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Biosynthesis of silver nano composite using agro waste and its larvicidal properties

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Abstract
Mosquito has attracted considerable attention in the medical and social regions of the world. They are the vectors of many diseases accounting for huge mortality and morbidity worldwide. In the present experiment we prepared a silver nano composite using paddy straw and tested its potential larvicidal activities against *Aedes aegypti* and *Anopheles gambiae* larvae at 25, 50, 100 ppm concentration. The synthesised nanoparticles using paddy straws were characterised by Ultra Violent resonance, Fourier transform infrared spectroscopy, Scanning Electron Microscopy and Energy-dispersive X-ray spectroscopy analysis. In larvicidal assay, the fourth instar larvae are exposed to different concentrations of silver nano composite aqueous extract for 24 hours. From the experiment it is revealed that the mean concentration to kill 50% of larval population is found to be 13.625 ppm for *Aedes aegypti* and 12.270 ppm for *Anopheles gambiae*.

Keywords: Silver nano composite, larvicidal, *Aedes aegypti* and *Anopheles gambiae*

1. Introduction
Mosquitoes widely transmit pathogens that spread endemic diseases among human beings and animals. It is reported that approximately 1.5 million new cases of malaria are recorded every year in India and it also ends up in nearly 1000 deaths [1]. According to 2006–2007 survey in India regarding the epidemics of mosquito-borne diseases concluded 1.4 million suspected and 2000 confirmed cases of Chikungunya virus and 1000 deaths from Japanese encephalitis [2]. So it is high time for Vector control by developing new and improved mosquito control methods that are economical and effective. The vector control methods should also be safe for non-target organisms and the environment. Nanoparticles are recognised for their unique optical properties. The metals like gold and silver have a broad absorption band in the visible region of the electromagnetic spectrum [3, 4]. The properties of these metals are used in advanced medicine, opto-electronics, chemical catalysis, sensors, drug delivery, and for etching and cutting as they are able to change their shape, size, and the surrounding medium [5,6,7,8,9,10].
To escape from the biting of mosquitoes people must use repellents and target larval stage of mosquito as it would be the better strategy to lower the incidence of mosquito-transmitted diseases. In larval stage have less mobility in breeding habitat, so planning control measures at this stage is comparatively easy [11]. The present practice to control mosquito larvae is the use of insecticides like carbamate, organophosphate and pyrethroids. Mosquitoes died for insecticides but the prolonged usage of it made mosquitoes get resistance towards insecticides [12]. Mosquitoes got varying amount of resistance to commonly used insecticides like temephos, fenthion, malathion and Dichloro Diphenyl Trichloroethane (DDT) [13]. Moreover, chemical insecticides remain a threat to non-target species, their environment as well as their food chain [14]. Considering this, insect control agents from biological sources would always be considered as a safe and effective way.
Several plants like *Azadirachta indica* [15], *Aloe vera* [16], *Plumeria rubra* [17], *Nelumbo nucifera* [18] and *Emblica officinalis* [19], *Medicago sativa* sprouts [20] are screened successfully for silver nanoparticles synthesis by various researchers. Nowadays, phytosynthesised silver nanoparticles as a mosquito larvicidal agent are gaining importance instead of chemical insecticide. This is because of their less harmful effect to non-targeted species and novelty in mechanism of action [21]. It is our dire need to control vector borne diseases as they are the major public health concern at global level.

Once a person is infected with malaria, it involves typical medical treatment and there is report of resurgence of malaria after eradication in many countries [10]. Hence the present study is undertaken to synthesize a nanocomposite using agricultural paddy straw and screen its larvicidal properties against *Aedes aegypti* and *Anopheles gambiae*.

2. Materials and Methods

2.1 Collection of plant materials and preparation of paddy straw extract

Fresh and healthy straw are collected locally and rinsed thoroughly with tap water followed by distilled water to remove all the dust and unwanted visible particles and are placed in a shadow and dried well. Then they were cut into small pieces and ground well to get a powder form.

2.2 Synthesis, characterization, and formulation of silver nanoparticles

10ml of paddy straw extract was added into 90 ml of aqueous solution of 1mM silver nitrate ($AgNo_3$) for the reduction of silver nitrate and kept in a dark room at 37 °C for 24 hours. After 24 hours, colour of the solution changed from green to dark brown indicating the formation of silver nanoparticles. The bioreduced silver nanoparticles solution was measured using UV- Visible absorbance spectrophotometer modified method of Joyti and Singh [22]. The surface group of the nanoparticles is qualitatively confirmed by using Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) to determine the crystalline structure and visible observation by Scanning Electron Microscopy.

2.3 Collection and rearing of Mosquito larvae

Larval strains of *Aedes aegypti* and *Anopheles gambiae* are collected from the Government research Institute of Indian Council for Medical Research, Madurai. Larvae are maintained in enamel tray containing dechlorinated tap water mixed with preparation of dog biscuits and yeast extracts under suitable conditions.

2.4 Mosquito larvicidal bioassay

For bioassay test, IVth instar larvae of *Aedes aegypti* and *Anopheles gambiae* are taken. The larvicidal activity is studied by the standard procedure recommended by WHO [23]. Green synthesized AgNPs are prepared into different concentrations of 25, 50 and 100 ppm with double distilled water. Twenty-five reared early fourth instar stage larvae are transferred by means of the dropper to (100 mL beakers) each concentration and the experiment is performed in three replicates under the laboratory conditions. Mortality of the larvae is calculated at 12 and 24 hr of exposure periods. During the both exposure periods, no food is supplied to the larvae and the percentage of mortality was calculated. The lethal concentrations in parts per million LC_{50} are also calculated using Probit analysis [24].

3. Results

Silver particles which are the primary result of this experiment changed its colour in various concentrations and the aqueous solution of silver ion complex started to change the colour from yellowish brown to dark brown. The synthesis of silver nanoparticles in the solution is further analysed and confirmed by UV-visible spectroscopy. The resonance is observed after 24 hrs at 431nm for the green synthesis.

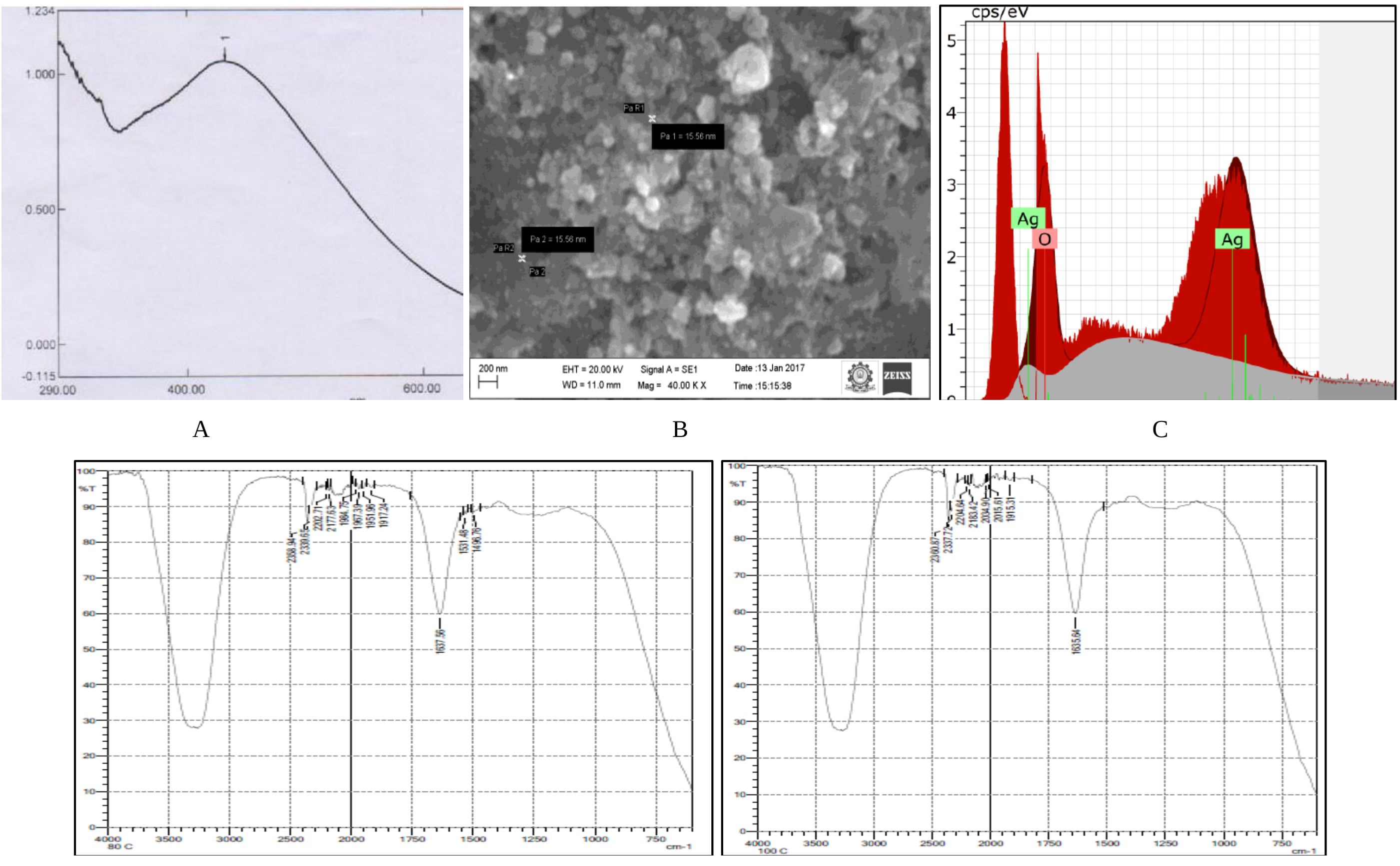

Plate 1: Observations of synthesised nanoparticle (A – UV light absorbance; B – Scanning electron micrograph; C – XRD analysis; D&E – FTIR peaks)

The silver composite prepared in this investigation is found to exhibit uniform sized cubic grains with a range of 15.56 nm particles. The presence of silver particles was also quantified using XRD pattern and obtained a peak at 3 keV. The bioreduction of silver nanoparticles is further confirmed by FTIR spectra of aqueous silver nanocomposite prepared from the paddy straw extract show transmittance peaks at 1496.76, 1637.56, 1917.24, 2202.71, 2339.65 /cm. These peaks indicate that the carbonyl group formed amino acid residues and that these residues capped the silver nanoparticles to prevent agglomeration, thereby stabilizing the medium. During the bioreduction there is a disappearance of many peaks due to the structural change in paddy straw. Different concentrations of 25, 50 and 100 ppm silver nano composite using paddy straw are prepared and observed for mortality of mosquito larvae of *Aedes aegypti* and *Anopheles gambiae*. From the experiment it is found that maximum number of mortality is found to be present in the concentration of 30 ppm in both the experimental larvae when compared to other concentrations (Table 1). No mortality is recorded in control sample. Synthesized nano particles from paddy extract are more active against the *Aedes aegypti* species presenting LC_{50} value of 13.625 when compared to LC_{50} value of 12.270 *Anopheles gambiae*.

4. Discussion

The typical Silver nanoparticles spectrum has a maximum absorption in the range of 420-450 nm. This absorption is a unique property of metal nanoparticles called SPR (Surface Plasmon Resonance) that arise due to conduction of electrons on the surface of silver nanoparticles. SPR for different metal nanoparticles synthesised using different substances are reported in previous studies such as, for gold nanoparticles it is around 540 nm Das *et al*. [25] and 315 nm for Zinc sulphide (ZnS) nanoparticles Hudlikar *et al*. [26]. In the present study the absorption spectrum is found to be 431 nm.

In the present study, the larvicidal activity of silver nitrate nanoparticle may be due to the direct attack mechanism on the larva and is supported by Naresh *et al.* [27] who used silver nanoparticles for the control of mosquito causing diseases like Malaria, dengue and filariasis. While comparing the prokaryotic systems, the silver nano particles have multiple targets for biocidal activity by causing structural damages to the organism Kim *et al.* [28], generation of reactive oxygen species and interfering with DNA replication (Liau *et al.* [29] and Feng *et al.* [30]. Administration of silver nanoparticles *in vivo* to Zebra fish embryos by Lee *et al.* [31] increased deformation rates and ultimately led to death. The mechanism which causes the death of the larvae could be the ability of the nanoparticles to penetrate through the larval membrane. The silver nanoparticles in the intracellular space can bind to sulphur containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes according to Rai *et al.* [32]. The mortality effect of silver nanoparticles on mosquito larvae may be enabled by the small size of the particles, which allows passage through the insect cuticle and into individual cells where they interfere with molting and other physiological processes.

5. Conclusion

From the experiment it is revealed that low concentration of the prepared nano composite can effectively kill the larvae of the mosquito but still field study is essential to confirm the activity. In the future, the mode of action of nanoparticles on the larvae can also be studied using latest methodologies.

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Table 1: Larvicidal activity of synthesized silver nanoparticles using paddy straw leaf extract against *Aedes aegypti* and *Aedes gambiae* larvae.

Mosquito species	Concentration (ppm)	Mortality	LC_{50}
Aedes aegypti	Control	0	13.625
	25	3±1.52	
	50	13±2.00	
	100	22.67±1.52	
Anopheles gambiae	Control	0	12.270
	25	3.67±1.52	
	50	17.33±2.51	
	100	23.66±1.52	

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