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BIOSYNTHESIS AND CHARACTERISATION OF SILVER (Ag) NANOPARTICLES USING *TAGETES ERECTA* LEAVES AND ITS LARVICIDAL EFFICACY AGAINST *ANOPHELES* MOSQUITOES

[1]*AYIM, T. P., [1]YORIYO, K. P., [2]AYIM, P., [3]AYIM, J. O. and OMBUGADU, A.[3]

[1]Department of Biological Science, Faculty of Science, Gombe State University, Gombe, Gombe State, Nigeria.

[2]Department of Chemistry, Faculty of Science, Gombe State University, Gombe, Gombe State, Nigeria.

[3]Department of Zoology, Faculty of Science, Federal University of Lafia, Lafia, Nasarawa State, Nigeria.

Corresponding Author: tessyamolegie@gmail.com

ABSTRACT

Vector control is an essential requirement in any attempt to curtail vector-borne diseases like; malaria, filariasis, dengue etc. that are particularly transmitted by different species of mosquitoes. Sequel to the above, the study evaluated the biosynthesized silver nanoparticles (AgNPs) and the aqueous extract of *T. erecta* for its efficacy against *Anopheles* mosquitoes following the World Health Organization guidelines for laboratory testing of mosquito larvicides. Synthesized silver nanoparticles (AgNPs) were characterized by UV-visible, FTIR spectroscopy and SEM. Larvae of mosquitoes were collected from their original breeding site between the months of June and July 2021. Mature fresh leaves of *T. erecta* were collected from Igwo village, Obudu Local Government of Cross River State, Nigeria. The leaves were cleaned, washed and cool-dried for two weeks at room temperature. The larvae were exposed to 20, 40, 60, 80 and 100 mg/ml concentrations at 24-, 48- and 72-hours exposure period. *Anopheles* larvae were susceptible (100 %) to both the aqueous extract and the biosynthesized silver nanoparticles (AgNPs) of *T. erecta* plant at the highest concentrations (100 mg/ml). There was a very high significance ($P<0.05$) in the mortality rates of the larvae of mosquitoes in relation to concentrations. LC50 and LC90 values of the aqueous extract for 72 hours were 5.13 mg/ml and 28.18mg/ml while the LC50 and LC90 values of the biosynthesized silver nanoparticles (AgNPs) for 72 hours were 12.59 mg/ml and 44.67 mg/ml. Hence, the aqueous extract revealed a superior efficacy against the larvae of *Anopheles* mosquitoes. The study, therefore, supports the use of the extracts of *T. erecta* as alternatives to synthetic insecticides in the control of *Anopheles* mosquitoes.

Keywords: Biosynthesis, Characterization, Silver Nanoparticles (AgNPs), Leaves, *Tagetes erecta,* Larvicidal efficacy, *Anopheles* mosquito.

INTRODUCTION

Vector-borne diseases have over the years remained the pestilence of man and animals resulting to over one million deaths each year (WHO, 2014). A good number of these diseases are vectored by blood sucking insects that are known to ingest pathogenic organisms from infected individuals during a blood meal and subsequently inoculate the pathogen into an uninfected individual during their next blood meal. Mosquitoes among other biological vectors are well-known vectors that have demonstrated such mode of feeding and transmission (WHO, 2017). It is in this regard that many insect-borne disease transmission of public health concern is linked to mosquitoes, including Malaria, dengue fever, lymphatic filariasis, yellow fever and

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Chikungunya. Consequently, mosquito vectors have through their wide range transmission coverage afflicted populace of many societies, especially those of the sub-tropical and the tropical regions. In 2019 for example, malaria ravaged about 87 countries with an estimated 229 million cases and about 409,000 death cases were recorded worldwide. The WHO African region carries a disproportionately high share of the global malaria burden. This region recorded about 94% of malaria cases and resultant deaths. In this same year, six countries accounted for almost half of the total malaria cases globally: Niger (4 %), Mozambique (4 %), Burkina Faso (4 %), United Republic of Tanzania (5 %), the Democratic Republic of Congo (11 %) and Nigeria (23 %). Children under 5 years of age remain the most vulnerable group affected by malaria. This group accounted for 67 % (274,000) of malaria deaths in the year 2019 (WHO, 2021). Emphatically, Nigeria suffers a great deal of malaria burden, with about 51 million cases and 207,000 deaths reported yearly. Malaria disease accounts for 60 % of outpatient visits to hospitals and have led to about 11 % maternal mortality and 30 % child mortality, especially among 0-5 years of age (WHO, 2019).

Vector control of mosquitoes has long been a critical part of the global strategy to manage mosquito-related diseases, and insecticides are the most important component in this effort. Pyrethroids are the most widely used insecticides for indoor spraying against mosquitoes worldwide, owing to their efficacy and safety. Even so, this insecticide partially controls the mosquito population only since it eliminates the adult flying insects but does not eliminate the breeding places. Pyrethroids are the only chemicals used in the treatment of mosquito nets (WHO, 2007; WHO, 2009), which is considered as an important tool for the prevention of malaria in Africa. However, mosquito-borne diseases are still on the increase, largely because of the insecticide resistance developed in mosquito vectors and the drug resistance of pathogens (Yan *et al*., 2012). Research on insecticide resistance in mosquitoes has proliferated since the 1950s, when the first report of mosquito resistance to chlorinated-hydrocarbon insecticides was published (Gjullen and Peters, 1952). Investigators worldwide are attempting to elucidate the mechanisms governing the development of insecticide resistance, a vital first step toward the creation of new, and more effective strategies to prevent resistance, control resistant mosquitoes, and, ultimately, reduce the prevalence of mosquito-borne diseases.

The utilization of natural products has delivered a potential strategy for identifying effective insecticide in the control of insects (Wafaa *et al.*, 2017). Plants produce numerous chemicals, many of which have medicinal and insecticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programs (Kumar *et al*., 2012). It is thus agreeable that the use of plant materials as insecticides is increasingly receiving attention as they produce no adverse effect on non-target organisms. The African marigold, botanically identified as *Tagetes erecta* is an ethnobotanically known plant that also possessed insecticidal characteristics (Gupter and Vasudeva, 2012). It shows different pharmacological activities like anti-bacterial activity, anti-microbial activity, hepatoprotective activity, insecticidal activity, mosquitocidal activity**,** nematicidal activity, wound healing activity, antioxidant and analgesic activity and Larvicidal activity (Priyanka *et al.*, 2013). A variety of chemical constituents have been isolated from *Tagetes* species and their structures elucidated. They belong to the classes as essential oils, thiophenes, flavonoids, carotenoids and phenolic compounds which have been

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describe as the phyto-active ingredients (Gupter and Vasudeva, 2012).

In recent days, the use of biosynthesized silver nanoparticles from plant extracts has gained momentum as bio control agents against mosquitoes and microbes. Silver nanoparticles are emerging as one of the fastest growing material due to their unique physical, chemical and biological properties, small size and high specific surface area (Sarah *et al.*, 2012). Vector control is an essential requirement in epidemic disease situations, it is therefore imperative to develop new and improved mosquito control measures that are economical and effective and also safe for non-target organisms and the immediate environment. Consequently, this study seeks explore alternative means of controlling and eradicating mosquito population with the focus on the larval forms, with the use of biologically synthesized silver nanoparticles of *Tagetes erecta* plant extracts.

MATERIALS AND METHODS

Plant Collection and Preparation

Matured fresh leaves of *T. erecta* were collected from Igwo village in Obudu Local Government Area, Cross River State. The authentication of the plant was done in the Department of Botany, Gombe State University, Gombe state, Nigeria.

The leaves of *T. erecta* were cleaned and washed of any visible debris and cool-dried in shade for two weeks at room temperature. The dried leaves were crushed and pounded using mortar and pestle, and it was sieved to obtain a fine powder, using a 0.9 mm mesh-sized sieve. The plant was prepared by warm maceration (Handa *et al*., 2008).

Biosynthesis of Silver (Ag) Nanoparticles

The methods described by Parashar *et al*. (2009) was used with some modifications. 20 g of finely cut leaves were placed in 300 mL of distilled water and boiled for 40 minutes with continuous stirring, then it was allowed to cool and was filtered into a conical flask using a Whatman filter paper No. 1. 5 mL of the plant extracts and 25 mL of the aqueous Silver Nitrate ($AgNO_3$) solution 1:5 W/V were measured and mixed; stirred for three hours (3 Hr) till the colour of solution changed from green to yellowish, thus indicating the formation of nanoparticles.

Characterization of Silver (Ag) Nanoparticles of *T. erecta*

The characterization of silver nanoparticles was done at Spectral Laboratory and Services, Tudun-wada, Kaduna South, Kaduna State. The synthesized silver nanoparticles (AgNPs) were identified by Ultraviolet-Visible (UV/VIS) spectroscopy (spectral scanning multimode reader, software version 2.4.5 Research Edition) at a slit width of 1 nm in the range of 200-600 nm. A filtrate containing AgNPs was used for Scanning electron microscopy (SEM), Energy dispersive X-ray spectrometry (EDX) and Fourier transform infrared (FTIR) spectrometry.

The morphology of the synthesized AgNPs was detected by a scanning electron microscope (SEM). The FTIR spectrometry was carried out using Perkin Elmer (Spectrum version 10.03.09) Spectrum One (Waltham, MA, USA) in the diffuse reflectance mode at a resolution of 4000-400 cm^{-1} in KBr pellets (Sutthanont *et al*., 2019).

Mosquito Larvae Collection

Larvae of mosquitoes were collected between the months of March and April 2021. *Anopheles* larvae were collected from rock pools and craters made because of quarrying activity and other construction activities around Gombe Metropolis. The collected larvae were colonized in the Department of Zoology Laboratory, Gombe State University, Gombe. Species identification was done with

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the help of a standard identification key (Potter and Beavers, 2005). The larvae were fed by adding finely ground powdered yeast on the surface of the water.

Preparation of Stock and Working Solution of Aqueous extracts of *T. erecta*

The stock solution was prepared using methods as suggested by Ibrahim, (2009) with some modifications, where 1 g of the aqueous extract was weighed using an Electronic Weighing Balance. The weighed extract was then added to 500 mL of distilled water and allowed to stand for 24 hours, with occasional agitation. The suspension was subsequently filtered into a volumetric flask using Whatman No. 1 filter paper and the residue washed three times with distilled water from the filter paper and added to the filtered portion in the volumetric flask. Final volume was adjusted to 1000 mL by adding distilled water to make the stock solution of 1000 mg/mL. From the stock solution, working solutions were prepared to obtain concentrations of 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL. Working solutions (diagnostic doses) was prepared using the formula:

$C_1V_1 = C_2V_2$

Where:

C_1= Stock concentration (beginning concentration)

V_1= Volume of stock required to prepare new solution

C_2= Concentration of new or working solution (desired concentration)

V_2= Volume of new solution desired

Larvicidal Assay

Bioassay method was carried out according to the recommended protocol of World Health Organization (2013) to susceptibility of first and second instar larvae of *Anopheles* exposed to five concentrations (20 mg/ml, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL) of the treatments (synthesized silver nanoparticles and the aqueous extracts of *T. erecta*). Four replicates were kept for each concentration, with a control for each experiment, and in each replicate, 20 early first and second instar larvae were released into disposable 250 mL bowls containing 100 mL of distilled water.1 mL of the solution of each diagnostic dose were dispensed in each bowl with the help of a syringe; the control were prepared as 100 mL distilled water only. Knockdown rate of larvae was recorded after 10 mins, 15 mins, 20 mins, 30mins, 40 mins, 50 mins and 60 mins. Larval mortality was recorded at 24-, 48- and 72-hours exposure period. Mortality was confirmed by gentle prick on the abdomen of the larvae with a needle (WHO, 2013a). Prevailing temperature and relative humidity values were observed and recorded accordingly, using a Thermometer and a Hygrometer.

Data Analysis

Data obtained was analyzed using R Console software (Version 4.1.1). Proportions of mortality rate of *Anopheles* larvae in relation to concentrations of extract and as well as the efficacy between the different synthesized silver nanoparticles and the aqueous extracts of *T. erecta* that was used for larvicidal test were compared using Pearson's Chi-square test. The *p*-values < 0.05 was considered statistically significant.

Test Analysis

The interpretation of the mortality rate of *Anopheles* larvae according to World Health Organization (2013) is as seen below:

1. Mortality rate between 98 – 100 % within the diagnostic time indicates susceptibility.

2. Mortality rate between 80 – 97 % suggest possible resistance.

3. Mortality rate < 80 % indicates resistance.

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Determination of Percentage Mortality

Mortality was corrected using Abbott's formula. Non-mobile and moribund larvae were recorded as dead. The mortality data was further subjected to Probit Analysis for estimating LC_{50} values using Finney method (Finney, 1971) as:

$$\% \text{ mortality} = \frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \times \frac{100}{1}$$

Correction for Mortality

The Corrected percentage mortality was used when a proportion of the larvae in the control batches died during the experiment. To correct this, Abbott's formula was used (Abbott, 1925), represented as:

$$P = \frac{\%Po - \%Pc}{100 - Pc} \times \frac{100}{1}$$

Where:
P = Corrected Mortality
Po = Observed Mortality
Pc = Control Mortality, all expressed in percentages

Determination of LC_{50} and LC_{90}

The 24-, 48- and 72-hours lethal concentration values (LC_{50}) was determined by Probit analysis as described by Finney (1971). Microsoft Excel regression analysis was employed, using the regression equation ($Y = a + bx$). Percentage mortalities were converted to probit by looking up the percentage in Finney's table, while calculating the log of concentrations. Regression line were derived by plotting a graph of probit versus the log of concentrations. Calculation using the regression equation was done after substituting the probit value of 5 for y-axis and values for slope and intercept on the x-axis. Taking the log inverse of the calculated value on the x-axis gave the LC_{50} (Same procedure for LC_{90}).

RESULTS

UV-visible Spectrophotometry Analysis

The Figures (1 and 2) shows the results obtained from the UV visible absorption spectra of the aqueous extract and synthesized silver nanoparticles using *Tagetes erecta* leaves.

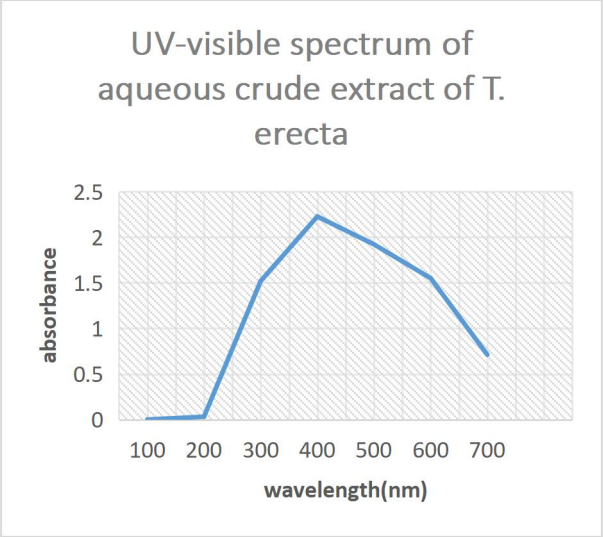

Figure 1: UV- Vis. absorption spectrum of aqueous extract of *Tagetes erecta* leaves

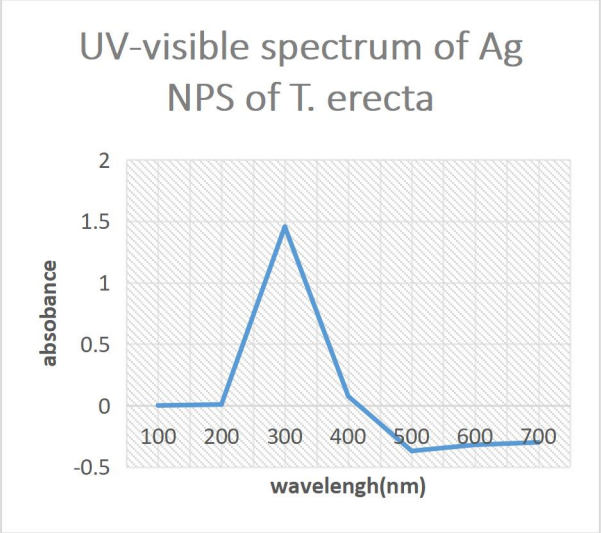

Figure 2: UV-Vis. absorption spectrum of synthesized silver nanoparticles of *Tagetes erecta* leaves

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Fourier Transmission Infrared (FTIR) Analysis

Figure 3 and 4 represents the results obtained from the FTIR analysis of the aqueous extract of *Tagetes erecta* leaves and synthesized silver nanoparticles using *Tagetes erecta* leaves (Table 1 and 2).

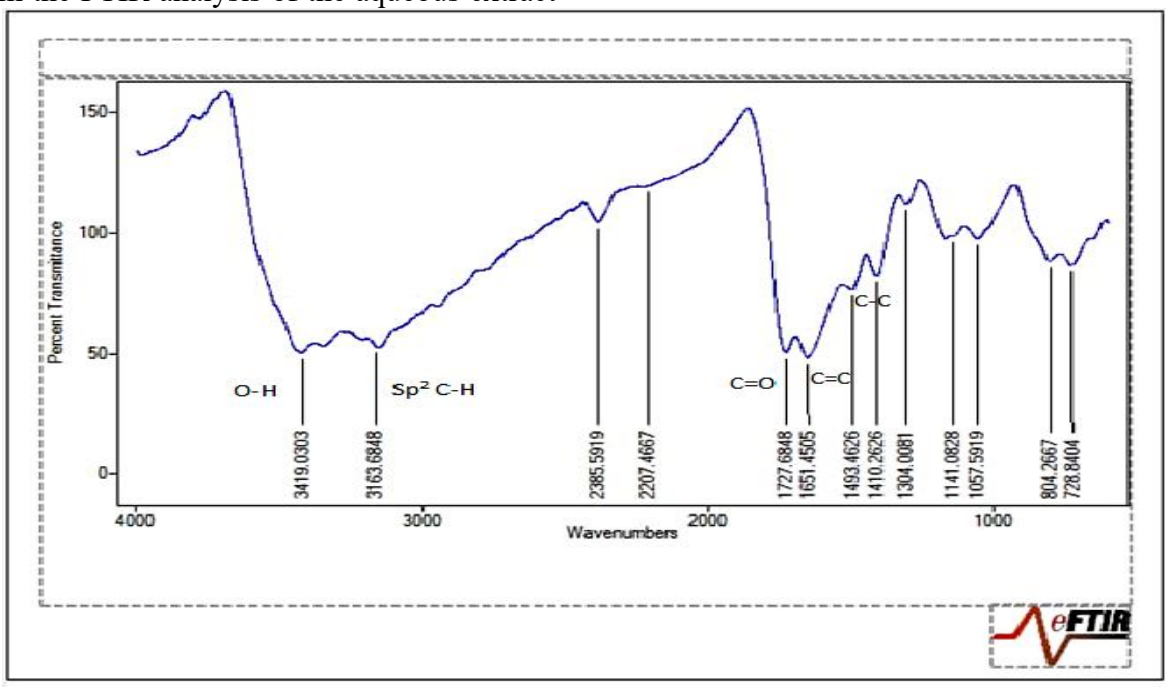

Figure 3: FTIR spectrum of aqueous extract of *Tagetes erecta* leave

Table 1: FTIR result for the leaf extract of *Tagetes erecta*

S/N	**Absorption Bands**	**Functional groups**
1	3419 cm^{-1}	O-H stretch (due to Carboxylic)
2	3163 cm^{-1}	Sp^2C-H stretch
3	1651cm^{-1}	C=C stretching
4	1727cm^{-1}	C=O (carbonyl stretch)
5	1410cm^{-1}	C-C stretching

Table 2: FTIR result of silver nanoparticle synthesized using *Tagetes erecta*

S/N	**Absorption Bands**	**Functional groups**
1	Around 3400cm^{-1}	-O-H stretch (due to carboxylic)
2	3170cm^{-1}	Sp^2 C-H stretching
3	1725cm^{-1}	-C=O (carbonyl stretch)
4	1649cm^{-1}	C=C stretching
5	1143cm^{-1}	C-O stretching
6	1051cm^{-1}	Phenolic or alcoholic group

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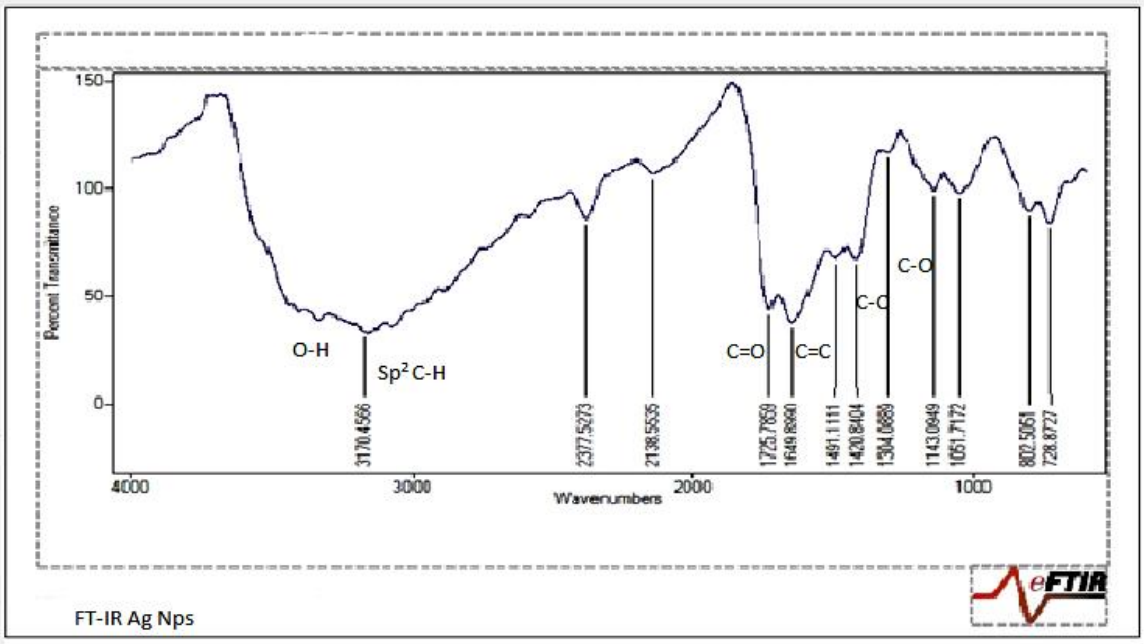

Figure 4: FTIR spectrum of synthesized silver nanoparticles using *Tagetes erecta* leaves

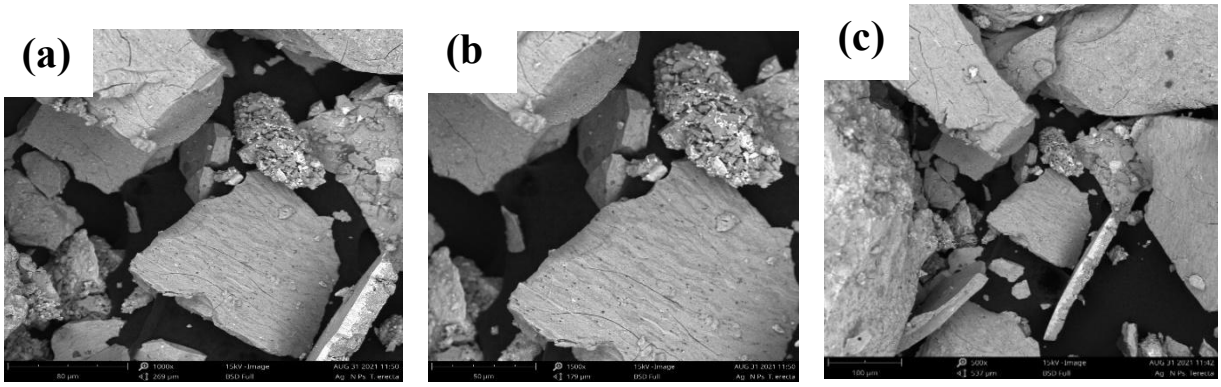

Figure 5: SEM image of synthesized AgNPs (a) 50 µm, **(b)** 80 µm and **(c)** 100 µm

Table 3. Elemental Composition of Biosynthesized AgNPs

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	48.97	14.21
8	O	Oxygen	32.26	12.48
82	Pb	Lead	7.16	35.86
47	Ag	Silver	6.91	18.03
91	Pa	Protactinium	1.34	7.51
84	Po	Polonium	1.15	5.80
14	Si	Silicon	0.94	0.64
86	Rn	Radon	0.85	4.56

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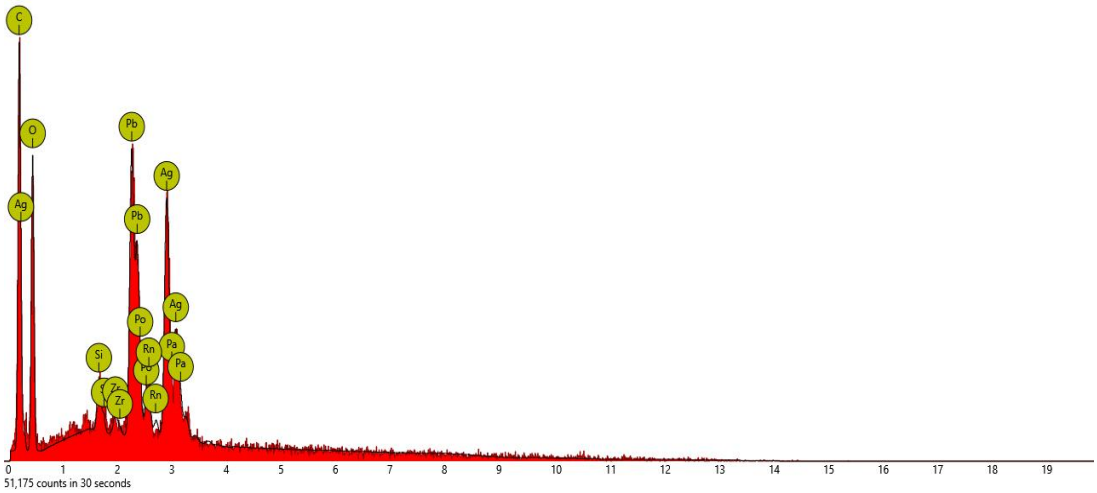

Figure 6: EDX spectrum of biosynthesized AgNP

Mortality Rate of the Larvae of *Anopheles* Mosquitoes Exposed to the Aqueous Extract of *T. erecta* in relation to time

The *Anopheles* larvae exposed to 100 mg/mL of the aqueous extract of *T. erecta* had the highest mortality rate of 97 % at 24 hours exposure period followed by 80 mg/mL which recorded a mortality rate of 93% while no mortality (0.00 %) was observed in the control (Table 4). Hence, there was a very high significant difference ($\chi^2 = 91.778$, df =5, $P < 0.0001$) in the mortality rate of *Anopheles* larvae in relation to the concentration of *T. erecta* at 24 hours. The mortality observed at 24 hours exposure time suggest a possible resistance of the larvae to the aqueous extracts.

Mortality rate of *Anopheles* larvae was the highest (100 %) at 100 mg/mL and mortality was the least (5 %) in the control (0 mg/mL) at 48 hours exposure time. The mortality rates at 48 hours exposure period showed a very high significance ($\chi^2 = 83.115$, df = 5, $P < 0.0001$) across concentrations. Mortality observed after 48 hours was within the diagnostic time, thus the larvae were susceptible to the extract.

At 72 hours exposure time, mortality rate of *Anopheles* larvae was the highest (100 %) at 100 mg/mL and mortality was the least (10 %) in the control (0 mg/mL). Therefore, the mortality in relation to the concentrations of *T. erecta* aqueous extracts also showed a very high significant difference ($\chi^2 = 73.47$, df =5, $P < 0.0001$). Again, mortality at 72 hours exposure time for the concentrations 20, 40, 60 and 80 mg/mL suggest possible resistance of the larvae to the extract. However, at 100 mg/mL for 72 hours, the larvae were susceptible to the aqueous extracts.

Table 4: Percentage Mortality of *Anopheles* Larvae Exposed to the Aqueous Extracts of *T. erecta* in Relation to Concentrations over Time

Exposure time (hr)	Corrected Mortality Across Concentrations (mg/ml)						χ^2	df	P-value
	0	20	40	60	80	100			
24	0	78	87	90	93	97	91.778	5	$p<0.05$
48	5	89	91	91	93	100	83.115	5	$p<0.05$
72	10	91	91	91	93	100	73.479	5	$p<0.05$

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Mortality Rate of *Anopheles* larvae Exposed to the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta* in Relation to Time

Anopheles larvae exposed to 100 mg/mL of the biosynthesized silver nanoparticles (AgNPs) of *T. erecta* had the highest mortality (100 %) at 24 hours exposure period and recorded no mortality (0.00 %) in the control) mg/ml (Table 5). Therefore, there was a high significant difference (χ^2 = 108.72, df = 5, P < 0.0001) in the mortality rate of the *Anopheles* larvae in relation to the concentration over time. Mortality rate observed after 24 hours exposure period for a concentration range of 20 to 80 mg/mL indicates that the larvae were resistant to the silver nanoparticles of *T. erecta.*

At 48 hours exposure time, mortality rate was the highest (100 %) at 100 mg/mL and mortality was the least (5%) in the control (0 mg/mL). The mortality rates at 48 hours exposure time varied significantly (χ^2 = 80.169, df = 5, P < 0.0001) across concentrations. After 48 hours, the larvae showed resistance at a concentration of 60 mg/ml and 80mg/ml respectively. But at 100 mg/mL concentration, the larvae were susceptible to the silver nanoparticles of *T. erecta.*

Mortality rate of *Anopheles* larvae was the highest (100 %) at 100 mg/mL concentration even at 72 hours exposure period. Within this period, the least mortality (15 %) was recorded in the control (0 mg/mL. Mortality across concentrations at 72 hours of exposure varied very significantly (χ^2 = 61.212, df = 5, P < 0.0001). Anopheles larvae were only resistant at 20 mg/ml concentration of the silver nanoparticles of *T. erecta.* Possible resistance was observed at 40, 60 and 80 mg/mL. While 100 mg/mL concentration revealed that larvae were susceptible to the silver nanoparticles of *T. erecta.*

Table 5: Percentage Mortality of *Anopheles* Larvae Exposed to the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta* in Relation to Concentrations over Time

Exposure time (hr)	Corrected Mortality Across Concentrations (mg/ml)						χ^2	df	P-value
	0	20	40	60	80	100			
24	0	52	73	67	35	100	108.72	5	p<0.05
48	5	75	77	86	88	100	80.169	5	p<0.05
72	15	79	84	86	88	100	61.212	5	p<0.05

Comparative Studies of Larvicidal Efficacy between the Aqueous Extracts and the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta* Against *Anopheles* Larvae

Results of the larvicidal efficacy between the aqueous extracts and the biosynthesized silver nanoparticles (AgNPs) showed that, for both samples used at the concentration range of 10 mg/mL to 60 mg/mL for 24, 48 and 72 hours, the mortality rates recorded for *T. erecta* aqueous extracts was relatively higher than the mortalities recorded for the biosynthesized silver nanoparticle. However, the difference in the mortality rate across concentrations over time did not show significance (P > 0.05). The difference in the mortality rate was only significant (P < 0.05) at 24-hour exposure period for both 20 mg/mL and 80 mg/mL concentrations. Generally, mortality rates using the aqueous extracts of *T. erecta* was appreciably higher than the use of the biosynthesized silver nanoparticles (AgNPs).

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Table 6: Comparative Larvicidal Efficacy between the Aqueous Extracts and the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta* Against *Anopheles* Larvae

Concentration (mg/mL)	Time of Exposure (hr)	Mortality of Crude Extract (%)	Mortality of Synthesized AgNPs (%)	χ^2	df	P-value
0	24	0	0	0.000	0	0
	48	5	5	0.000	1	1.000
	72-	10	15	1.000	1	0.317
20	24	78	52	5.200	1	0.023
	48	89	75	1.195	1	0.274
	72	91	79	0.847	1	0.357
40	24	87	73	1.225	1	0.268
	48	91	77	1.167	1	0.280
	72	91	84	0.280	1	0.597
60	24	90	67	3.369	1	0.066
	48	91	88	0.050	1	0.823
	72	91	86	0.141	1	0.707
80	24	95	35	27.69	1	$P < 0.05$
	48	98	89	0.433	1	0.510
	72	93	88	0.138	1	0.710
100	24	97	100	0.046	1	0.831
	48	100	100	0.000	1	1.000
	72	100	100	0.000	1	1.000

Lethal Concentration (LC_{50} and LC_{90}) of *T. erecta* Aqueous Extracts that Exhibits Larvicidal Activity Against *Anopheles* Larvae in Relation to Exposure Periods

Lethal Concentration of *T. erecta* aqueous extracts that exhibits larvicidal activity against 50 % and 90 % of *Anopheles* larvae at 24 hours exposure time is 6.91 mg/ml and 53.70 mg/ml respectively. The aqueous extracts of *T. erecta* required at 48 hours exposure time to exhibit larvicidal activity against 50 % and 90 % of *Anopheles* larvae is 6.92 mg/ml and 31.62 mg/ml respectively. After 72 hours exposure time, the lethal concentration of *T. erecta* required to exhibit larvicidal activity against 50 % and 90 % of *Anopheles* larvae is 5.13 mg/ml and 28.18 mg/ml respectively.

Lethal Concentration (LC_{50} and LC_{90}) of the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta* that Exhibits Larvicidal Activity Against *Anopheles* Larvae in Relation to Exposure Periods

Lethal concentration of the biosynthesized silver nanoparticles (AgNPs) of *T. erecta* that show larvicidal activity against 50 % and 90 % of Anopheles larvae at 24 hours exposure time is 17.78 mg/mL and 75.86 mg/mL respectively. At 48 hours exposure time, the biosynthesized silver nanoparticles (AgNPs) of *T. erecta* required to show larvicidal activity against 50 % and 90 % of *Anopheles* larvae is 15.49 mg/mL and 48.98 mg/mL respectively. After 72 hours exposure time, the lethal concentration of biosynthesized silver nanoparticles (AgNPs) of *T. erecta* required to exhibit 50 % and 90 % larvicidal activity against *Anopheles* larvae is 12.59 mg/mL and 44.67 mg/mL respectively.

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Table 7: Lethal Concentration (LC_{50} and LC_{90}) of *T. erecta* aqueous extracts that Exhibits Larvicidal Activity against *Anopheles* Larvae in Relation to Exposure Periods

Time of exposure (hr)	LC_{50} (mg/mL)	LC_{90} (mg/mL)
24	6.91	53.70
48	6.92	31.62
72	5.13	28.18

Table 8: Lethal Concentration (LC_{50} and LC_{90}) of the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta* that Exhibits Larvicidal Activity Against *Anopheles* Larvae in Relation to Exposure Periods

Time of exposure (hr)	LC_{50} (mg/mL)	LC_{90} (mg/mL)
24	17.78	75.86
48	15.49	48.98
72	12.59	44.67

DISCUSSION

UV-visible, FTIR Spectroscopy and SEM-EDX analysis

Figure 1 showed UV-visible absorption spectrum results of the aqueous extract of *Tagetes erecta* leaves. The absorption peak was found at 400 nm due to surface plasmon resonance of the plant extract. Meanwhile, Figure 2 presents UV-visible absorption spectrum result of the synthesized AgNPs of *Tagetes erecta.* The formation of Silver Nanoparticle was indicated by a color change from dark green to light colloidal green. The absorption maxima were found to be 300 nm this was due to surface plasmon resonance of the AgNPs. This is in line with the report of kiranmai *et al.* (2017) which showed that the highest absorption peak was at 300 nm and 650 nm which suggest that the variation depends on the reducing agent and the type of metal salt used as a precursor.

The FT-IR spectrum for the aqueous crude extract as presented in the figure 3 above shows prominent peaks at 3419 cm^{-1}, 3163 cm-^{1}, 1651 cm^{-1}, 1727 cm^{-1} and 1410 cm^{-1}. These absorption peaks indicate the presence of essential secondary metabolites with distinct functional groups as -O-H stretching, (due to carboxylic acid, SP^2 due to C-H stretch, C=C double bond stretching, C=O carbonyl stretch and C-C stretching vibration present in the aromatic ring respectively.

FTIR analysis figure 4 identified all the possible biomolecules responsible for the reduction of Silver and capping of silver nanoparticles .The FT-IR spectrum for the silver nano particles showed similar bands of absorption with slight variation as presented in figure 3. The major peaks around 3400 cm^{-1}, at 3170 cm^{-1}, 2377 cm^{-1}, 2138 cm^{-1}, 1725 cm^{-1}, 1649 cm^{-1}, 1143 cm^{-1}, and 1051 cm^{-1}.The broad absorption band around 3400 indicated an O-H stretching due to carboxylic group, at 3170 cm^{-1} represents SP^2 stretch, 1725 cm^{-1} indicates -C=O (carbonyl stretch), The absorption bands ranging from 1640-1650 cm^{-1} indicates C=C stretching where 1649 cm^{-1} falls in the spectrum, then the peaks at 1143 cm^{-1}, corresponds to C-O stretching, and 1051 cm^{-1} indicates phenolic or alcoholic group which is in agreement with the report of Kuchekari *et al*. (2018).

The surface morphology of the silver nanoparticles was investigated by scanning electron microscopy (SEM) method. The SEM micrograph showed a crystalline form of AgNPs with regular shape and smooth surface at 50 µm, 80 µm and 100 µm as shown in figure 5. SEM-EDX presents the elemental composition and their weight concentrations of the synthesized AgNPs. Table 3 represents the elemental composition of biosynthesized AgNPs. The result revealed that that carbon, oxygen, lead, and silver has the weight concentrations of 14.21, 12.48, 35.86,

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18.03 % respectively. However, other elements present were in small proportion and may have been from the soil.

Mortality Rate of the Larvae of *Anopheles* Mosquitoes Exposed to the aqueous extract of *T. erecta*

The results obtained revealed a progressive increase in percentage mortality as concentrations increased after 24- and 48-hours exposure period. Percentage mortality across concentration remained stable even after 72 hours of exposure. This is congruent with the study by Tribaskoro *et al.* (2018) who assessed the mosquitocidal activity of 100 % essential oil against *Aedes aegypti* and *Culex quiquefasciatus*. The stable mortality rates demonstrated after 48- and 72-hours exposure time for a concentration rage of 20 to 80 mg/ml could possibly be that some of the larvae developed some level of resistance to the doses after tolerating the aqueous extracts for 24 hours. However, they lost their resistance at increased/higher concentrations. As increased concentration are known to accumulate plant toxin in the larval tissues and the active conversion of active substances into more toxic metabolite's concomitant with longer exposure (Nikkon *et al.*, 2011). Conversely, an earlier study on the larvicidal activity of the oils of three species of marigold (*T. patula, T. erecta and T. minuta*) against *Aedes aegypti*. This study observed that *T. erecta* oils did not exhibit mortality even after 24 hours, mortality was observed within 24 hours only among the larvae treated with the oils of *T. minuta*; it was also found that even higher concentrations of the oils lost their activity within 24 hours after dispersal in water (Green *et al.,* 1991). This is therefore a reflection on the comparative study of the utilization of the aqueous extracts, essential oils, and the other metal nanoparticles of *T. erecta* for their mosquitocidal activities.

Mortality Rate of the Larvae of *Anopheles* Mosquitoes Exposed to the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta*

After 24 hours of larval exposure to the biosynthesized silver nanoparticles (AgNPs), the result revealed a relatively slow increase in the percentage mortality as concentrations increased. The highest mortality (100 %) was recorded at the highest concentration (100 mg/ml). This agrees with the recent report of Alhassan, and Yoriyo, (2021) on larvicidal activities of biosynthesized zinc and nickel nanoparticles using *Allium sativum* (garlic) oil against the larvae of malaria *Anopheles spp.* However, an irregular pattern (drastic decline in mortality) of mortalities at 80mg/ml (35 %) and 60 mg/ml (67 %) were observed. Even so, there was a steady decline as concentrations were reduced to 40 mg/ml (73 %) and 20 mg/ml (52 %). This pattern of larval mortality gave an undulating mortality curve pattern.

After 48 hours, there was a progressive mortality rate pattern. The percentage mortality rate remained constant after 72 hours exposure period with 100 mg/ml recording the peak mortality among the concentrations. This observation is in fair alignment with that of Idowu *et al*. (2021) who compared the larvicidal potential of two silver nanoparticles (*Moringa oleifera* and *Ficus exasperate)* against some strains of *Anopheles gambia*e. Mortality rates after 24 and 48 hours remained constant with a progressive increase as concentrations increase. This implies that, the peak mortality was reached at 24 hours for the highest concentration used. The variations in the results may be associated to the difference in experimental conditions and plant species.

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Larvicidal Efficacy between the Aqueous leaf Extract and the Biosynthesized Silver Nanoparticles (AgNPs) of *T. erecta*

The current study revealed that aqueous leaf extracts of *T. erecta* showed a promising larvicidal efficacy against *Anopheles* larvae. This is in tandem with the study by Marques *et al*. (2011) who evaluated the larvicidal activity of *T. erecta* against *A. aegypty*. A contrary observation was recorded by Nikkon *et al.* (2011) where *Culex quinquefasciatus* demonstrated a considerable tolerance against *T. erecta*. This could be because of difference in the position of the siphon of *Culex quinquefasciatus* in water.

The biosynthesized silver nanoparticles (AgNPs) used in this study also showed an appreciable larvicidal efficacy, especially at higher concentrations. Similar utilization of silver nanoparticles (AgNPs) against the larvae of *Aedes* species also showed an impressively promising larvicidal efficacy at a highest concentration (Amarasinghe *et al*., 2020). This is an affirmation that larvicidal efficacy increases with an increase in concentration (Finney, 1971).

Generally, the aqueous leaf extract had a better impact on the mortalities of *Anopheles* larvae when compared to the effect of the silver nanoparticles. More so, the silver nanoparticles exhibited a moderate effect on the mortality of *Anopheles* mosquito larvae, thus revealing a lower efficacy than the aqueous leaf extracts. The moderate effect of the silver nanoparticles against *Anopheles* larvae could be that the introduction of silver nitrate as caused a reduction in the charge and the toxicity potential of the plant. This finding agrees with the study by Khader *et al.* (2017) who observed a higher efficacy on the use of selected medicinal plant extracts than the use of the green synthesized nanoparticles against the larvae of mosquito species. Nonetheless, the study is in contrast with the findings of Morejon *et al*., (2018) and Amarasinge *et al*. (2020) that recorded a better efficacy on the use of green synthesized silver nanoparticles (AgNPs) against Aedes species.

Lethal Concentration (LC_{50} and LC_{90}) of the Aqueous Extracts and the Biosynthesized Silver nanoparticles (AgNPs) of *T. erecta*

Lethal concentrations (LC_{50} and LC_{90}) are the concentrations of the extract that can kill 50 % and 90 % of the larvae population. It is therefore worthy of note that the lower the LC_{50} and LC_{90}, the more effective the larvicidal activity of the test substance.

The results of the lethal toxicology revealed that the concentration of aqueous extracts of *T, erecta* capable of killing 50 % and 90 % *Anopheles* larvae populations is 5.13 mg/mL and 28.18 mg/ml after 72 hours. The lower LC_{50} and LC_{90} values observed is in resemblance with the studies by Marques and colleague (2011), they evaluated *T. erecta* for its larvicidal activity against *A. aegypti*. In their findings, *T. erecta* extracts proved to be effective at LC_{50} of 79.78 µg/mL and LC_{90} of 100.84 µg/mL. Nonetheless, opinions from another research holds that the extracts of *T. erecta* was effective against *Anopheles stephensi* with LC_{50} of 76.24 ppm and LC_{90} of 194.5 (Jayaram *et al.,* 2015).

The lethal concentration of the biosynthesized silver nanoparticles of *T. erecta* that exhibit 50 % and 90 % larvicidal activity against *Anopheles* larvae was 12.59 mg/mL and 44.67 mg/mL after 72 hours exposure respectively. This finding disagrees with similar study by Hajra *et al*. (2016) who evaluated the larvicidal activity of Cadmium nanoparticles of *T. erecta*. They recorded an LC_{50} of 5 ppm and LC_{90} of 10 ppm after 24 hours: thus, demonstrating a better efficacy against mosquitoes. The variation in the result may be

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because of the difference in the type of synthesis

CONCLUSION

The study was able to biosynthesize and characterized silver nanoparticles of *T. erecta* for its larvicidal activity against *Anopheles* mosquitoes. The results obtained showed that both the aqueous leaves extracts and the biosynthesized silver nanoparticles of *T. erecta* can be used to eliminate the larval population of *Anopheles* mosquitoes, especially at a dose of 100 mg/mL. However, the aqueous extracts of *T. erecta* demonstrated a superior efficacy than the biosynthesized silver nanoparticles. Therefore, the extracts of *T. erecta* has potentials to be used as an alternative and biofriendly insecticide. More so, investigations using different metal nanoparticles for its larvicidal or mosquitocidal activity should be explored. Again, the effect of the extracts on non-target organisms should also be studied before its recommendations for commercial production.

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