Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 12(X), 2023:
http://dx.doi.org/10.7770/safer-V12N1-art2734

Efficacy of Biosynthesized Silver Nanoparticles using Leaf extract of *Melia azedarach* against *Spodoptera frugiperda* (Lepidoptera: Noctuidae).

Eficacia de nanopartículas de plata biosintetizadas usando extracto de hoja de Melia azedarach contra Spodoptera frugiperda (Lepidoptera: Noctuidae).

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ABSTRACT

The present investigation was carried out to the study of biosynthesized AgNPs using leaf extracts of *M. azedarach* in *S. frugiperda*. The UV spectrum of the aqueous medium containing silver nanoparticles showed an absorption peak at around 400 nm. FTIR was performed to identify the functional groups related to different chemical compounds. XRD pattern confirmed the formation of nanocrystals in nature. SEM has been used to investigate the morphology of prepared AgNPs. Third instar larvae were exposed to different concentrations of synthesized AgNPs for 24 hrs. The highest mortality was observed in aqueous extract and synthesized AgNPs against third instars larvae of *S. frugiperda* of LC50 and LC90 values of 36.83(28.05-57.78), 85.71(55.65-425.73) and 25.44(18.27-33.60), 58.65(41.82-143.87) respectively. The silver nanoparticle was found to larvicidal activity against *S. frugiperda.*

Key words: *M. azedarach*, UV, FTIR, XRD, SEM.

RESUMEN

La presente investigación se dedicó al estudio de AgNPs biosintetizadas utilizando extractos de hojas de M. azedarach en S. frugiperda. El espectro UV del medio acuoso que contenía nanopartículas de plata mostró un pico de absorción en torno a los 400 nm. Se realizó FTIR para identificar los grupos funcionales relacionados con diferentes compuestos químicos. El patrón XRD confirmó la formación de nanocristales en la naturaleza. SEM se ha utilizado para investigar la morfología de AgNP preparados. Las larvas de tercer estadio se expusieron a diferentes concentraciones de AgNP sintetizadas durante 24 horas. La mayor mortalidad se observó en extracto acuoso y AgNPs sintetizados contra larvas de tercer estadio de S. frugiperda con valores de CL50 y CL90 de 36.83(28.05-57.78), 85.71(55.65-425.73) y 25.44(18.27-33.60), 58.65(41.82-143.87) respectivamente. Se encontró que la nanopartícula de plata tiene actividad larvicida contra S. frugiperda.

Palabras clave: *M. azedarach*, UV, FTIR, XRD, SEM.

INTRODUCTION

The Fall Army Worm *S. frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a migratory insect pest known to cause massive destruction of maize crops under warm and humid conditions in the Americas. In particular, this Army worm has been causing severe damage to irrigated maize crops in southern India, especially in Tamil Nadu, Kerala and Karnataka since 2018, causing great economic loss to farmers. In maize growing areas, the fall army worm spreads rapidly over a distance of 200 to 500 km with the help of front winds, during which the impact of pests is greatest from March to September. Bio-pesticides have a broad spectrum of activity and are therefore considered to be relatively safe for the environment and living organisms in their mode of operation (Thakkar *et al*., 2009). Nanotechnology is a recent discipline which has been also used in pest control.The $AgNO_3$ nanoparticles have been identified as a viable replacement for synthetic chemical insecticides because they are less likely to harm the environment. In this study, the larvicidal effect of synthesized AgNPs using the leaf extract of *M. azedarach* indicating the industrial prospects for its large scale and low cost synthesis to control pest.

MATERIALS AND METHODS

Collection and extraction of plant materials: The present investigation was carried out at P.G & Research Department of Zoology, Raja Doraisingam Govt. Arts College, Sivagangai, Tamil Nadu and India from September 2019 to March 2020. The *A.indica* plant was selected for the study on the basis of availability, free from the insect attack and pungent smell. Leaves of selected plants were collected in the month of September 2019 from Anaimavali village, Sivagangai district. The Plant was identified in Herbarium Centre, Dept. of Botany, Raja Doraisingam Govt. Arts College, Sivagangai and Tamilnadu. The leaves were shade-dried at room temperature and coarsely powdered in a powder machine. 10g of dried leaf powder was subjected to Soxhlet extraction using 90ml different solvents. The extraction of each plant samples was done in about 12 hrs. After the period of extraction, the content was filtered through Whatman No.1filter paper and solvent was removed by using the rotary vacuum evaporator at 40°C. The crude extract was obtained and stored in refrigerator at 4°C until further use.

Biosynthesis of Silver Nanoparticles: 20gm powder of selected leaf powder was taken separately in 500ml conical flask along with 200ml of distilled water and then boiled in mixture of 40°C in Soxhlet apparatus. Filtered extracts were collected from it. Silver nitrate is prepared from (1mM Ag solution) 180ml and 20ml leaf extract. This solution was transferred to 250ml of conical flasks and kept in shaker for 24 hrs. The colour change indicates the synthesis of silver nanoparticles. The samples were subjected to centrifugation process of 12000rpm for 20minutes. The supernant was discarded. The pellet was collected and kept in oven for powdering process. Plant mediated silver nanoparticles using the leaf extract upon evaluation resulted in silver nanoparticles formation and was confirmed by different characterization techniques.

Characterization of Silver Nanoparticles

UV-visible spectroscopy analysis: Synthesis of AgNPs can be easily reducing the respective metal ion solution by water leaf extract may be easily detected by UV-vis spectroscopy (Shimadzu; UV 1700 Double Beam Spectrophotometer). The absorption spectra in both extraction and metal ion concentration were measured in 304-700nm range. Synthesized AgNPs provided peaks in visible region of the electromagnetic spectrum around 400nm, which confirmed the formation of nanoparticles.

Measurement by FT-IR: FTIR, the reaction mixture was centrifuged at 6000rpm for 15 minutes. The pellets were washed for three times with 20ml of distilled water to get rid of other compounds. The sample was dried and thoroughly ground with KBr and then analysed. Shimadzu 8400S was used for the measurement of the samples and using a spectral range of 500-4000 m−1 with a resolution of 4cm−1. The FTIR spectrum of leaf extract measured before and after the synthesis of NPs was then analysed for analysis of potential functional groups for the synthesis of AgNPs.

X-Ray diffraction measurements: Structural analysis of synthesized nanoparticles using XRD. Nanoparticles were loaded in XPERTO-PRO X-Ray diffractometer operating at 40kV and 30mA current. The scanning was carried out with 1θ angle from 20° to 80° at 0.02°/min, with 2θ time constant. The crystalline domain size was calculated from the width of the XRD peaks, assuming that they are free from non – uniform strains, using the Debye –Scherrer's formula.

$$D = K\lambda/\beta Cos\theta.$$

When D is a average particle size, K is Scherrer constant (0.94), λ is the X – ray wavelength radiation (1.5406A^{o}), β is the Full width at Half Maximum (FWHM) of the peak. θ is the diffraction angle.

SEM analysis

Morphological study of synthesized silver nanoparticles was done using high resolution SEM analysis (SEM Zeiss EVO 40). The sample was coated on a clean glass by a simple drop coating with suspended silver solution. Then solvent (water) was allowed to evaporate. Then sample was left to dry at room temperature.

Insect culture

Larvae of *S. frugiperda* (Lepidoptera: Noctuidae) were collected from the infested maize field of Saloor village, Sivagangai district, Tamil Nadu, India, and cultured at room temperature (27 ± 2ºC) in the insectary. The pest was identified with the help of Zoological Survey of India (ZSI), Calcutta. The larvae were fed with a standard artificial diet. The laboratory-reared third instar larvae were used for the bioassay test.

Larvicidal activity of *S. frugiperda*

The Larvicidal activity was examined using the leaf dip method (Basker *et al.,* 2010). The maize leaf discs were dipped in different concentrations of synthesized AgNPs in *A.indica*. After 24 hrs of treatment, the larvae were continuously maintained on the non-treated fresh maize leaves. Diet was changed every 12 hrs. Larval mortality was recorded after 24 hrs of treatment. Five replicates were maintained for each treatment with 10 larvae per replicate (Abbot, 1925).

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead Larvae}}{\text{Initial Number of Larvae}} \times 100$$

Data analysis

The average larval mortality data were subjected to probit analysis for calculating LC50 and LC90 values were calculated by using the Finney (1971) method. SPSS software package 26 versions were used. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

UV analysis:

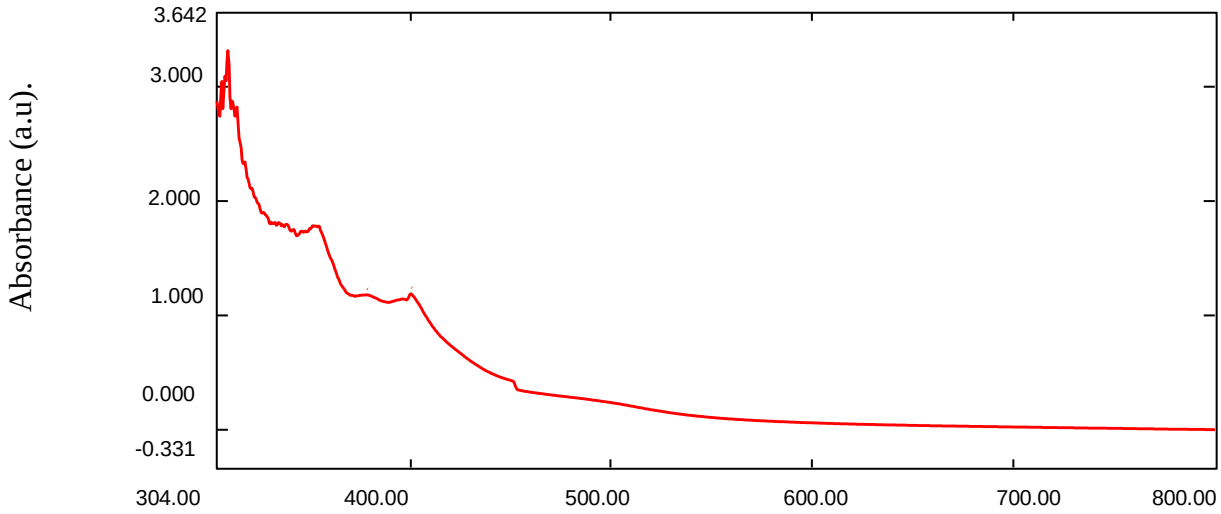

Figure 1: UV- VIS Absorption Spectra of AgNO3 Synthesized from *M. azedarach*

The analysis of UV-Visible spectroscopy data revealed an appearance of surface Plasmon resonance peak in the UV-vis absorption spectra of the silver nanoparticles synthesized by biological reduction showed maximum absorption of peak at 400 nm in wavelength in *M. azedarach*(Fig: 1). The aqueous $AgNO_3$ solution turned into brown colour within 1 hrs of addition of *M. azedarach* leaf extract. In UV spectral analysis, the generation of color was due to excitation of surface Plasmon in metal nanoparticles (Mulvaney 1996).

FTIR Analysis

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the silver nanoparticles synthesized by plant leaf extract. The FT-IR spectrum of *Melia azedarach* confirmed the presence of peaks at 3144.12 cm^{-1} and 1644.20 cm^{-1} correspond to the O-H Stretch in carboxylic acids and C=0 stretch of amides. The band at and 1514.98 cm^{-1} and 1401.19 cm^{-1} to assigned the N-H bend amides and C=F Stretch in Alkyl & Aryl Halides. The peaks at 1123.46 cm^{-1} and 1101.28 cm^{-1} can be assigned to C=O stretch alcohols, The peaks at 751.20 cm^{-1},646.11 $cm^{-1}$and 1216.71 cm^{-1} observed to the C-H bend aromatic compounds,= C-H bend Alkyne and –C-Br stretch alkyl halides in respectively(Fig: 2).

XRD analysis

The analysis of structure and crystalline size of the synthesized silver nanoparticles were carried out by XRD. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peak at 2θ value of 38.11º, 44.31º, 64.51º and 76.63º could be attributed to (111),(200),(220) and (311) crystallographic planes of Ag crystals, correspondingly(Fig:3). By comparing JCPDS (file no: 89-3722), the typical pattern of bio-synthesized AgNPs is possess an fcc structure. The XRD peaks indicated that the synthesized nanoparticles were of clear crystalline nature. The peaks can be assigned to the planes. In addition of two unidentified peaks appeared at 32.22º and 46.54º. This

may be due to some bio organic compounds occurring on the surface of the $AgNO_3$. They are unexpected crystalline structure are also present and might be due to the organic compounds in the leaf extract (Anandalakshmi *et al.* 2016; Suvith and Philip 2014). A similar results were observed by Kumar and Yadav (2009) and Jeeva *et al*. (2014b).

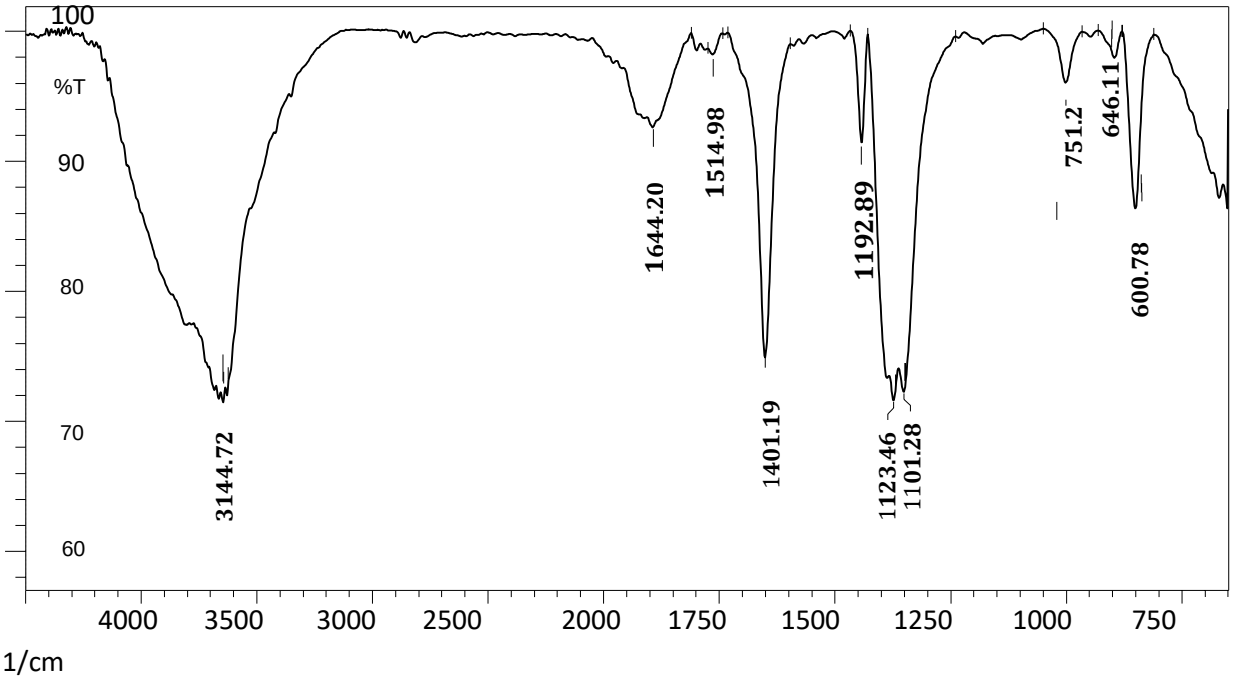

Figure: 2: FTIR Spectrum of Synthesized AgNPs Using Aqueous Extract of *M. azedarach.*

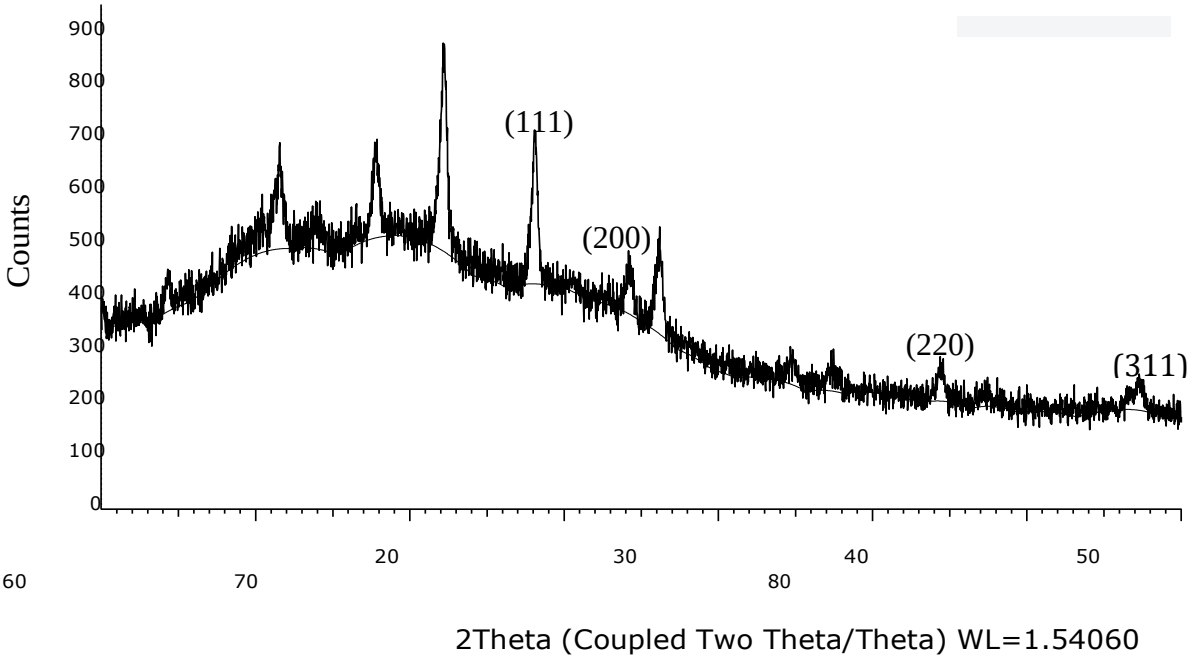

Figure 3: XRD Patterns of AgNPs Synthesized using aqueous extract of *M. azedarach*

SEM Analysis

The SEM analysis shows triangular and spherical structures. SEM image revealed the AgNPs produced by using the extract of the *M. azedarach* leaves are in the range of 6 to 12 nm (Fig: 4). The size of the AgNPs changes as the nanoparticles form over different periods of time (Ibrahim.,2015). The grain size of the AgNPs of the SEM supports the findings of the XRD analysis.

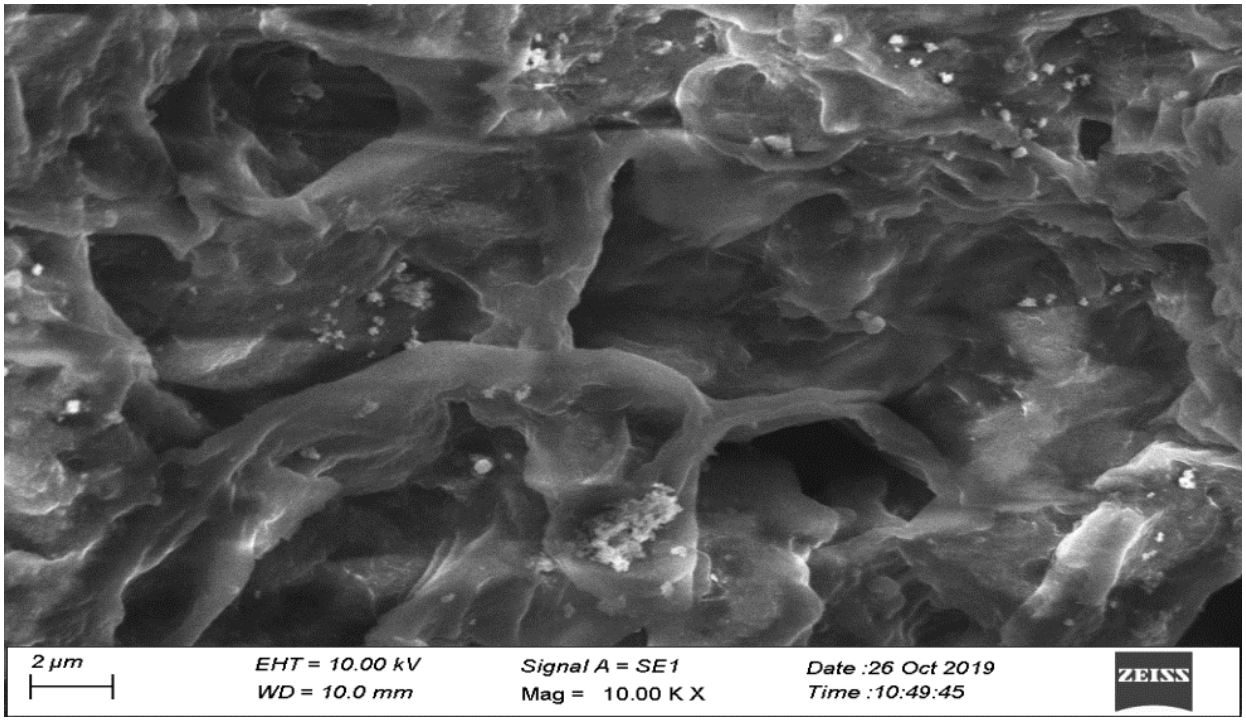

Figure: 4 SEM Image of AgNPs Synthesized by *M. azedarach*

Larvicidal Activity of Synthesized AgNPs of *M. azedarach* against third instar larvae of *Spodoptera frugiperda*

The larvicidal activity of *M. azedarach* AgNPs (10, 20, 30, 40 and 50 mg/l) were used against third instar of *S. frugiperda*. Larval mortality of Third instars larvae of *S. frugiperda* found to be decreased with increasing concentration of aqueous extract and Synthesized AgNPs of *M. azedarach* LC50 and LC90 values of 36.83(28.05-57.78), 85.71(55.65-425.73) and 25.44(18.27-33.60), 58.65(41.82-143.87) was respectively (Table:1). The larval mortality of the third instar confirmed the effectiveness of synthesized nanoparticle technique in biological control. We are avoiding the use of hazardous and toxic solvents in current practices. This nanostructure showed excellent larvicidal activity against *S. frugiperda*. Therefore $AgNO_3$ integrated using this plant extract provides a promising optimal approach to the large scale industry in nanomaterials for pest control.

Table: 1 Larvicidal Activity of Synthesized AgNPs of *M. azedarach* against third instar larvae of *Spodoptera frugiperda*

M. azedarach	Concentration (mg/ml)	% Mortality (mg/ml)	LC50	LCL-UCL	LC90	LCL-UCL	Chi-square	Regression Equation
Plant extracts	Control	0.0	36.83	28.05-57.78	85.71	55.65-425.73	0.274	Y=4.285X+6.28
	10	02.3						
	20	17.7						
	30	37.7						
	40	54.9						
	50	67.8						
AgNPs	Control	0.0	25.44	18.27-33.60	58.65	41.82-143.87	0.791	Y=4.285X+5.78
	10	07.0						
	20	35.5						
	30	59.9						
	40	75.6						
	50	85.0						

Control (Distilled water); Nil mortality; Mean value of five Replicates ± SD

As conclusion the present study was carried out successfully synthesized the Ag nanoparticles in *M. azedarach* extract. These were synthesized AgNPs were characterized using UV, XRD, FTIR and SEM. All synthesized AgNPs were evaluated for larvicidal activity against *S. frugiperda*. Furthermore, the effect of synthesized AgNPs was tested against lepidopteran pest. From this study we are concluded that the silver AgNPs are non-toxic, low cost, eco-friendly and insect repellent.

ACKNOWLEDGEMENTS

The authors express sincere thanks to the head of the PG and Research Dept. of Zoology, RD Govt. Arts College. Sivagangai, Tamilnadu for the facilities provided to carry out this research wok.

Conflicts of Interest

The authors declare no conflict of interest.

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Received: 28th December 2021; Accepted: 10th February 2022; First distribution: 20th March 2022.