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Effect of Different Light Regimen on Larvicidal Activities of *Vitellaria paradoxa* Leaf Mediated Silver Nanoparticles against Malaria Vector, *Anopheles gambiae* s. l.

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Abstract

Mosquitoes have been known to spread diseases of public health concerns to human of which malaria fever is the most prevalent globally. In order to develop environmentally friendly vector control method, plant-based nanoparticles have attracted considerable attention in recent times. Research has shown that environmental factors such as light duration during development interfere with insects' development and responses. In this study, different light regimen was explored in testing the effect of different light regimen on larvicidal potential of synthesized silver nanoparticles (AgNPs) solution of *Vitellaria paradoxa* (Shea butter) leaf. This was tested against 3rd to 4th larval stages of *Anopheles gambiae* sensu lato. Zeta potential shows the average size of synthesized silver nanoparticle to be 62.36 nm with 1 mM of AgNO₃ at 5 minutes. UV spectrometry shows its absorbance at the wavelength of 411.5 peaking at 1.702 absorbance. Thermo-gravimetric and Differential Thermal Analysis revealed an initial weight loss at 110°C and further degradation between 440°C and 700°C which shows its stability. Larvicidal bioassay showed that the mean mortality was not significant at $p < 0.05$ for larvae reared under 6 hours and 18 hours of light. The mean mortality was significant for those reared under 12 hours of light with LC₅₀ and LC₉₀ of 24.99 and 49.88 mg/l respectively, R² value being 0.99. Therefore, through this study, it could be deduced that light duration has a significant effect on the responses of *Anopheles gambiae* s. l. and there is the possibility of enhancing larvicidal efficacy of plant mediated silver nanoparticles by alteration of light duration on the developing larvae.

Keywords: Light regimen, silver nanoparticles, larvicide, *Anopheles gambiae*, *Vitellaria paradoxa*

Introduction

Diseases that are transmitted by vectors have become a threat to human health. Most common of these vectors include mosquitoes. Mosquitoes are known to transmit malaria, filariasis, encephalitis, dengue and yellow fever [12]. Over half of the world's population is at risk of Malaria fever and there were about 229 million cases of malaria across the world in 2019 [41] leading to millions of death. 94% of cases and death occur in sub-Saharan Africa, 23% of which occur in Nigeria, where malaria accounts for 13% to 50% of all medical reasons for school absenteeism. [41] *Anopheles* mosquitoes are the culprits when it comes to the transmission of malaria parasite, plasmodium, which has been plaguing many countries in different parts of the world. *Anopheles* mosquito undergo complete metamorphosis with for larval stages designated as L1-L4

which is the most vulnerable of the life stages [37] and is thereby focused on for delivery of an effective control [22]. Several methods have been employed in larviciding in the bid to control malaria vector as a preferred option in vector control because larvae occur in specific areas and can thus be more easily controlled. Treatment of mosquito breeding sites provides control over the larvae before the biting adults appear and are dispersed from such sites. [32]. Control of vectors has been majorly by using synthetic chemicals. This method though effective has not been potent in ameliorating the disease burden caused by these vectors [36] because they have proven not to be environmentally friendly. It has led to resistance in mosquitoes, mammalian toxicity and accumulation of residue in the food chain and thereby affecting non target organisms [42, 12].



As there is a rising need for an environmentally friendly, cost effective and fast method of control, nanotechnology has become hopeful. The use of biological methods in synthesizing nanoparticles would help to remove harsh, rigorous and expensive processing conditions of chemical methods by enabling the synthesis at physiological pH, temperature, pressure, and at the same time at lower cost [39] and also fulfill the disadvantages of the synthetic insecticides. Silver nanoparticles (AgNPs) have been synthesized using different organisms belonging to four kingdoms out of five kingdoms of living organisms i.e., Monera (prokaryotic organisms without true nucleus), Protista (unicellular organisms with true nucleus), fungi (eukaryotic, saprophyte/parasite), plantae (eukaryotic, autotrophs) and animalia (eukaryotic, heterotrophs). Nanoparticles synthesized by plant extracts are of more focus because the production of these are not time consuming, are cost effective and poses less threat to the environment [39].

Generally, plants that are used in synthesizing silver nanoparticles are those that have been identified as having phytochemicals which are capable of reducing silver nitrate to produce nanoparticles. More than 80 plants species have been employed in the successful synthesis of nanomoscitocides, with particular reference to larvicidal purposes and more researches are still underway [9]. This study focused on using *Vitellaria paradoxa* in the synthesis of silver nanoparticles.

Vitellaria paradoxa (Gaertn F.), commonly known as Shea tree, is locally called 'kareje' in Fulfulde, 'kadanya' in Hausa, 'okwuma' in Igbo, 'ikini' in Taroh, 'munameng' in Cham [6] and 'igi emi' in Yoruba language. It is a member of the Sapotaceae family and the only species of the genus *Vitellaria* [3]. The Shea tree grows naturally in the wild in the dry savannah belt of West Africa [11] and is abundant in Nigeria in the derived Savannah zones, particularly near towns and villages. In Africa, where the species occurs predominantly, the seed fat is used for cooking, in lighting of lamps, soap and pomade preparations as well as for medicinal purposes [17, 2]. Other parts of the plant have also been reported to possess various medicinal properties [25].

In using plant-based silver nanoparticle as larvicide, there is need to consider the interaction with their environmental. Some factors like light, temperature, pH play a vital role in development of insects. Research has shown that light influences growth of insects [20] most especially at the larval stage. Studies have shown the differential responses of insects to photoperiodic conditions indicating that some insect species show faster growth under short day length [27] while others may experience diapauses. Ukubuiwe et al. reported that some insects may show no changes to the varying photoperiod and some exhibit various developmental times at different photoperiodic conditions. Those raised at shorter day lengths (0 and 6hL) had faster growth while those reared at longer day lengths (18 and 24hL) had slower growth [36]. Therefore, since

photoperiod affects the metabolic rate of mosquitoes and consequently their rate of growth and teneral reserves, information on the impact of environmental manipulations on the biological and physiological responses of mosquito larvae to synthesized larvicide may be very critical in developing efficient and cost-effective control program.

The aim of this study is to synthesize silver nanoparticles from *Vitellaria paradoxa* leaf and assess the effect of light on its larvicidal activities against *Anopheles gambiae* s. l.

Materials and Methods

Materials

Fresh leaves of *Vitellaria paradoxa* were collected from Shiroro local government of Niger State Nigeria. They were identified by botanists in the department of Plant Biology, Federal University of Technology, Minna, Niger state, Nigeria. Silver nitrate crystal was acquired from the Biochemistry department laboratory of Federal University of Technology, Minna Niger state.

Rearing of larvae.

First instar *Anopheles gambiae* s. l. larvae (L1) were collected from Bosso dam, Minna Niger State, using a scoop and a bowl and properly identified by the entomologists in the Department of Animal Biology, Federal University of Technology Minna, Niger State. The larvae were brought to the laboratory and transferred into a one-liter capacity bowl with 200ml of distilled water for rearing. 40 larvae were reared per bowl under varying photoperiod of 0 hour, 6 hours, 12 hours, 18 hours and the prevailing photoperiod which was 13 hours. LED lamp was used in varying the light, the intensity being maintained at 250-300 lx throughout the exposure time. The mosquito larvae were fed with yeast in ratio 1:3 daily until the larvae developed into late third and early fourth instar ready for experiment.

Preparation of stock solution solution from *Vitellaria paradoxa* leaves

Vitellaria paradoxa leaves were washed with distilled water and then dried in room temperature for two weeks to obtain a crispy texture. A decoction was prepared by placing 40 g of finely grinded leaves in 400 mls distilled water, boiled for 30 minutes and then filtered through Whatman No. 1 filter paper. The filtrate was evaporated into a paste. 1 g of the paste was dissolved in 10 ml distilled water to form the stock solution [40].

Preparation of silver nitrate solution

To prepare silver nitrate solution, 0.017g of silver nitrate crystals was dissolved in 100 ml of distilled water in an Erlen Meyer flask and incubated at room temperature to make 1 mM solution of silver nitrate. The solution was stirred well continuously until the silver nitrate dissolved completely [21].



Synthesis of silver nanoparticles

In synthesizing silver nanoparticles from aqueous leaf extract of *Vitellaria paradoxa*, 1ml of the stock solution of plant extract was added by drops to 99 ml of 1 mM Silver Nitrate (AgNO_3) solution [1, 16] and stirred well continuously with a stirring rod under room temperature. A control was maintained alongside the experimental set with the crude aqueous leaf extract in distilled water without silver nitrate solution [7]. The reduction of Ag^+ was confirmed from the UV-Vis spectrum of the solution at different times – 5 mins, 6 hours, 12 hours and 24 hours. A stabilizer was added to the synthesized silver nanoparticle to be taken for characterization to prevent it from further aggregation which will lead to an increase in particulate size [4, 14].

Characterization of synthesized silver nanoparticles

UV-VIS spectrophotometer of UV-1800 series was used to monitor the formation of the nanoparticles with absorbance in the range of 200-800 nm at room temperature, operated at a resolution of 1nm with fast scanning speed under x5 dilution. The weight loss and the reaction type of synthesized AgNPs was examined by Thermogravimetric and Differential thermal Analysis (TGA/DTA) in temperature range of room temperature to 1000°C where Al_2O_3 was used in heating measurement, carried out in air atmosphere at 20°C/min heating rate to determine the weight loss. The nanoparticle and zeta potential of AgNO_3 was analyzed on particle size analyzer system (Zeta sizer, Malvern Instruments Ltd, USA). Zeta potential cell was washed with ethanol and de-ionized water followed by AgNPs sample. The average distribution of nanoparticles based on intensity was observed [7].

Larvicidal bioassay

Preliminary screening was first carried out to determine the lethal concentrations of the synthesized silver nanoparticles. Mosquito larvae were exposed to a range of concentrations from 0.01% - 1.5% of synthesized silver nanoparticle representing 10 mg/l – 150 mg/l respectively and maintained under the same light duration in which they were reared for 24 hours in plastic containers of 250 ml capacity [36].

Batches of 20 healthy mosquito larvae ranging from late third instar to early fourth instar were transferred by means of a strainer into the various test concentrations. Four replicates were set up for each concentration with an equal number of controls simultaneously using distilled water as the positive control. The negative control was set up using 1 ml of 1 mM AgNO_3 solution in 99ml of distilled water [7]. The larvae were not fed during treatment [40].

Data analysis

All experiments were carried out in four replicates and results expressed in Mean \pm S.D with $p < 0.05$. Probit regression analysis was used for the determination of LC_{50} and LC_{90} value from mortality rates. Effects of

concentrations and photoperiod were subjected to analysis of variance (ANOVA). Statistical differences between treatments were determined by Duncan multiple range test using statistical packages for social sciences (SPSS 2116.0, 23rd version).

Results and Discussion

Synthesis and characterization of silver nanoparticles

The result showed a colour change from light yellow of the aqueous leaf extract to dark brown (Figure 1) which signifies the reduction of Ag^+ in silver nitrate (AgNO_3) to Ag^0 by the phytochemicals in the aqueous leaf extract. This indicates the formation of silver nanoparticles. The appearance of a brown colour may be as a result of excitation of Surface Plasmon Resonance (SPR) typical of AgNPs [1]. An increase in the contact time increases the intensity of the localized surface Plasmon resonance, reflecting an increase in the concentration of the nanoparticles up to a point before the concentration decreases. With UV-VIS spectrophotometer, localized Surface Plasmon Resonance band which was visible at 5 mins showed maximum absorbance at 411.5nm (Plate I). Sujitha et al. reported a peak at 410 nm wavelength with Aloe vera extract. The wavelength at which the SPR appears varies with type of plant and the part of the plant being used [35].

As the contact time increases, there was a shift of the peak to the right to 434.5nm, 444.0 nm and 453.0 nm by 6 hours, 12 hours and after 24 hours of incubation respectively as presented in plates II, III and IV respectively. This shift indicates a larger size of nanoparticle which means that as the time increases, the nanoparticles being formed becomes larger because of aggregation. This is in agreement with the report in Elias et al. [7]. In this study, the finest nanoparticle was formed at 5 mins of incubation with smallest peak wavelength of 411 nm.

The broadness of the peak is good indicator of the size of the nanoparticles as shown in Figure 1. As the size increases, the peak becomes broader with an increased bandwidth. This trend is in agreement with the work of other authors characterizing silver nanoparticles synthesized using aqueous plant extracts [24, 19]. It is generally recognized that the UV-VIS spectroscopy could be used to examine the size and shape-controlled nanoparticles in aqueous suspensions [30].

There was no need of adding any buffer, capping agent or stabilizing agent as usually is with chemical method of nanoparticle formulation and some other plant-based synthesis. Srika et al. reported that phytochemicals in plants or other constituents present in the cells act as stabilizing and capping agents, so there is no need of adding capping and stabilizing agents from outside [34]. Rao et al. also reported that plant extracts played dual role of potential reducing and stabilizing agents with an exception in few cases where external chemical agents like sodium-do-decyl sulphate were used for stabilization of the AgNPs [26].



Plate I: Synthesis of silver nanoparticles as shown by colour change at 5 minutes.



Plate II: Synthesis of silver nanoparticles as shown



late III: Synthesis of Silver nanoparticle shown by colour change 12hours



Plate IV: Synthesis of Silver nanoparticle shown by colour change 24 hours

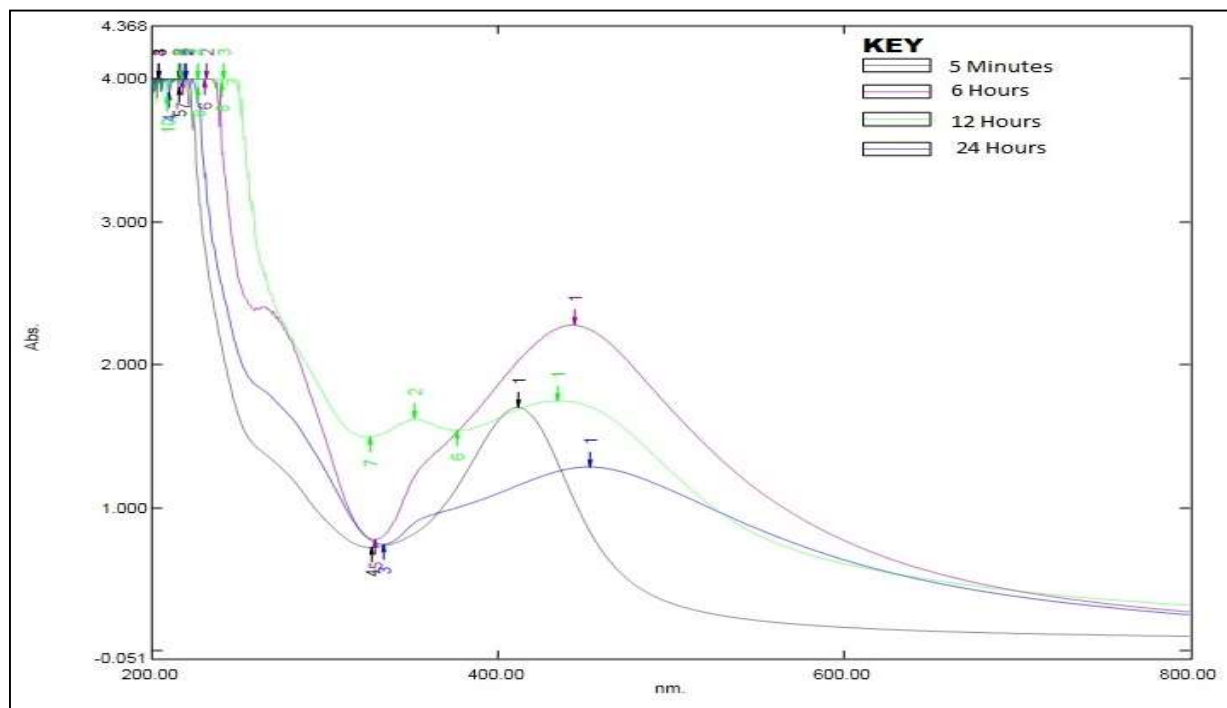


Figure 1: UV-Visible spectrum of synthesized AgNPs at 5 mins, 6 hrs, 12 hrs and 24 hrs of incubation

Thermo-Gravimetric Analysis and Differential Thermal Analysis (TGA/DTA) is presented in Figure 2. The DTA plot displays an intense exothermic peak between 220 and 420°C which is mainly attributed to the crystallization of silver nanoparticles. The DTA profile shows that complete thermal decomposition occurs simultaneously with the weight loss. There was a percentage weight loss between 30°C and 900°C. The initial weight loss of about 2% at the temperature of 110°C was due to loss of water molecules from the AgNPs. The weight loss proceeded in two major steps; the first occurred between 110°C and 190°, while further degradation was observed between 290°C to 400°C. Similar report was presented by Khan et al, 2011 in which the peak temperature was between 220 and 420°. Slight degradation still occurred between temperature 440°C and 700°C. There is almost no weight loss between temperature 27.29°C and 100°C and also above 700°C. This report indicates that the nanoparticles synthesized using aqueous leaf extract of *V. paradoxa* are quite stable. The pattern of weight loss and degradation presented by the

sample generally characterizes stability of metallic nanoparticles [8].

Figure 3 shows the Zetasizer distribution of the synthesized AgNPs by intensity. The average size is 62.36 nm with a single peak at 69.65 nm. Figure 4 shows the Zetasizer distribution of the synthesized AgNPs by volume. It also shows a good average size of 62.36 nm with a single peak at 52.24 nm. The Zetasizer provide an average figure which is the intensity weighted mean hydrodynamic size of the ensemble collection of particles measured by dynamic light scattering (DLS) with average size by intensity and by volume less than 100 nm (62.36 nm). Similar result was reported in other works [33, 15]. A size range of 50-150 nm have been reported for *Eucalyptu hybrid* peel [5] and 50-100 nm for *Alternanthera dentate* leaf [23] and 25-80 nm for *Nelumbo nucifera* leaf [29]. Nano-sized particles of less than 100 nm in diameter are currently attracting increasing attention for the wide range of new applications in various fields of industry [31].

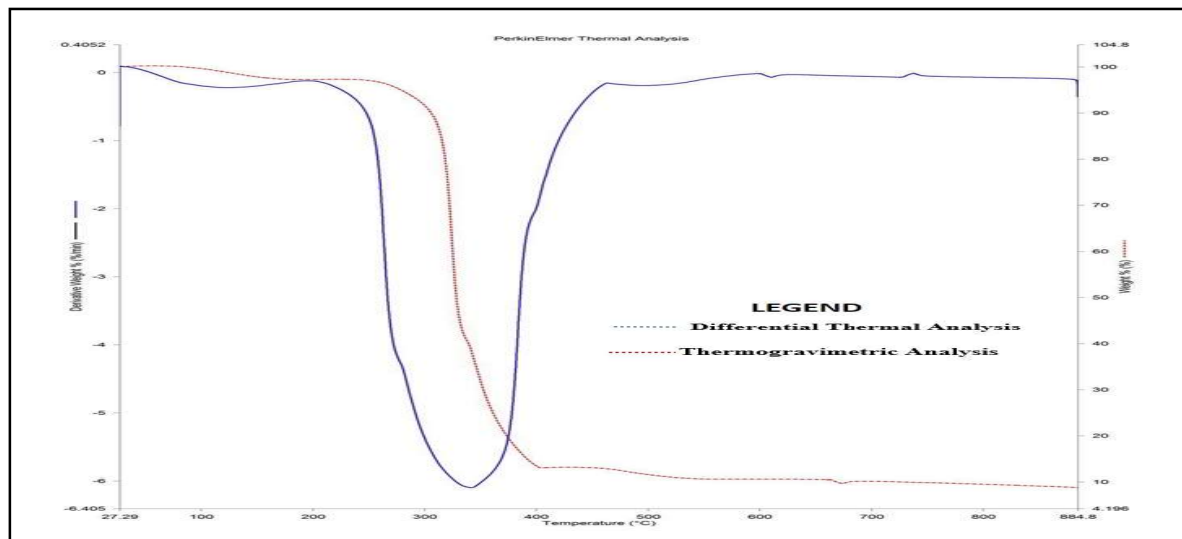


Figure 2: Thermo-gravimetric and Differential Thermal Analysis of synthesized AgNPs of *V. paradoxa* aqueous leaf extract

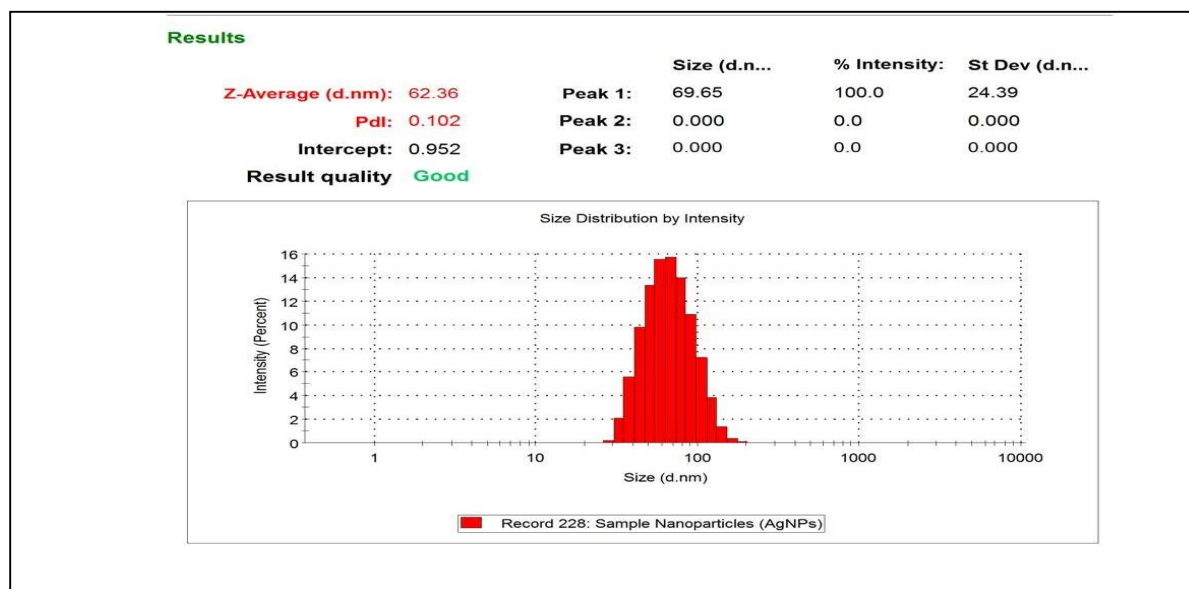


Figure 3: Size distribution of synthesized AgNPs of *V. paradoxa* by intensity

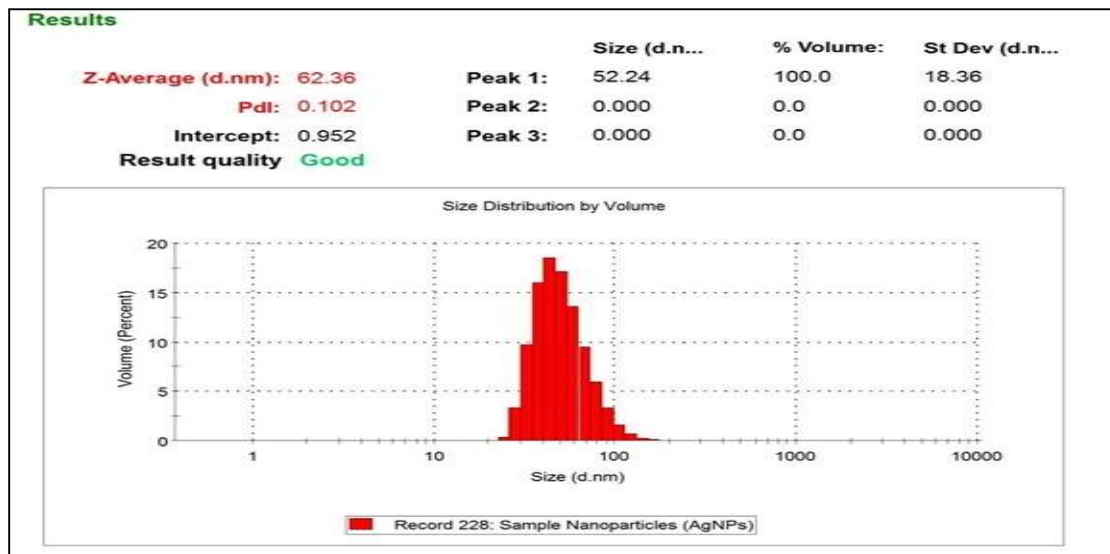


Figure 4: Size distribution of synthesized AgNPs of *V. paradoxa* by volume

Larvicidal activities of synthesized AgNPs under prevailing light duration (13 hours)

Larvicidal activity of synthesized AgNPs against 3rd to 4th instar larvae of *An. gambiae* s. l. within 24 hours exposure at prevailing light hours is presented in Table 1 and Figure 5. The synthesized AgNPs became lethal at 5.00 mg/l and mortality increases with concentration. Larvicidal activity was observed first at 18hrs, 12hrs, 6hrs, 3hrs, 2hrs and 1hr of exposure with 5.00 mg/l, 10.00 mg/l, 30.0 mg/l, 50 mg/l, 100 mg/l and 150 mg/l respectively. This confirms that mortality increases with concentration and it is dose dependent. This result was supported by other researchers [7].

It was also observed that the rate of mortality peaks at a point during treatment with silver nanoparticles. By 12 hours of exposure, majority of the larvae had died after which the rate of mortality gradually drops. There was no mortality in water (positive control), whereas there was 100% mortality within 1 hour of exposure to 1mM silver nitrate solution (AgNO₃). Table 2 shows the percentage

mortality as the concentration of the nanoparticle increases. 100% mortality was observed at 12 hours with 150 mg/l concentration. No mortality was observed in the distilled water which is the control and 1mg/l concentration of synthesized silver nanoparticle (Table 2).

The LC₅₀ and LC₉₀ were 66.294 and 135.108 mg/l as shown in Table 4. This is different from what was reported by Sivapriyajothi *et al.* in which the LC₅₀ and LC₉₀ were reported to be 22.10 and 41.28 ppm respectively for *Ae. Aegypti*, 19.17 and 37.23 ppm respectively for *An. stephensi* with *Leucas aspera* leaf mediated silver nanoparticle under 14 hours of light [33]. This difference may be due to species of *Anopheles* species and the plant used in synthesizing the silver nanoparticles. Increasing concentration of the silver nanoparticles is significant on the mortality of the larvae at $p > 0.05$. There was no significant difference between the mortality at 18 hour and 24 hours. This may be as a result of aggregation of the nanoparticles over time [7, 10] Utpal *et al.* also reported a drop in the mortality rate with time [38].



Table 1: Larvicidal Activities of Synthesized Silver Nanoparticle (AgNPs) against *An. gambiae* s. l. at 13 hours light (Prevailing light hours)

TIME	30mins	1hr	2hr	3hr	6hr	12hr	18hr	24hr
Concentration (Mg/l)								
1.00	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
5.00	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.25±0.18 ^b	2.00±0.01 ^b
10.00	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.25±1.31 ^b	2.50±0.64 ^b	3.75±0.07 ^b
30.00	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.00±0.01 ^b	1.75±0.05 ^b	3.00±0.32 ^c	2.75±0.21 ^b
50.00	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.50±0.05 ^a	1.75±0.05 ^b	1.75±0.57 ^b	3.00±0.01 ^c	6.25±0.23 ^c
100.00	0.00±0.00 ^a	0.00±0.00 ^a	4.00±0.03 ^b	8.25±0.03 ^b	10.75±1.25 ^c	13.00±0.41 ^c	13.25±0.18 ^d	14.75±0.21 ^d
125.00	0.00±0.00 ^a	0.00±0.00 ^a	6.00±0.14 ^c	9.75±0.12 ^b	13.00±1.16 ^d	15.00±0.08 ^d	16.25±0.41 ^c	17.50±0.31 ^e
150.00	0.00±0.00 ^a	8.00±0.12 ^b	14.5±1.21 ^d	17.50±1.01 ^c	19.00±1.17 ^e	20.00±0.00 ^e	20.00±0.00 ^f	20.00±0.00 ^f
(control)AgNO₃ 1.70	0.00±0.00 ^a	20.00±0.00 ^c	20.00±0.00 ^e	20.00±0.00 ^e	20.00±0.00 ^f	20.00±0.00 ^e	20.00±0.00 ^f	20.00±0.00 ^f
(control)Water	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

*Values are represented in Mean ± SE

*Values on the same column with same alphabet are not significant at $p > 0.05$

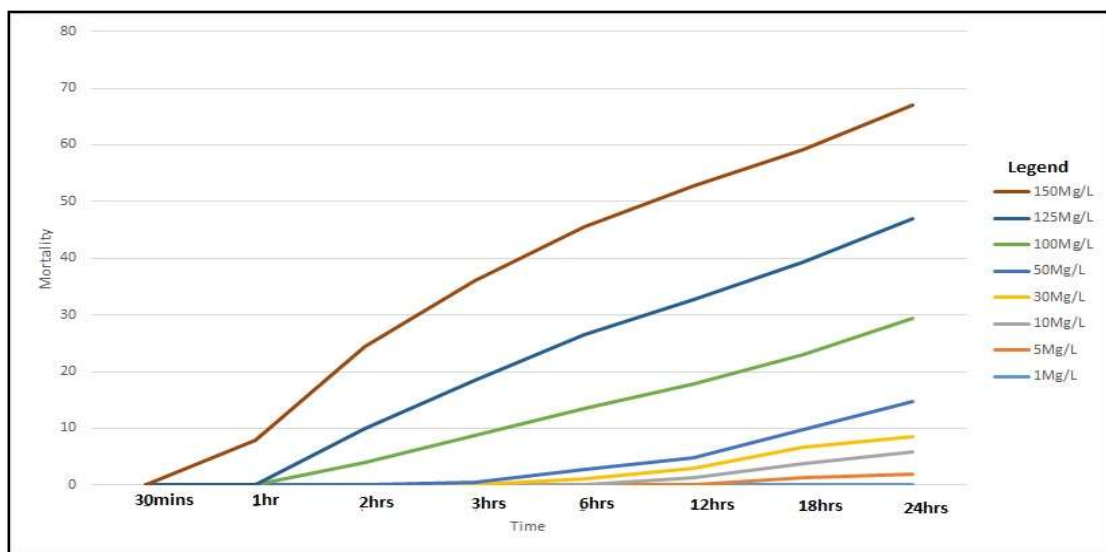


Figure 5: Larvicidal Activities of Synthesized Silver Nanoparticle (AgNPs) against *An. gambiae* s. l. at prevailing light duration (13 hours)



Table 2: Percentage mortality *An. gambiae* s. l. larvae in synthesized AgNPs of *V. paradoxa* at 12hrs and 24hrs exposure (13 hours of light)

EXPOSURE TIME	CONCENTRATION (mg/l)							
	1.0	5.0	10.0	30.0	50.0	100.0	125.0	150.0
12HR	0.00±0.00 ^a (0.00)	0.00±0.00 ^a (0.00)	1.75±0.05 ^b (6.75)	1.75±0.05 ^b (8.75)	2.75±0.21 ^b (2.50)	5.75±0.25 ^d (31.25)	4.00±0.03 ^c (75.00)	10.50±1.25 ^e (100.00)
24HRS	0.00±0.00 ^a (0.00)	3.00±0.32 ^b (10.00)	8.75±0.03 ^c (18.75)	11.00±1.25 ^d (13.75)	13.5±0.41 ^e (31.23)	18.25±1.16 ^f (73.75)	19.5±1.17 ^f (87.50)	20.00±0.00 ^g (100.00)

*Values in parenthesis represent the percentage mortality.

* Values with different superscript alphabet are significant at $p > 0.05$.

Larvicidal activities of synthesized AgNPs against *An. gambiae* s.l. at different light regimen

Table 3 presents the percentage mortality of *An. gambiae* s.l. with synthesized AgNPs at different light regimen. It was observed that larvae reared and tested at 12 hours of light experienced 100% mortality for all test concentrations as represented in Figure 8. Those reared at 0, 6 and 18 hours of light showed 100% mortality with only 50 and 100 mg/l concentrations of synthesized AgNPs while those reared under the prevailing light hours only showed 73.75% mortality at the highest concentration after 24 hours. This shows that larvae reared and tested at 12 hours of light were most affected by the synthesized silver nanoparticles. The LC_{50} and LC_{90} were very low at 24.99 and 49.88 mg/l respectively. Those reared at prevailing photoperiod were the most tolerant to the synthesized

silver nanoparticle with LC_{50} and LC_{90} of 66.249 and 135.10 mg/l respectively (Table 4). There was no mortality in the positive control, there was 100% mortality in $AgNO_3$ being the negative control within 1 hour of exposure.

To the best of the researcher's knowledge, larvicidal activities have not been carried out under varying photoperiod but other researchers have investigated larvicidal activities under specific light duration. Utpal *et al.* reported that under 14:10 (light: dark) condition, *An. stephensi* had an increased mortality which gradually reduced with time [38]. Toxicity of *Leucas aspera* mediated silver nanoparticle against fourth instar larvae of *Ae. Aegypti* was reported to show LC_{50} and LC_{90} of 22.10 and 41.28 ppm and *An. stephensi* showed 19.17 and 37.23 ppm respectively under a light duration of 14 hours (14:10 Light:Dark) [33].



Table 3: Percentage mortality of *An. gambiae* s. l. with synthesized AgNPs of *V. Paradoxa* Leaf after 24 hours exposure at varying light hours

AgNPs conc. (mg/l)	0hr	6hrs	12hrs	Prevailing Photoperiod (13 hrs)	18hrs
10	10.50±0.65 ^c (52.50)	2.25±0.63 ^a (11.25)	20.00±0.00 ^d (100.00)	4.50±1.32 ^b (22.50)	10.50±1.04 ^c (52.50)
50	20.00±0.00 ^b (100.00)	20.00±0.00 ^b (100.00)	20.00±0.00 ^b (100.00)	6.25±0.75 ^a (31.25)	20.00±0.00 ^b (100.00)
100	20.00±0.00 ^b (100.00)	20.00±0.00 ^b (100.00)	20.00±0.00 ^b (100.00)	14.75±0.25 ^a (73.75)	20.00±0.00 ^b (100.00)
Water	0.00±0.00 ^a (0.00)	0.00±0.00 ^a (0.00)	0.00±0.00 ^a (0.00)	0.00±0.00 ^a (0.00)	0.00±0.00 ^a (0.00)
AgNO₃	20.00±0.00 ^a (100.00)	20.00±0.00 ^a (100.00)	20.00±0.00 ^a (100.00)	20.00±0.00 ^a (100.00)	20.00±0.00 ^a (100.00)

*Values in parenthesis represent the percentage mortality.

*Values with the same superscript alphabet along the same row are not significant at $p < 0.05$

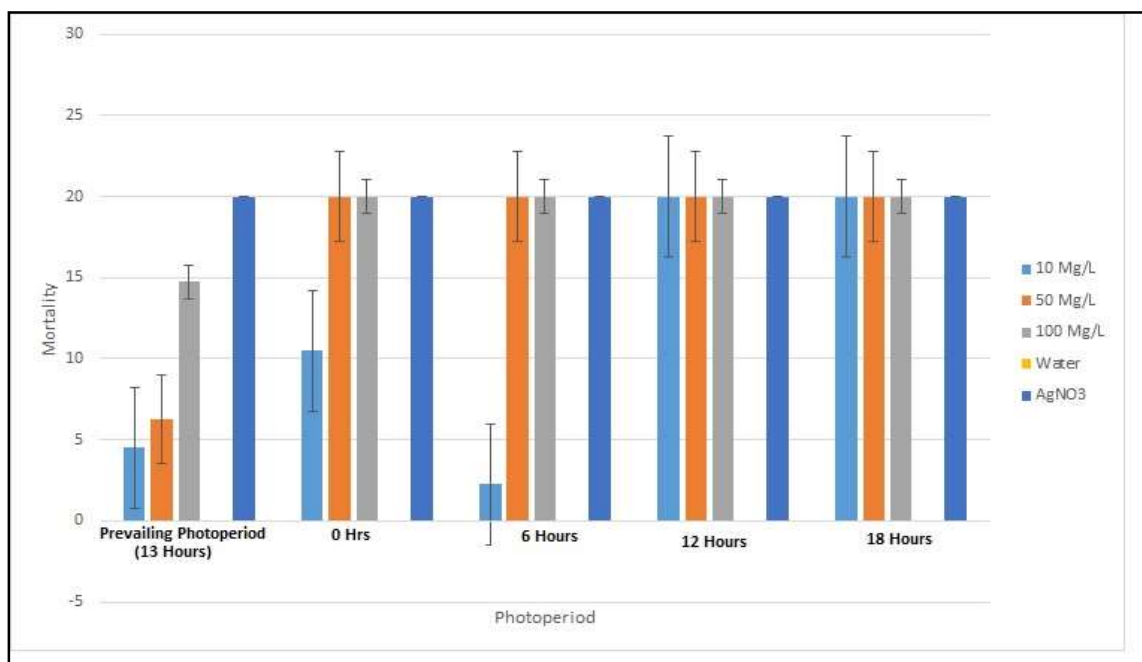


Figure 6: Mortality rate of *An. gambiae* s. l. in different concentrations of AgNPs under different light regimen.



Table 4: LC₅₀, LC₉₀ and Regression for Synthesized AgNPs of *V. paradoxa* against *An. gambiae* s.l. at different light regimen

	0hr	Photoperiod 6hrs	12hrs	Prevailing Photoperiod 13 Hours	18hrs
LC ₅₀	14.1604	31.746	24.99	66.249	13.40
LC ₉₀	64.8597	74.045	49.88	135.108	65.49
R ²	0.6928	0.6927	0.9959	0.9131	0.7095
Regression equation	y=0.5062x+57.168	y=0.9457x+19.976	y=0.0002x+99.978	y=0.5809x+11.516	y=0.507x+56.795

- LC₅₀ is the median lethal concentration.
- LC₉₀ is the upper lethal concentration.

Conclusion

In this study, *Vitellaria paradoxa* leaf was used for the reduction of silver nitrate to form silver nanoparticles. This was due to its readily availability in the savanna belt of Africa. It was proven of being capable of delivering nanoparticles within a short time without any buffer or capping agent. The silver nanoparticles so synthesize have been proven to be stable and are effective as a larvicide against *Anopheles gambiae* s. l. as it is a biological way of getting environmentally friendly and effective control and a shorter

time of delivering a high mortality rate at low lethal concentrations. However, the effectiveness of the synthesized silver nanoparticles as larvicide is significantly affected by the duration of light to which the larvae has been exposed during their development and treatment. Therefore, this information is critical for its use for an effective mosquito control approach.

Declaration of conflicting interests

The author declared no potential conflicts of interest

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