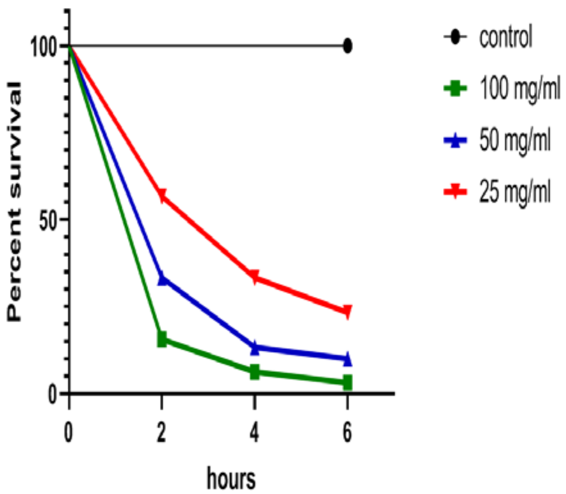

Figure 4. Showing the Percentage Survival of *Anopheles* Mosquito Larva Expose to Different Concentration of *Lantana camara* Methanolic Extract.

3.1.5. Percentage survival of *anopheles* mosquito larva exposed to *T. diversifolia* aqueous extract

At $p<0.05$, there was a significant difference in the mortality rate of mosquito larva with different concentration and time.

The survival curve of the mortality rate of anopheles mosquito larva exposed to different concentration of *T. diversifolia* aqueous extract shows that at two hours, 64%, 75%, and 87% of the larvae died at 25mg/ml, 50mg/ml and 100mg/ml respectively. After four hours, all the larvae at 100mg/ml had died and similarly, after six hours, all the larvae at 50mg/ml had died with 10% survival at 25mg/ml.

For the lethal time (LT_{50}), it took one hour thirty minutes, one hour twenty-five minutes and one hour fifty minutes to have 50% larva mortality at 100mg/ml, 50mg/ml and 25mg/ml respectively (Figure 6).

The graph shows probit mortality plotted against log concentration and the LC50 evaluated from the graph is 13.50 (Figure 5).

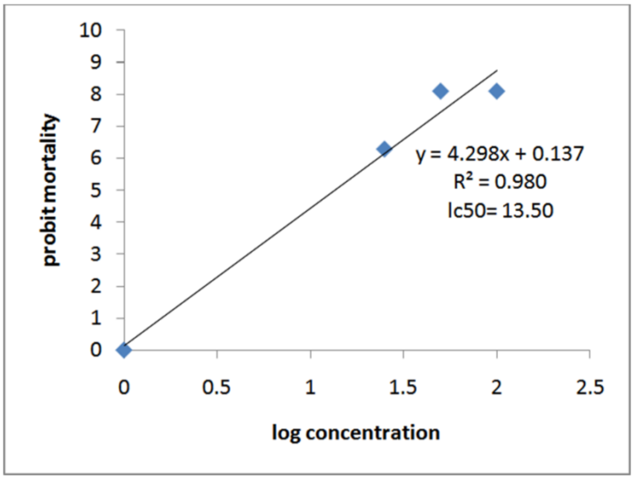

Figure 5. Showing the lethal concentration of *Tithonia diversifolia* (aqueous extract) (LC_{50}= 13.50)

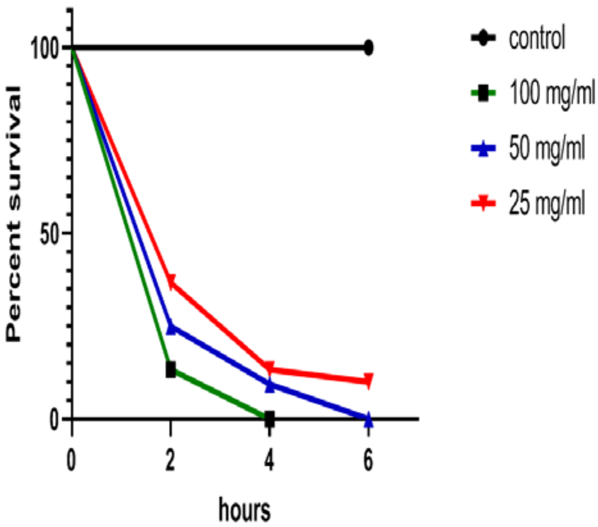

Figure 6. Showing the Percentage Survival of *Anopheles* Mosquito Larva Exposed to Different Concentration of *Tithonia diversifolia* Aqueous Extract.

3.1.6. Percentage survival of *anopheles* mosquito larva exposed to *T. diversifolia* methanolic extract

At $p<0.05$, there was a significant difference in the mortality rate of mosquito larva with different concentration and time.

The survival curve of the mortality rate of anopheles mosquito larva exposed to different concentration of *T. diversifolia* methanolic extract shows that at two hours, 40%, 65%, and 87% of the larvae died at 25mg/ml, 50mg/ml and 100mg/ml respectively. At the end of six hours, 3.3%, 9.67% and 30% of the larvae survived at 100mg/ml, 50mg/ml and 25mg/ml respectively. For the lethal time (LT_{50}), it took one hour twenty minutes, one hour forty minutes and three hours twenty seven minutes to have 50% larva mortality at 100mg/ml, 50mg/ml and 25mg/ml respectively (Figure 8).

The graph shows probit mortality plotted against log concentration and the LC50 evaluated from the graph is 28.84 (Figure 7).

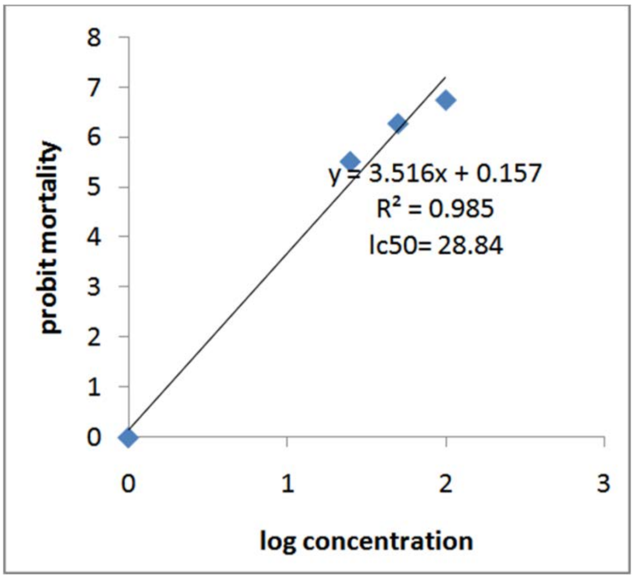

Figure 7. Showing the lethal concentration of *Tithonia diversifolia* (methanolic extract) (LC_{50}= 28.84)

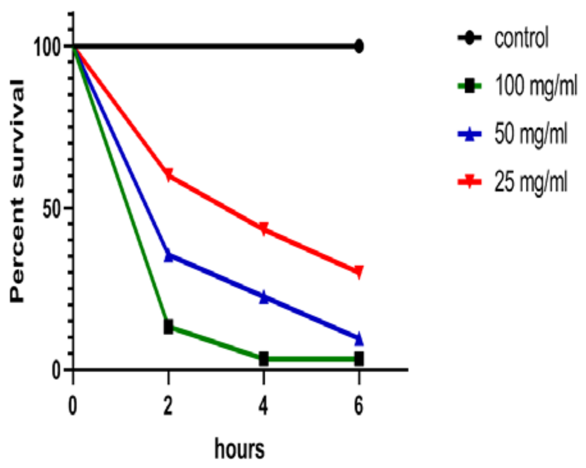

Figure 8. Showing the Percentage Survival of *Anopheles* Mosquito Larva Expose to Different Concentration of *Tithonia diversifolia* Methanolic Extract.

4. Discussion and Conclusion

4.1. Discussion

The larvicidal activities of aqueous and methanolic leaf extracts of *Tithonia diversifolia* and *Lantana camara* showed that the plant extracts have insecticidal properties on the targeted organism. Mortality of *Anopheles* mosquito larvae exposed to the plant extracts increased with exposure and concentration of extracts. The higher the concentration, the higher the mortality rate of the larvae. Going by the above deductions, it can be rightly put that the effect of the extract is dose and time dependent as evident by increase in the percentage mortality with increasing concentration and time. This result is in agreement with the works of [30] and [8] which reported that larvae of *Culex quinquefasciatu* exposed to extracts of *Nerium indicum* and *Euphorbia royleana* showed high mortality with increased time of exposure and concentration of extracts. Mortality of Anopheles mosquito larvae exposed to the plant extracts increased with exposure and concentration of extracts as was also reported for larvae of *Anopheles gambiae* exposed to extracts of *Anarcardium occidentale* [22]. The survival curves in the result (Figure 3, Figure 5, Figure 7 and Figure 8 above) shows that the aqueous extract of *Tithonia diversifolia* had the highest larva mortality, followed by that of the aqueous extract of *Lantana camara*, followed by the methanolic extract of *Lantana camara* and then the methanolic extract of *Tithonia diversifolia*. The lethal concentrations LC_{50} (Figure 2, Figure 4, Figure 6 and Figure 8 above) shows that the aqueous extract of *Tithonia diversifolia* has the lowest LC_{50} which is 13.50 followed by the aqueous extract of *Lantana camara* which is 17.38. The methanolic extract of *Lantana camara* has a higher LC_{50} which is 22.91 and the methanolic extract of *Tithonia diversifolia* has the highest LC_{50} which is 28.84.

The presence of active phytochemicals like alkaloids, terpenoids, flavonoids, tannins and saponins in the plants account for the insecticidal and larvicidal properties of the plants (Pedro *et al*., 2014). All the plant extracts showed larvicidal activity against *Anopheles* mosquito larvae at 0.05 level of significance. However, the aqueous leaf extract of *Tithonia diversifolia* was the most effective mosquito larvicide which is manifested by the highest percentage mortality and lowest LC_{50}. The high larvicidal property of the aqueous extract of *Tithonia diversifolia* leaves is supported by the abundance of phytochemicals which show synergistic effects in terms of larvicidal action to mosquito larvae [5].

4.1.2. Conclusion

Lantana camara and Tithonia diversifolia are plants known for their insecticidal properties against mosquito larvae [41]. These plant extracts are also environmentally friendly as opposed to their chemical counterpart that can be very hazardous to the environment. With *Tithonia diversifolia* aqueous leaf extract, a significantly 100% mortality of *Anopheles* was recorded after 4 hours at 100mg/ml as compared to other treatments.

4.1.3. Recommendation

From this study, it is concluded that *T. diversifolia and L.camara* has potential bioactive compounds against larvae of *Anopheles* mosquito, and that the extract from the plant could be used in stagnant water bodies and drainages which are known to be breeding sites for mosquitoes. These extracts can be used to flush drainages and the plants can also be planted around residential areas to prevent breeding of these anopheles mosquitoes. However, further research on the bioactive compounds found in the flowers, bark, root and stems of the plant are highly recommended as this study focuses on the leaves of the plant only. Also, more research work should be done on the toxicity of *Tithonia diversifolia and Lantana camara.*

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