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Original Article

Larvacidal efficacies of *Terminalia catappa* and *Alstonia boonei* synthesized silver, iron and copper nanoparticles against *Culex quinquefasciatus*.

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ABSTRACT

The excessive dependence on chemical-based mosquito larvicides has resulted in ecological harm, development of resistant mosquitos' populations and potential health hazards, creating an urgent need for sustainable solutions. Green-synthesized nanoparticles present a viable environmentally conscious alternative plant-based larvicidal agent demonstrating significant effectiveness while causing substantially less environmental damage than their synthetic counterpart. In this study, the potency of three different, green-synthesized nanoparticles were experimented on the larvae of *Culex quinquefasciatus*. The different nanoparticles (silver, iron and copper) were green-synthesized with two different plant (*Alstonia boonei* and *Terminalia catappa*) leaf extract, the characteristics functional group of active constituents were identified using UV-visible spectrophotometer, Fourier Transform Infrared spectrophotometer and scanning Electron Microscope analysis. Larvicidal activities were screened using five different concentrations (50, 100, 150 200 and 250 mg/mL) following WHO standard protocol and mortality was recorded after 15 and 20 minutes and also 12 and 24 hours respectively. Silver nanoparticles of *Terminalia Catappa* (TCA) and *Alstonia boonei* (AA) showed the highest mortality in the shortest exposure time with both having 100 % mortality at 20 minute in the highest concentration (250 mg/mL) followed by iron nanoparticles of *Terminalia Catappa* (TCF) and *Alstonia boonei* (AF) and copper nanoparticles of *Terminalia catappa* (TCC) and *Alstonia boonei* (AC) which performed at same duration of exposure time of 12 and 24 hours respectively. TCF and TCC had 100 % mortality at 24 hours while AF and AC had 98.3 % and 96. 7 % mortality respectively. In conclusion, this study established the lethal efficacy of all the nanoparticles as promising alternative plant-based and eco-friendly larvicide against *Cx. Quinquefasciatus*.

Keywords: *Cx. quinquefasciatus,* nanoparticles, larvicides and vector control.

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INTRODUCTION

Culex quinquefasciatus is a major vector of diseases such as lymphatic filariasis, Japanese encephalitis, West Nile virus, Mansonella filariasis, and St. Louis encephalitis, affecting millions globally [1]. Mosquito population control is crucial in combating these diseases, with insecticidal treatments being central to control efforts [2]. Larvicides target mosquitoes during their developmental stages, preventing them from maturing into adults and reducing disease transmission [3]. Since mosquito larvae are habitat-bound, larvicides offer a more straightforward and effective control method [4]. However, the overuse of synthetic chemicals has led to resistance and environmental pollution [2-4]. This has prompted the search for eco-friendly alternatives, particularly plant-based compounds, which are less harmful, economically viable, and help reduce the emergence of resistant strains [5, 6, 7-8,]. Plant-derived insecticides target mosquito larvae at their breeding sites, disrupting their growth in water and providing effective control [5].

Green-synthesized nanoparticles have emerged as a promising eco-friendly alternative to conventional control methods, which are becoming less effective due to resistance [9, 10]. Advances in nanotechnology have enabled the creation of materials at the nanoscale with precise control over size, distribution, and morphology [11, 12]. Plant extracts are particularly effective for synthesizing nanoparticles, which have shown great promise in medical applications and are increasingly seen as vital for future treatments [13-14, 15]. These nanoparticles, including silver (Ag), copper (Cu), and iron (Fe), synthesized using plant extracts such as *Terminalia catappa* and *Alstonia boonei*, offer an environmentally friendly approach to mosquito control. Recent studies emphasize the effectiveness of plant-based metal nanoparticles in controlling mosquito larvae, presenting a viable alternative to chemical insecticides and potentially reducing vector-borne disease transmission [16]. As research advances, the potential of nanoparticles for plant-based mosquito control solutions continues to grow [17].

MATERIAL AND METHODS

Collections of Plant material

Fresh *T. catappa* and *A. boonei* leaves were collected in their full thriving stage around Omun, Coker Osogbo (Longitude: E 4.60388, Latitude: N 7.76117). They were validated in the taxonomy section of the Department of Plant Biology of Osun State University, Osogbo. The plants were washed to remove dust, dried under room temperature for 7-14 days, the leaves were then collected and blended separately into fine particles and preserved in different airtight container till use. Other parts (Leaves, fruits, flowers and bark) of the plants were collected and taken to the Department of Plant Biology Laboratory, Osun state University, Osogbo were it was further identified correctly

Preparation of plant extract

To prepare the aqueous extract, 10 grams of each of the already blended leaf extract was diluted with 1000 ml of distilled water, stirred allowed for 24 hours under room temperature, the solution was then be filtered using Whatman filter paper and refrigerated till use.

Green-synthesis of nanoparticles

Green synthesis of silver (AgNPs), iron (FeNPs) and copper (CuNPs) using *A. boonei* and *T. catappa* leaf extract was done following the procedures reported by Lateef et al. (2016). The reduction of silver ions was achieved by adding 7 mL of the extract with 293 ml of $AgNO_3$ solution (1mM), while the reduction of ferrous ions was achieved by adding 10 mL of the extract with 1 ml of $FeSO_4$ solution and both were incubated at room temperature The transparent brown color seen indicates the formation of silver nanoparticles while the amber colour in FeNPs gives the formation of iron nanoparticles. The solution was stored in a clean air-tight container and refrigerated until use.

Characterization of Nanoparticles

UV-visible spectroscopy

The synthesized *T. catappa*-AgNPs (TCA), *A. boonei*-AgNPs (AA), *T. catappa*-iron (TCF), *A. boonei*-iron (AF), *T. catappa*-copper (TCC) and *A. boonei*-Copper (AC) nanoparticles were analyzed for Surface Plasmon Resonance with wavelength ranging from 200 - 1000 nm at room temperature using a UV-Visible spectrophotometer (Biobase BK-UV1900 P spectrometer, China).

Fourier transform-infrared spectrometer (FTIR) analysis

The FTIR spectrum (TCA, AA, TCF, AF, TCC and AC) of the synthesized nanoparticles were mediated to identify the characteristic functional groups of bioactive components using an infrared spectrum analyzer (SHIMADZU FTIR-8400S). The spectra were measured between 400 and 4000 cm1 in frequency.

Scanning electron microscope (SEM) analysis

SEM (Phenom PRO X SEM-MVE01570775) was used to determine (TCA, AA, TCF, AF, TCC and AC) synthesized nanoparticles size and shape. The constituent of the nanoparticles was analyzed with Energy Dispersive X-ray Fluorescence (EDXRF) Spectrum (ARL QUANT'X EDXRF Analyser. Serial No.9952120).

Larvae Collection and maintenance

The third and fourth larvae instars of *Culex quinquefasciatus* were collected from in and around Freedom Park area of Osogbo, Osun state, with the help of "O" type brush. These larvae were brought to the laboratory and maintained in 500 ml of water. Further identification was carried out at the Department of Animal and Environmental Biology, Osun State University, Nigeria.

Preparation of plant extract for larvicides

To prepare the aqueous extract, 50 g of powdered leaf and 500 mL of distilled water was heated for 15 min using a magnetic stirrer/hot plate at 70°C (78HW-1, JINOTECH). The extract was filtered and refrigerated in an airtight bottle for preservation until use.

Larvicidal bioassay

The larvicidal activity was evaluated following the standard protocol of WHO, (2005). From the stock solution, different concentrations of aqueous extracts of nanoparticles: 50, 100, 150, 200 and 250 mgL1 were prepared and 25 fourth-instar larvae of *Cx. Quinquefasciatus* was exposed to each concentration. Larvae in the control was exposed to 250 ml of distilled water. Three replicates were performed for each test. After exposure, immovable larvae was considered dead and their number were recorded respectively.

Data Analysis

Data from all replicates will be pooled for analysis. LC_{50} and LC_{90} values are calculated from a log dosage–probit mortality regression line using SPSS version 25.0. Standard deviation or confidence intervals of the means of LC_{50} values was calculated and recorded on a form. A test series is valid if the relative standard deviation (or coefficient of variation) is less than 25% or if confidence limits of LC_{50} overlap (significant level at $P < 0.05$). The potency of the chemical against the larvae of a particular vector and strain can then be compared with the LC_{50} or LC_{90} values of other insecticides.

RESULTS AND DISCUSSION

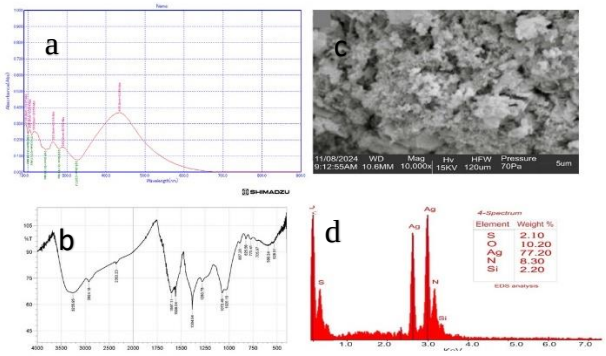

Fig.: 1a-d **a**. UV- spectrum; **b.** FTIR Spectroscopy; **c.** SEM image; **d.** EDS spectrum of AgNPs of *A. boonei*

Green-synthesis and characterization of AgNPs synthesized from both *A. boonei* and *T. catappa*

After an incubation period, a color change from yellow to transparent brown was seen, which implies bio-reduction of Ag^+ to Ag^0. The change in the appearance of the solution is a function of different bio-active constituents in the extract which serve as a reducing agents. According to Kanwal *et al.*, (2019), the many stages of nucleation and development that occur during synthesis are visible in the color variation that occur overtime.

The spectrum of the biosynthesized AgNPs with an aqueous extract of *A. boonei* was monitored under UV-visible spectra at a wavelength between 190- 700 nm. The absorption peak is characteristics to Surface Plasma Resonance (SPR) was recorded at 435 nm. The peak is indicative of reduced Ag and falls within the range characteristics of AgNPs (Figure 1a). The functional groups of bioactive constituents in the biosynthesized AgNPs are shown in Figure 1d. The prominent bands observed were 3256, 3924, 2353, 1559, 1385 and

1072 cm^{-1}. This bands were related to O-H stretching of alcohols, C-H alkane (CH stretching), C≡C, C=C stretching characteristics of alkenyl, CH_3 bending absorption of methylene group and nitrogen group respectively (Figure 1c). The biosynthesized nanoparticles were capped and synthesized by these functional groups. Fine whitish-ash cloudy pattern of AgNPs was observed in the SEM-EDs images, with the major components of the EDs patterns as Ag (77.20 %), O (10.20 %), N (8.30 %), Si (2.20 %) and S (2.10 %). The obvious presence of Ag in the EDs pattern implies the formation of AgNPs was confirmed (Figure 1d).

Green-synthesis of *T. catappa* with $AgNO_3$

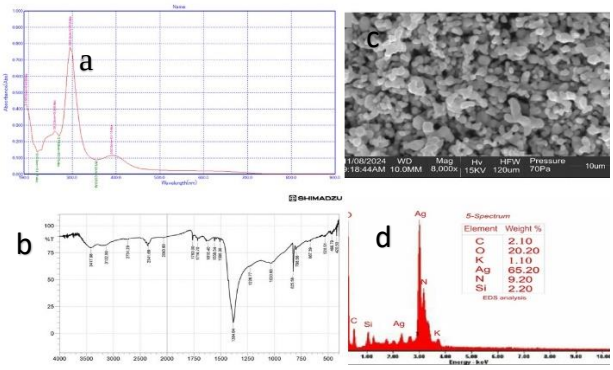

Fig.: 2a-d **a.** UV- spectrum; **b.** FTIR Spectroscopy; **c.** SEM image; **d.** EDS spectrum of AgNPs of *T. catappa*

Upon incubation visual observation of gradual color change from light yellowish brown to dark brown was seen. This observation showed bio-reduction of Ag^+ to Ag^0. The change in the appearance of the solution is a function of the different bioactive constituent in the extract which serve as reducing agents.

The spectrum of the biosynthesized AgNPs with an aqueous extract of *T. catappa* was monitored under the UV-Visible spectra at a wavelength between 190 - 700 nm (figure 2a). The absorption peak characteristics of surface plasmon resonance (SPR) was recorded at 295 nm. This peak is indicative of reduced Ag and falls within the range characteristic of AgNPs (Azeez *et al* 2022: Aremu *et al.*, 2023). It has been previously reported that an SPR peak between 410 and 450 nm as obtained in this study is related to spherical AgNPs (Figure 1a).

The functional groups of bioactive constituents in a biosynthesized AgNPs are shown in (Figure 2b). The prominent bands observed were 3418, 3133, 2342, 1763 and 1395 cm^{-1}. These bands were related to O-H stretching and bending of primary and secondary alcohol, C-H stretching (alkane), C=O polyphenol and nitrate group respectively. The bio synthesized nanoparticles were stabilized and capped by these functional groups (Figure 2b). The SEM-EDS images showed free irregular shape patterns of AgNPs, the main component of EDS patterns are Ag (65.20 %), O (20.20 %), N (9.20 %), Si (2.20 %), C (2.10 %), K (1.10 %). The overwhelming presence of Ag in the EDS pattern indicates the formation of AgNPs (Figures 2c and d).

Green-synthesis and characterization of FeNPs synthesized from both *A. boonei* and *T. catappa*

Green-synthesis of *A. boonei*

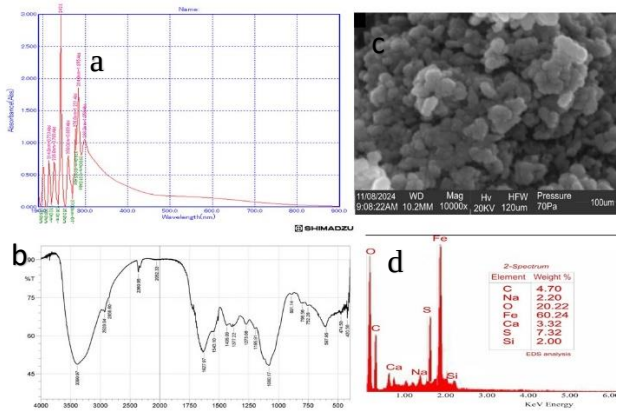

Fig.: 3a-d **a.** UV- spectrum; **b.** FTIR Spectroscopy; **c.** SEM image; **d.** EDS spectrum of FeNPs of *A. boonei*

After incubation over a period of time, the color change from light golden- yellow to deep amber transparent brown was observed which suggest bio-reduction of Fe^{+} to Fe^{0} the change in the appearance of the solution is a function of the different bioactive constituent in the extract which serve as reducing agents. The many stages of the nucleation and development that occur during synthesis are visible in the color variation that occur over time. The spectrum of the biosynthesized FeNPs with an aqueous extract of *A. boonei* was monitored under UV-visible spectra at a wavelength of between 190-900 nm (figure 3a-b) the absorption peak is characteristics to surface plasma resonance (SPR) was observed at 242 nm. This peak is indicative of reduced Fe and falls within the characteristics of FeNPs. The functional groups of bioactive constituents in the synthesized FeNPs are shown in (Figure 3c). The prominent bands observed were 3391, 2928, 1628 and 1080 cm^{-1}. These bands were related to OH stretching of alcohol with C-H broad stretch of alkane, C=C and sulphate group, the presence of polyphenol protein as the functional group is responsible for capping and stabilizing. The SEM-EDS image showed bunches of clouded patterns of FeNps. The major components of the EDS pattern are Fe (60.24 %), O (20.22 %), S (7.32 %), C (4.70 %), Ca (3.32 %), Na (2.20 %) and Si (2.00 %). The eminence of Fe in the EDS pattern indicates the formation of FeNps.

Green-synthesis iron sulfate ($FeSO_4$) with *T. catappa*

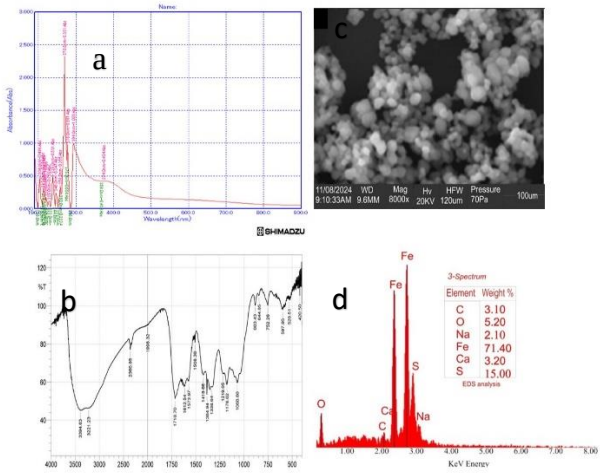

Fig.: 4a-d **a.** UV- spectrum; **b.** FTIR Spectroscopy; **c.** SEM image; **d.** EDS spectrum of FeNPs of *T. catappa*

After the incubation period, a color change from deep yellow/ burnt orange color to blackish-brown deep was seen which implies biosynthesis of Fe. The change in the appearance of the solution is a function of the different bioactive constituents in the extract which serve as reducing agents. The UV-visible spectra was observed at a wavelength of between 190-900 nm. The absorption peak is characteristic to

surface resonance (SPR) was observed at 270 nm (figure 4a). This peak is indicative of reduced Fe and falls within the characteristic of FeNPs. FTIR spectrum for *T. catappa* medicated FeNPs manifested strong peaks at 3394.83 and 1716.70cm^{-1} implicating OH and C=O stretch of an ester or a carboxylic acid, The 1176.62cm^{-1} is a C-O stretching vibration while 1068.60 is a sulphate group respectively (figure 4c). The SEM-EDS image revealed a clustered cloudy pattern of FeNps. The main components of the EDS patterns are Fe (71.40 %), S (15.00 %), O (5.20 %), Ca (3.20 %), C (3.10 %) and Na (2.10 %). The strong presence of Fe in the EDS pattern relates to the presence and formation of FeNPs (figure 4c and d

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Green-synthesis of Copper sulphate (CusSO$_4$) with *A. boonei*

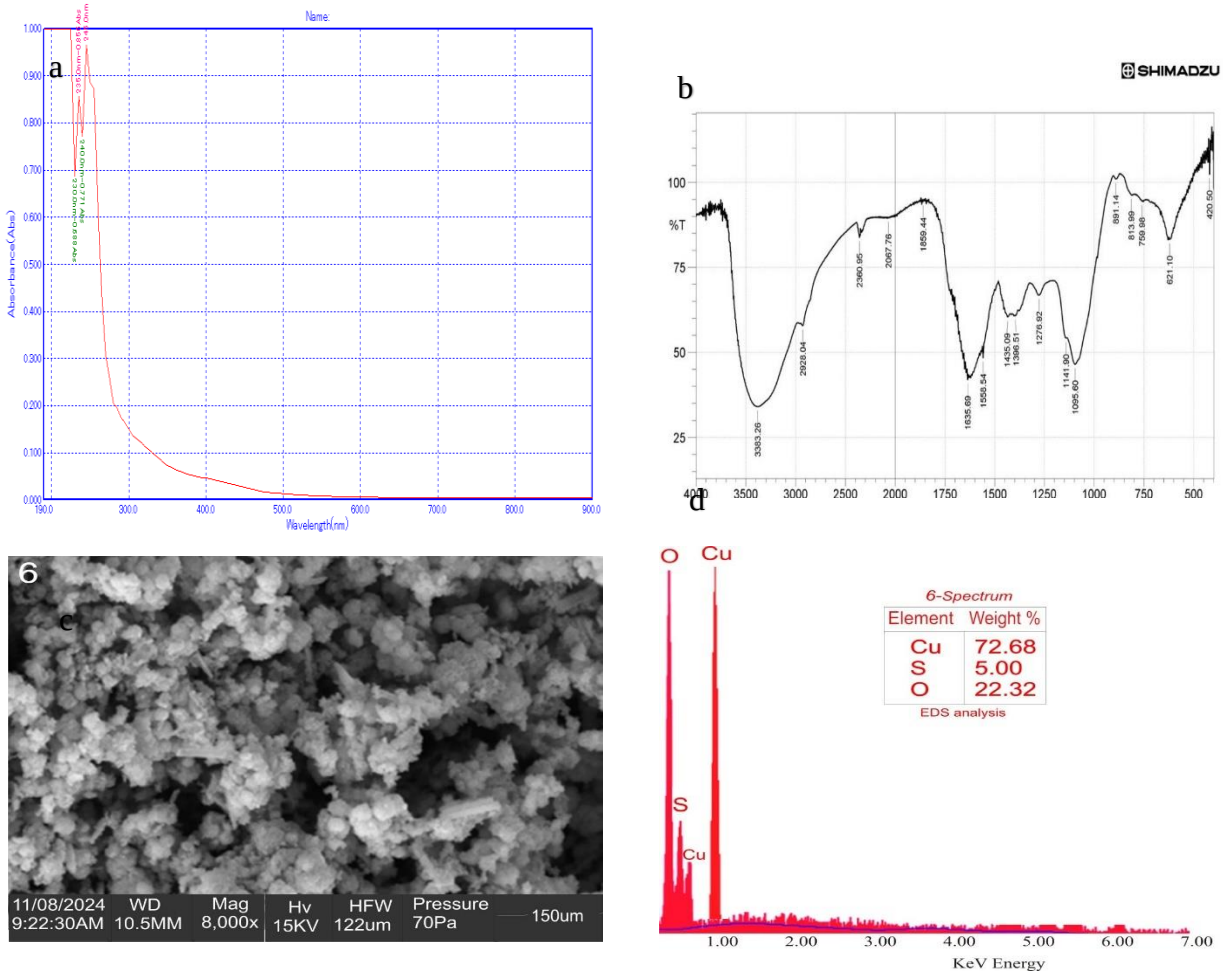

Fig.: 5a-d **a.** UV- spectrum; **b.** FTIR Spectroscopy; **c.** SEM image; **d.** EDS spectrum of CuNPs of *A. boonei*

There was an obvious color change which was observed upon incubation over a period of time, the color change from light golden yellow to greenish-black was seen with maximum absorption at 245 nm (Figure 5a). This peak is characteristic of CuNPs and it occurred within the range of 391–460 nm mostly reported for AgNPs. The FTIR absorption spectrum showed distinct peaks at 3383, 2928, 1636 and 1096. The band 3383 and 2928cm^{-1} refer to the binding OH and C-H stretch, indicating polyphenol responsible for capping and stabilization of the biomolecules in the synthesis of CuNPs. Band 1636 is of C=C and 1096 of sulphate group respectively. Images from SEM-EDS revealed whitish- cloudy fine patterns of CuNPs. The major components of thus EDS patterns are Cu (72.68 %), O (22.32 %) and S (5.00 %) (figure 5c and d). The strong presence of Cu in the EDS pattern indicates the formation of CuNPs.

Green-synthesis of Copper sulphate ($CuSO_4$) from *T. catappa*

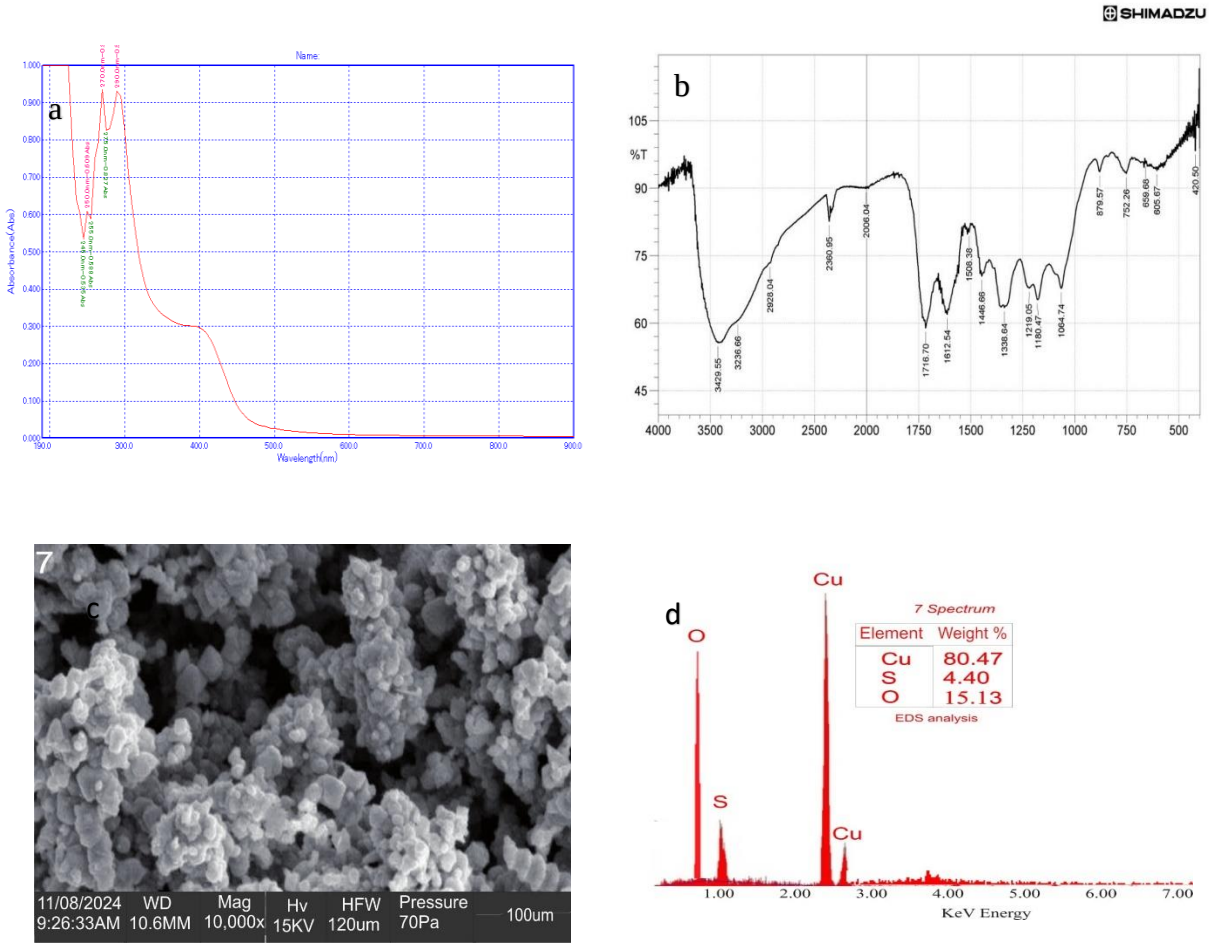

Fig.: 6a-d **a.** UV- spectrum; **b.** FTIR Spectroscopy; **c.** SEM image; **d.** EDS spectrum of CuNPs of *T. catappa*

The color change was seen by visual observation in the *T. catappa* leaf extracts when incubated with $CuSO_4$ solution. The color of the extract changed to light brown within an hour and later it changed to deep dark brown during a few hours of incubation period after which no significant change occurred. The brownish colloidal CuNPs absorbed maximally at 290 nm (Figure 6a). The FTIR showed involvement of OH stretch in band 3429.55cm^{-1}, C=C at 1612.54cm^{-1} with a double peak of sulphate groups at band 1338.64 and 1180.47 respectively. The SEM-EDS image revealed clusters of spherical particle pattern of CuNPs (6c). The main components of EDS pattern are Cu (80.47 %), O (15.13 %) and S (4.40 %) respectively (Figure 6d). The obvious presence of Cu in the EDS pattern attest the formation of CuNPs.

Table 1. Larvicidal activity of *Terminalia catappa and Alstonia boonei* silver nanoparticle against *Culex quinquefasciatus* larvae upon exposure at 15 and 20 minutes respectively

Time taken	Extract	Mortality (%)		LC_{50} (50 %CI)	LC_{90} (90%CI)	LC_{95} (95%CI)	X^2	*P* value
		Conc. (mg/g)	Mean ± SD					
15mins	TCA	Control	0.0 ± 0.0					
		50	40.0 ± 10.0^{c}	43.5 (<<0.1 to 83.1)	698.7 (269.9 to >>1000)	1796.5 (452.1 to >>1000)	4.27	0.039
		100	55.0 ± 5.0^{b,c}				.	.
		150	71.7 ± 10.4^{a,b}				.	.
		200	66.7 ± 15.3^{a,b}				.	.
		250	81.7 ± 10.4^{a}				.	.
20mins	TCA	Control	0.0 ± 0.0	22.4 (<<0.1 to 43.5)	70.9 (22.6 to 157.1)	104.9 (65.1 to >>1000)	5.03	0.025
		50	61.7 ± 10.4^{b}					
		100	91.7 ± 5.8^{a}				.	.
		150	95.0 ± 8.7^{a}				.	.
		200	96.7 ± 2.9^{a}				.	.
		250	100.0 ± 0.0^{a}				.	.
15mins	AA	Control	0.0 ± 0.0	48.7 (<<0.1 to >>1000)	1732.8 (<<0.1 to >>1000)	5840.3 (<<0.1 to >>1000)	2.79	0.095
		50	11.7 ± 7.6^{c}					
		100	58.3 ± 10.4^{b}				.	.
		150	66.7 ± 7.6^{a,b}				.	.
		200	70.0 ± 5.0^{a,b}				.	.
		250	73.3 ± 5.8^{a}				.	.
20mins	AA	Control	0.0 ± 0.0	26.4 (0.7 to 46.9)	88.1 (52.3 to 200.5)	132.9 (87.1 to 850.7)	6.62	0.010
		50	43.3 ± 16.1^{b}					
		100	86.7 ± 5.8^{a}				.	.
		150	90 ± 8.7^{a}				.	.
		200	98.3 ± 2.9^{a}				.	.
		250	100.0 ± 0.0^{a}				.	.

LC_{50}; Lethal concentration for 50% mortality of larvae, LC_{90}; Lethal concentration for 90% mortality of larvae, LC_{95}; Lethal concentration for 95% mortality of larvae, 95% CI; 95% confidence interval, X^2; Chi square test, >>; much greater than, <<; much less than, SD; Standard deviation. Means with different superscripts are statistically

different (P<0.05) based on Duncan's multiple range tests

Table 2. Larvicidal activity of *Terminalia catappa and Alstonia boonei* Copper nanoparticle against *Culex quinquefasciatus* larvae upon exposure at 12 and 24 hours respectively

Time taken	Extract	Mortality (%)		LC_{50} (50%CI)	LC_{90} (90%CI)	LC_{95} (95%CI)	X^2	*P*value
		Conc. (mg/g)	Mean ± SD					
12hours	TCC	Control	0.0 ± 0.0	85.3 (46.8 to 115.7)	389.2 (240.3 to >>1000)	652.1 (342.4 to >>1000)	12.88	0.003
		50	35.0 ± 5.0^{c}					
		100	38.3 ± 7.6^{c}				.	.
		150	56.7 ± 7.6^{b}				.	.
		200	68.3 ± 10.4^{b}				.	.
		250	83.3 ± 7.6^{a}				.	.
24hours	TCC	Control	0.0 ± 0.0	28.4 (1.7 to 47.6)	84.8 (53.4 to 178)	123 (83 to 577.5)	7.11	0.008
		50	76.7 ± 7.6^{c}					
		100	85.0 ± 5.0^{b,c}				.	.
		150	90.0 ± 5.0^{a,b}				.	.
		200	96.7 ± 5.8^{a}				.	.
		250	100 ± 0.0^{a}				.	.
12hours	AC	Control	0.0 ± 0.0	265.5 (<<0.1 to >>1000)	11208.9 (<<0.1 to >>1000)	40026.3 (<<0.1 to >>1000)	2.43	0.119
		50	20.0 ± 5.0^{c}					
		100	30.0 ± 5.0^{b,c}				.	.
		150	38.3 ± 2.9^{b}				.	.
		200	40.0 ± 5.0^{b}				.	.
		250	53.3 ± 10.4^{a}				.	.
24hours	AC	Control	0.0 ± 0.0	38.3 (7.0 to 61.0)	163.6 (111.6 to 498.2)	268 (162.4 to >>1000)	9.51	0.002
		50	56.7 ± 10.4^{c}				.	.
		100	63.3 ± 10.4^{c}					
		150	80.0 ± 5.0^{b}				.	.
		200	88.3 ± 7.6^{a,b}				.	.
		250	96.7 ± 5.8^{a}				.	.

LC_{50}; Lethal concentration for 50% mortality of larvae, LC_{90}; Lethal concentration for 90% mortality of larvae, LC_{95}; Lethal concentration for 95% mortality of larvae, 95% CI; 95% confidence interval, X^2; Chi square test, >>; much greater than, <<; much less than, SD; Standard deviation. Means with different superscript are statistically different (P<0.05) based on Duncan's multiple range tests

Table 3. Larvicidal activity of *Terminalia catappa and Alstonia boonei* Iron nanoparticle against *Culex quinquefasciatus* larvae upon exposure at 12 and 24 hours respectively

Time taken	Extract	Mortality (%)		LC_{50} (50%CI)	LC_{90} (90%CI)	LC_{95} (95%CI)	X^2	*P* value
		Conc. (mg/g)	Mean ± SD					
12hours	TCF	Control	0.0 ± 0.0	43.9 (5.2 to 71.8)	273.1 (165.6 to >>1000)	508.7 (250.6 to >>1000)	8.06	0.005
		50	53.3 ± 7.6[d]					
		100	61.7 ± 7.6[c,d]				.	.
		150	65.0 ± 0.0[b,c]				.	.
		200	73.3 ± 5.8[b]				.	.
		250	96.7 ± 2.9[a]				.	.
24hours	TCF	Control	0.0 ± 0.0	27.4 (0.5 to 51.7)	137.2 (87.6 to 607.1)	237.3 (138.9 to >>1000)	6.64	0.010
		50	70.0 ± 8.7[d]					
		100	80.0 ± 5.0[b,c]				.	.
		150	78.3 ± 2.9[c,d]				.	.
		200	88.3 ± 2.9[b]				.	.
		250	100.0 ± 0.0[a]				.	.
12hours	AF	Control	0.0 ± 0.0	451.7(<<0.1 to >>1000)	22947.1 (<<0.1 to >>1000)	87266.6 (<<0.1 to >>1000)	2.02	0.156
		50	20.0 ± 5.0[b]					
		100	21.7 ± 7.6[b]				.	.
		150	31.7 ± 7.6[a,b]				.	.
		200	36.7 ± 10.4[a]				.	.
		250	45.0 ± 5.0[a]				.	.
24hours	AF	Control	0.0 ± 0.0	41.9 (9.9 to 64.7)	177.8 (121.5 to 535.7)	290.6 (175.2 to >>1000)	10.26	0.001
		50	56.7 ± 2.9[c]					
		100	63.3 ± 2.9[c]				.	.
		150	80.0 ± 5.0[b]				.	.
		200	81.7 ± 7.6[b]				.	.
		250	98.3 ± 2.9[a]				.	.

LC_{50}; Lethal concentration for 50% mortality of larvae, LC_{90}; Lethal concentration for 90% mortality of larvae, LC_{95}; Lethal concentration for 95% mortality of larvae, 95% CI; 95% confidence interval, X^2; Chi square test, >>; much greater than, <<; much less than, SD; Standard deviation. Means with different superscript are statistically different ($P<0.05$) based on Duncan's multiple range tests

DISCUSSION

The current focus on managing mosquito populations has shifted towards targeting the larvae stages, as these stages are more accessible in their natural habitats. This has led to the development and incorporation of eco-friendly, non-toxic, and plant-based larvicides in mosquito control programs [11, 12]. Several studies have highlighted the insecticidal potential of specific plants against mosquito populations [5]. For example, [3] reported

the larvicidal effects of crude and acetone extracts of *Rhizophora mucronata* against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*, with LC_{50} values of 0.13, 0.11, and 0.34 mg/mL, respectively. Similarly, a study [18]. demonstrated the larvicidal properties of silver nanoparticles (AgNPs), synthesized using the leaf extract of *Azadirachta indica*, against late fourth instar larvae of *Culex quinquefasciatus*, with LC_{50} and LC_{90} values of 85.56 ppm and 231.51 ppm, respectively.

In the present study, we investigated the larvicidal effects of green-synthesized plant leaf extracts combined with three different salts (silver, iron, and copper) against *Culex quinquefasciatus* larvae at different exposure times. Among the various treatments, silver nanoparticles synthesized with *Terminalia catappa* (TCA) and *Alstonia boonei* (AA) showed the most potent larvicidal activity. For 15 and 20-minute exposures (table 1), the concentrations of 50–250 mg/gL yielded mortality rates of 40.0±10.00, 55.0±5.0, 71.7±10.4, 66.7±15.3, and 81.7±10.4% at 15 minutes, and 61.7±10.4, 91.7±5.8, 95.0±8.7, 96.7±2.9, and 100±0.00% at 20 minutes. The LC_{50} value for the 15-minute exposure was 43.5%, with LC_{90} and LC_{95} values of 698.7% and 1796.5%, respectively. In comparison, the LC_{50} values for the 20-minute exposure were 22.4%, with LC_{90} and LC_{95} values of 70.9% and 104.9%, respectively. This indicates a significant increase in larvicidal efficiency with prolonged exposure times.

Copper nanoparticles, synthesized with *Terminalia catappa* (TCC) and *Alstonia boonei* (AC), exhibited lower larvicidal activity compared to silver and iron nanoparticles. After 12 and 24 hours of exposure to concentrations ranging from 50–250 mg/gL, mortality rates ranged from 35.0±5.0% to 83.37±7.6% for the 12-hour exposure, and 76.7±8.7% to 100±0.00% for the 24-hour exposure. The LC_{50} for the 12-hour exposure was 85.3%, with LC_{90} and LC_{95} values of 389.2% and 652.1%, respectively, while the 24-hour exposure yielded LC_{50}, LC_{90}, and LC_{95} values of 28.4%, 84.8%, and 123%, respectively.

For iron nanoparticles, *Terminalia catappa* (TCF) and *Alstonia boonei* (AF) were tested at concentrations of 50–250 mg/gL over 12 and 24-hour exposure periods. Mortality rates ranged from 53.3±7.6% to 96.7±2.9% for the 12-hour exposure, and 70.0±8.7% to 100±0.00% for the 24-hour exposure. The LC_{50} for the 12-hour exposure was 43.9%, with LC_{90} and LC_{95} values of 273.1% and 1796.5%, respectively, while the 24-hour exposure yielded an LC_{50} of 27.4%, with LC_{90} and LC_{95} values of 137.2% and 237.3%, respectively.

Overall, the results demonstrated that all the green-synthesized nanoparticles exhibited larvicidal activity, which was concentration-dependent. These findings align with previous studies [18]. who also observed increased larvicidal potential with higher concentrations of green-synthesized silver nanoparticles. In the present study, the larvicidal effects of silver, iron, and copper nanoparticles synthesized from *Terminalia catappa* and *Alstonia boonei* against early fourth instar larvae of *Culex quinquefasciatus* suggest that the size of the nanoparticles plays a significant role in their effectiveness. The small size of the particles facilitates penetration through the larval cell membrane or interaction with S-containing proteins and DNA, leading to the denaturation of proteins and nucleic

acids. This results in severe damage to cellular membranes, disruption of proton motive force, and ultimately, cell death.

CONCLUSION

This findings in this study has revealed the larvicidal potential of different nanoparticles with silver nanoparticles (TCA and AA) showing the highest lethal effect on larvae of *Cx. Quinquefasciatus* in the shortest of exposure time. These larvicidal effect observed in all the nanoparticles may be as a result of the presence of bio-active compounds that caused significant level of changes in the larvae. These nanoparticles are a potential alternative for the development of eco-friendly larvicide against *Cx. quinquefasciatus*.

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