Egyptian Journal of Soil Science
http://ejss.journals.ekb.eg/

Efficiency of Green Synthetic Silver and Iron Nanoparticles of *Acacia nilotica* Pods Against Plant Pathogenic Bacteria and Land Snails

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IT BECAME necessary to use safer and more efficient alternatives to control pathogenic bacteria and land snails due to the increasing problems caused by these snails and bacteria on various crops. Green nanoparticles have been used because of their stability, low cost, feasibility and antimicrobial characteristics and snails. The current work intends to investigate the effectiveness of green synthetic silver and iron oxide nanoparticles of an aqueous extract of *Acacia nilotica* pods Ag-NPs (AEANP-AgNPs) and FeO-NPs (AEANP-FeONPs) against two identified plant pathogenic bacterial strains (*Xanthomonas euvesicatoria* and *Pseudomonas syringae* pv. *tomato*) and two land snails (*Monacha cartusiana* and *Eobania vermiculata*) collected from sandy loamy sand. Different concentrations of AEANP-AgNPs (1700, 3400, and 5100 ppm) and AEANP-FeONPs (2700, 5400, and 8100 ppm) were tested. The concentration (5100 ppm) of Ag-NPs was the highest in inhibiting *P. syringae* pv. *tomato* while (5400 ppm) and (8100 ppm) concentrations of FeO-NPs were equal in inhibiting *X. euvesicatoria*. *In planta*, the treated strain of *X. euvesicatoria* exhibited low severity while the treated strain of *P. syringae* pv. *tomato* didn't exhibit a significant difference in severity. Also, *M. cartusiana* snail was more sensitive to the toxic effect of Ag-NPs and FeO-NPs than the adult snail, *E. vermiculata*. The mortality of adult snails was increased with increasing concentration of both nanoparticles. The LC_{50} concentrations of Ag-NPs for adult snails *M. cartusiana* and *E. vermiculata* were ($1.885\times10^3$and 7.618×10^3) respectively and (4.066×10^3 ppm and 10.776×10^3 ppm) respectively for FeO-NPs.

Keywords: Ag-NPs, FeO-NPs, *Xanthomonas euvesicatoria*, *Pseudomonas syringae* pv. *tomato*, *Monacha cartusiana*, *Eobania vermiculata*.

1. Introduction

As a source of food and revenue for many nations, agriculture plays a crucial role in society. The majority of rural residents depend on agricultural production for their living; this percentage is around 86% (Atiq et al., 2020). Currently, using agrochemicals is essential for disease control (Sarkar et al., 2020) but because of their toxicity and systemic mode of action, agrochemicals can have adverse effects on non-target living organisms (Sebastian et al., 2020) despite having many positive benefits, such as quick action, dependability, and high availability (Sule et al., 2022), many harmful effects of agrochemicals. These effects include disruption of metabolite levels in the biosynthetic pathway of aromatic amino acids within soil microorganisms, development of microbial resistance, and a resurgence in population of pests and environment (Zaller and Brühl, 2019).

Searching for safer alternatives and more accurate became a necessary matter (Mohamed et al., 2022; Ghoname et al., 2024). Nanotechnology is a rapidly developing field of study that deals with extremely small particles and has many applications in innovation, development, and agricultural activities. At the same time, nanotechnology also shows promise in the fight against agricultural pests like fungi, bacteria and snails (Ali et al., 2015; Helmy et al., 2022). The application of nanopesticides and other nanotechnology-based methods can improve the sustainable management of agricultural pests, effectiveness, and accuracy (Rani et al., 2023).

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Received: 22/04/2024; Accepted: 29/05/2024
DOI: 10.21608/EJSS.2024.283948.1752

Green synthesis techniques, are cost low, safe, biocompatible, and most importantly environmentally benign, and offer an excellent alternative to existing approaches of nanoparticles (Malhotra and Alghuthaymi, 2022; Britto-Hurtado and Cortez-Valadez, 2022; Abdalla et al., 2023). Furthermore, green nanoparticles have high catalytic properties, minimizing the need for harmful chemicals (Patel, 2022). The improved characteristics of green nanomaterials have led to increased use of green synthesis techniques and several nanoparticles have been made from plant extracts over a long time (Vivek et al., 2020). The biosynthetic technique employing diverse plant extracts for NP synthesis is of special interest and numerous transition metal-based NPs such as Fe, Ag, Au, Zn, Mn, Cu, and Ti were produced utilizing diverse plant extracts (Nasrollahzadeh et al., 2019). Also, nanoparticles have many useful effects for plants *i.e.* tolerance the stress, supply the nutrients, antimicrobial activities and protection of the plant and reduction of the harmful effects of pathogens (Wohlmuth et al., 2022; Tariq et al., 2022; Tehri et al., 2022; Taha et al., 2024). Green nanoparticles are stable, low cost and have antimicrobial characteristics, anti-insects and anti-snails (Helmy et al., 2022; Samuel et al., 2022; Maxwalt et al., 2022).

Silver nanoparticles, loaded on oak leaves and fruit extracts, effectively control plant pathogenic bacteria without causing toxicity (Chahardooli et al., 2014; Ayisigi et al., 2020; Akshaya et al., 2022, Abdelhady et al., 2024). Elkobrosy et al. (2023) found that increasing silver nanoparticles concentrations with *Ficus sycomorus* leaf extract, inhibited plant pathogenic bacterial growth. Ghazy et al. (2021) demonstrated the antibacterial activity of silver nanoparticles against sugar beet soft rot. Additionally, ferric oxide nanoparticles and silver oxide nanoparticles are used in tomato fertilization to combat wilt and reduce the pathogenic bacteria (*Xanthomonas vesicatoria* and *Pseudomonas syringae* pv. *tomato*), they enhance antioxidant enzymes and defence-enzyme activity (Ghazy et al., 2021; Elbasuney et al., 2022; Parveen and Siddiqui, 2023). Nanocomposites containing copper or silver at different concentrations were significantly suppressed the disease severity of pepper bacterial spot which caused by *Xanthomonas euvesicatoria in vitro* and *in planta* (Bytešníková et al., 2022).

Several studies have highlighted the significant molluscicidal effects of nanoparticles. Iron oxide (Fe_2O_3) nanoparticles have been molluscicidal properties (Caixeta et al., 2021). Similarly, Zinc oxide (ZnO), Cerium oxide (CeO_2), and copper oxide (CuO) nanoparticles have also demonstrated molluscicidal activity (Saad et al., 2019; Yusof et al., 2019; Ibrahim et al., 2021; Mohammad et al., 2021; Tadros et al., 2021). Besides, Helmy et al. (2022) found that ZnO and F-ZnO NPs reduced *Monacha cartusiana* snail populations with a lower cost than commercially recommended molluscicides. Also, Morsy and Mohamed (2022) reported that chitosan and silver nanoparticles might be alternatives to the traditional insecticides against the black cutworm, *Agrotis ipsilon*, and the land snail, *Monacha obstructa*. These findings collectively emphasize the potential of binary metal oxide nanoparticles in controlling mollusk populations. According to Khidr (2018) and Kandil et al. (2020), nano-chitosan has demonstrated potential as an effective substance for controlling land snails such as *Eobania vermiculata* and *Monacha obstructa*. Ali et al. (2015) found that silver nanoparticles induced changes in the digestive gland of the land snail, *E. vermiculata*.

Many plant compounds found in fruits, leaves, pods, and bark have been used to fight plant-pathogenic bacteria, snails, and insects (Gohar et al., 2014; Al-Khayri et al., 2023). These natural substances offer an alternate material of controlling pests. Their application in the production of green nanoparticles enhances the significance and efficacy of their impact (Hano and Abbasi, 2021; Singh et al., 2023).

One of the plant parts that has active chemicals that enable the creation of green mineral nanoparticles that can be utilized to fight plant diseases is the seedless pod of *Acacia nilotica* (Edison and Sethuraman, 2013; Da'na et al., 2018). *Acacia nilotica* is one of the traditional medicinal plants. The bods and other parts of *Acacia nilotica* are used as a pharmaceutical tool and have antimicrobial activity (Ali et al., 2012; Jame, 2018).

The current work intends to investigate the AEANP-AgNPs and AEANP-FeONPs generated in an environmentally acceptable way utilizing an aqueous extract of abundant *A. nilotica* seedless pods to control two identified pathogenic bacterial strains (*Xanthomonas euvesicatoria* and *Pseudomonas syringae* pv. tomato) *in vitro* and *in planta* and control two land snails (*Monacha cartusiana* and *Eobania vermiculata*) *in vitro*.

2. Materials and Methods

2.1. Chemicals and reagents

The chemical materials were brought from El-Gomhouria Company for Chemicals, located in Cairo, Egypt, which included $AgNO_3$, $FeCL_3$, NaOH, $MgSO_4$, K_2HPO_4 and glycerol.

2.2. Plant materials

The seedless pods of *Acacia nilotica* were collected from Halayeb and Shalatine region of Upper Egypt.

Dust and unwanted particles were removed from the pods by exposing the pods to running water for

10 minutes, then drying them away from sunlight for 7 days.

2.3. Pathogenic bacterial strains source

Two identified pathogenic bacterial strains were used in this study. The strains were isolated and identified in previous studies. Strains included *Xanthomonas euvesicatoria* 1X-MAS (accession number MZ540733 in GenBank https://www.ncbi.nlm.nih.gov) which caused bacterial spot disease on pepper plants (Soliman, 2022) and *Pseudomonas syringae* pv. *tomato* Pst1-MAS (accession number OQ117369.1 in GenBank https://www.ncbi.nlm.nih.gov) which caused tomato bacterial speck disease on tomato plants (Soliman, 2023).

2.4. Land snails source

Adult snails of glassy clover snails, *Monacha cartusiana* and chocolate band land snails, *Eobania vermiculata* were collected from mango nurseries of Abu Rawash village, Kerdasa City, Giza, Egypt from two sites of loamy sand soil (30.056534ºN, 31.094160ºE and 30.05695ºN, 31.086540ºE) for testing under lab conditions. Some soil chemical and physical properties were determined according to Page et al. (1982) and Klute (1986). Particle size distribution of the soil sample (Table 1) and the chemical analysis of saturated soil paste (Table 2). The snails were transferred in plastic bags to the lab. Then they were put in plastic boxes filled with the soil, from which land snails were collected, with suitable temperature and moisture. They were supplied with green lettuce leaves for feeding.

Table 1. Some physical properties of the studied soil samples.

Saturation percentage, %	Coarse sand	Fine sand	Silt	Clay	Texture class
40.0	35.6	38.50	19.60	6.30	Loamy sand soil

Table 2. Some chemical properties of the studied soil samples.

pH	EC, dS m^{-1}	Soluble cations, $mmol_c$ l^{-1}				Soluble anions, $mmol_c$ l^{-1}			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
8.01	5.45	13.50	10.30	25.30	0.90	0.00	1.90	43.60	4.50

2.5. Preparation of aqueous extract of *Acacia nilotica* pods (AEANP)

The aqueous extract was prepared from dry *A. nilotica* pods powder according to Ferraz et al., 2022 with some modifications, the extraction ratio was 1:10 (w: v) for each of the pods powder and distilled water respectively. The suspension was stirred by a magnetic stirrer for one hour at 70◦C. The temperature increased gradually by 10 until reached to 80°C for another hour. The suspension was filtrated by Whatman No. 1 filter paper and the extract was maintained at 4°C until use.

2.6. GC mass analysis (scan)

Each fraction was concentrated before being analyzed using the GC-MS according to Said et al., 2023. To facilitate fraction separation, hexane, a nonpolar solvent, was utilized as the injection solvent. ADB5 ms ultra-inert capillary column (0.25 mm ×30 m ×0.25 µm) was used to separate the samples with a 1µL injection volume. Under the following conditions, the analyte was identified using a Thermo TRACE™ 1300 gas chromatography equipped with a PTV mode splitless injector: temperature 75°C, splitless time 2 minutes, purge flow 5 mL/min, carrier gas saver flow 20 mL/min for 5 minutes, transfer temperature delay 1 minute, injection pressure 70 kPa for 6 s, transfer pressure 210 kPa, transfer rate 2.5 °C/s, transfer temperature 280°C, transfer time 3.0 minutes, cleaning rate 2.5°C/s, cleaning temperature 300°C, and cleaning time 5.0 minutes. For data gathering, reprocessing, and report generation, Thermo Scientific Xcalibur was employed.

2.7. Preparation of aqueous extract of *Acacia nilotica* pods nanoparticles

2.7.1. Preparation of aqueous extract of Acacia nilotica pods with silver nanoparticles (AEANP-AgNPs)

Silver nanoparticles were prepared according to Zubair et al., 2022 with some modifications, 100 ml of the aqueous extract was properly and continuously mixed with 900 ml of AgNO3 solution (0.01, 0.02 and 0.03 M). Materials were mixed with a magnetic stirrer for 30 minutes at 60 °C in a dark setting. The mixture was centrifuged at 13,000 rpm for 30 minutes and then washed with deionized water to remove water-soluble contaminants. The suspension was centrifuged again at 13,000 rpm for 30 minutes. The solution was filtered using filter paper (Whatman No. 1) to remove any remaining contaminants and dried for one day at 70 °C. After drying, pure Ag-NPs powder was produced and utilized for further investigation. Then, three different concentrations

of AEANP-AgNPs (1700, 3400, and 5100 ppm) were tested against plant pathogenic bacteria and land snails.

2.7.2. Preparation of aqueous extract of Acacia nilotica pods with iron oxide nanoparticles (AEANP-FeONPs)

According to Da'na et al. (2018), and Altaf et al. (2021), Fe_2O_3 NPs were synthesized with some modifications by adding a concentration (0.01, 0.02 and 0.03M) $FeCL_3$ $6H_2O$ solution to an *A. nilotica* pods extract at volume ratios (1:1) and temperatures (60-70°C) on a hot plate with a light magnetic stirrer. This was followed by addition of 0.1 M NaOH solution to adjust the pH to 6.0, and the development of a black precipitate verified the formation of FeO-NPs. Magnetite (Fe_3O_4) black precipitates were cleaned 5:8 times with deionised water and dried for 8h at 80°C in the oven. The particles were produced and kept for characterization and experimental usage. Then, three different concentrations of AEANP-FeONPs (2700, 5400, and 8100 ppm) were tested plant pathogenic bacteria and land snails.

2.8. Characterization of AEANP-AgNPs and AEANP-FeONPs

Transmission electron microscopy (TEM), Zeta Potential, Fourier transform infrared (FT-IR) spectroscopy, and energy dispersive X-ray (EDX) spectroscopy were used to investigate AEANP-AgNPs and AEANP-FeONPs.

2.8.1. TEM analysis

A transmission electron microscope (TEM) was used to determine the average size of NPs. For three-dimensional imaging. The inside of the sample is observed in transmission electron microscopy by passing an electron beam through a tiny specimen. This approach is regarded as one of the most effective methods for obtaining information on nanoparticles (Alduraihem et al., 2023).

2.8.2. Zeta Potential

To investigate the stability of the biosynthesized NPs and the degree of electrostatic or charge repulsion/attraction between the NPs, Zeta potential was measured by the dynamic light scattering (DLS) method by using a PSS-NICOMP 380-ZLS, US, apparatus (Mohamed et al., 2022).

2.8.3. FTIR analysis

The chemical structure of *A. nilotica* pod extracts AEANP-AgNPs and AEANP-FeONPs were analysed in the liquid phase. Fourier-transformed infrared spectroscopy (FTIR) is commonly used for investigating various bio-reducing functional groups in materials, A Vertex 70 Bruker Transform Infrared spectrophotometer was used to capture the spectra (Nagy et al., 2022).

2.8.4. EDX analysis

EDX was utilized to gather qualitative information about elements in nanoparticles since it is one of the most practical, quick, and appropriate analytical procedures for multi-element determination. In addition to pictures of NPs, nanostructure and morphology were captured using a scanning electron microscope SEM (FEI Quanta FEG 450) equipment (Mohamed et al., 2022).

2.9. Bacterial cultivation and preparation of the inocula

To prepare the bacterial inoculum, the bacterial strains were cultured on King's B medium (20g proteose peptone, 1.5g $MgSO_4$. $7H_2O$, 1.5g K_2HPO_4, 15ml glycerol and 15g bacteriological agar then completely the volume to 1 Liter by distilled water with final pH (7.2± 0.2) and incubated at 28ºC ±1 for 48h. The bacterial growth for the strains were harvested and suspended in sterilized distilled water to 10^8 CFU/ml concentration using a spectrophotometer (JENWAY 6305 UV/Vis. Spectrophotometer) at OD_{600} nm (Jones et al. 2000).

2.10. The laboratory evaluation with plant pathogenic bacteria

Petri plates 9 cm diameters contained on 20 ml of KB medium were inoculated by 10 µl of bacterial suspension 10^8 CFU/ml and spread by sterilized swab on the medium surface. AEANP-AgNPs concentrations (1700, 3400, and 5100 ppm) and AEANP-FeONPs concentrations (2700, 5400, and 8100 ppm) were tested by diffusion method using sterilized filter paper discs according to Balouiri et al. (2016).

Sterilized filter paper discs 6 mm in diameter were soaked in 5 µl of tested material concentrations and dried for 3 min at a laminar flow cabinet and then put on the surface cultures. Sterilized filter paper discs were soaked in 5 µl of 500 ppm streptomycin antibiotic as an antibiotic control. Others were soaked in sterilized distilled water as a negative control and then put on the surface cultures. The cultures were incubated at 28 ºC ±1 for 3 days. Three replicates of each treatment were used in the experiment. The inhibition zones were measured by millimetre using a ruler and the mean of the inhibition percentage (the efficiency) was calculated according to Naz and Bano (2012) using the formula:

Inhibition percentage (efficiency) = 100 × (X-Y) / (Z-Y).

Where: (X) is inhibition by tested concentration of material, (Y) is inhibition by negative control, and (Z) is inhibition by positive control (antibiotic).

2.11. Treated the pathogenic strains

The inhibitor concentrations to bacterial growth among the tested concentrations (8100 ppm AEANP-FeONPs with *Xanthomonas euvesicatoria* 1X-MAS and 5100 ppm AEANP-AgNPs with *Pseudomonas syringae* pv. *tomato* Pst1-MAS) were used in this test. Four milliliters of a KB liquid medium were inoculated by 5µl of pathogenic bacterial suspension (10^8 CFU/ml) with 10µl of sterilized inhibitor concentrations. Other treatments were inoculated by 5µl of pathogenic bacteria only and used as a positive control. The cultures were incubated at 28 ºC overnight with shaking. After the growth, the cultures were centrifugated at 5000 rpm for 10m. The supernatants were discarded and the pellets were suspended by 100 ml of sterilized water.

2.11.1 Pathogenicity test for the treated strains (in planta)

Seedlings of pepper (*Capsicum annuum* cv. Top star) and tomato (*Solanum lycopersicum* cv. Super strain B) plants were used as hosts for bacterial strains. Pepper and tomato plants 6 weeks old were grown in 20 cm pots (plant/pot) containing 2 kg of peat moss and sandy (1:1) with regular irrigation and suitable fertilization. Artificially inoculation was performed in the greenhouse conditions, (16 h of light with 28 ºC and 8 h of darkness with 22 ºC and 65% humidity) for pepper plants and (16 h of light with 24 ºC and 8 h of darkness with 20 ºC and 65% humidity) for tomato plants.

The spraying method according to Soliman (2022) was used to evaluate the efficiency of tested concentrations in the reduction of the pathogenicity for the bacterial strains on host plants. Approximately 100 ml of the tested suspensions (bacteria treated by test concentrations) were used to treat the plants. Other plants from pepper and tomato were inoculated with 100 ml of pathogenic bacteria suspension only and served as a positive control and others were treated with 100 ml of sterilized water and served as a negative control (healthy). Three replicates were performed for each treatment. The plants were covered by transparent plastic bags to increase the humidity (Wreikat et al., 2006). After 2 days, the bags were removed. Plants were observed for 14 days and the symptoms were recorded.

2.11.2. Pathogenicity symptoms

The typical symptoms of pepper bacterial spots include water-soaked with yellowish hallo, then turning to necrotic spots and finally turning to dark brown spots. With high disease severity, the infected leaves defoliated (Potnis et al., 2015; Osdaghi et al., 2021; Soliman, 2022). The typical symptoms of tomato bacterial specks include necrotic dark brown to black specks (1-3mm) surrounded by chlorotic yellowish halos on tomato leaves (Soliman, 2023).

2.11.3. Pathogenicity estimation

Exhibiting the symptoms on the plants is considered a positive result and disease severity was estimated. Visual scales were used for disease severity estimation, with pepper bacterial spot Abbasi *et al*. scale (Abbasi et al., 2002) used (1-degree meaning, symptomless on treated plants, 2-degree meaning, a few necrotic spots on a few leaflets of treated plants, 3-degree meaning, a few necrotic spots on many leaflets of treated plants, 4-degree meaning, many spots with coalescence on few leaflets of treated plants, 5-degree meaning, many spots with coalescence on many leaflets of treated plants, 6-degree meaning, severe disease and leaf defoliation of treated plants and 7-degree meaning, treated plant dead). While with tomato bacterial speck, the disease severity was estimated according to (Soliman, 2023) using the mean of the speck numbers on tomato leaves.

The percentage of disease reduction was calculated according to **Aliye et al., 2008** using the formula:

$$PR = \frac{PC - PT}{PC} \quad X\ 100$$

Where:
PR = Disease percentage reduction.
PC = Percent of the pathogen control ratio.
PT = percent of the treated ratio.

2.12. Tested land snails

For 14 days, the snails were housed in boxes to allow them to acclimatize. The studies started with the snails being fasted for a full day (Bashandy and Raddy, 2021). No dead or sick snails were employed in our tests; instead, five healthy snails per replicate were used to investigate the toxicity of produced nanomaterials at varying doses (Abdel-Halim et al., 2021).

2.13. Tested materials activity by contact technique

Three various concentrations of AEANP-AgNPs (1700, 3400, and 5100 ppm) and (2700, 5400, and 8100 ppm) of AEANP-FeONPs were prepared. To ensure equal distribution of the different amounts, three milliliters of each dosage were carefully swirled in a circular motion in the bottom of nine-centimeter-diameter plastic cups (Ascher and

Eliyahu, 1981). After allowing the solvents to drain, the treated land snails were placed into plastic vials, which had a screened plastic top and measured 9cm diameter × 15cm height. Twenty animals were per treatment and four replicates per dose. Some snails were treated with water only as a control. Death of land snails was observed for 48 hr. after treatment.

2.14. Statistical Analyses

Abbott's formula (Abbott, 1925) was used to calculate the death rate. Using (Bakr, 2000) "Ldp line" program, the lethal concentrations (LC_{50} values) and slope values were determined according to (Finney, 1971) technique. One-way randomized analysis of variance (ANOVA) was used to examine the experiment data and using Student-Newman-Keuls's test (Abdi and Williams, 2010) to compare between means.

3. Results

3.1. Characterization of aqueous extract of *Acacia nilotica* pods with AEANP-AgNPs and AEANP-FeONPs

3.1.1. GC mass analysis

The spectrogram obtained from the GC-mass spectrometry analysis (Fig. 1) facilitated the concurrence of several compounds (Table 3). A total of distinct peaks, representing different yet closely related plant compounds, were observed in both time and space dimensions. These compounds primarily consist of Decamethylcyclopentasiloxane, Pyrocatechol, Phenol, 4-tert-butyl-2,6-dinitro-, 4-Methylcatechol, Pyrogallol, 1,1,3,3-Tetramethyl-2,3-dihydro-1H-naphtho1,8-de1,3 disiline, Hexadecamethyl-cyclooctasioxane and 3-O-Methylhexose.

Table 3. GC analysis of aqueous extract of *A. nilotica* pods.

RT	Formula	MW	Name
5.37	$C_{10}H_{30}O_5Si_5$	370	Decamethylcyclopentasiloxane
5.97	$C_6H_6O_2$	110	Pyrocatechol
6.57	$C_{10}H_{12}N_2O_5$	240	Phenol, 4-tert-butyl-2,6-dinitro-
6.94	$C_7H_8O_2$	124	4-Methylcatechol
7.90	$C_6H_6O_3$	126	Pyrogallol
8.24	$C_{15}H_{20}Si_2$	256	1,1,3,3-Tetramethyl-2,3-dihydro-1H-naphtho[1,8-de][1,3]disiline
11.06	$C_{16}H_{48}O_8Si_8$	592	Hexadecamethyl-cyclooctasioxane
12.16	$C_7H_{14}O_6$	194	3-O-Methylhexose

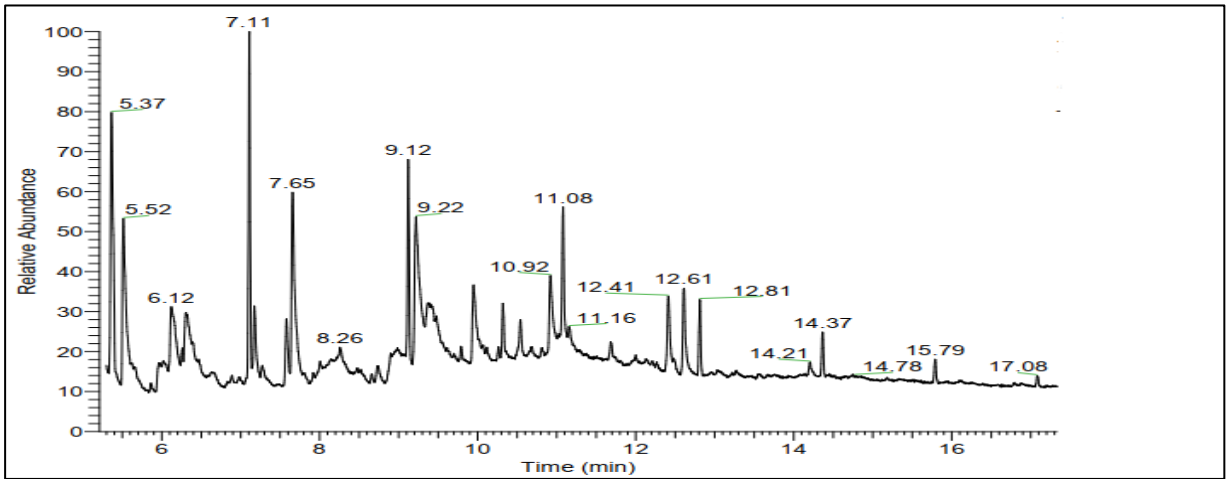

Fig. 1. GC chromatogram of aqueous extract of *A. nilotica* pods.

3.1.2. TEM analysis

The study investigated the size and composition of synthetic AEANP-AgNPs and AEANP-FeONPs extracts using high-resolution TEM. The TEM photograph shows the globular morphology of the NPs with a middle diameter of 23.2 nm in AEANP-AgNPs and 71.9 nm in AEANP-FeONPs (Fig. 2a, b).

3.1.3. Zeta analysis

The determination of zeta potential for AEANP-AgNPs and AEANP-FeONPs were synthesized through biological means. The outcomes obtained from this analysis reveal that the surfaces of these nanoparticles have a negative charge, specifically -14.5 mV and 12.9 mV in AEANP-AgNPs and AEANP-FeONPs respectively and are evenly dispersed within the surrounding medium (Fig. 3a, b).

IR analysis

FTIR analysis revealed distinct bands when examining AEANP-AgNPs, AEANP-FeONPs and aqueous extract of *Acacia nilotica* pods (Fig. 4). FTIR analysis shows the existence of absorption peaks at certain wave numbers, namely 3435.87 cm^{-1}, 2066.10 cm^{-1}, 1634.23 cm^{-1}, 1450.66 cm^{-1} and

1350.87 cm^{-1} in aqueous extract of *Acacia nilotica* pods, 3435.90 cm^{-1}, 2077.82 cm^{-1}, 1636.14 cm^{-1} and 684.19 cm^{-1} in Ag-NPs and 3563.93 cm^{-1}, 2065.68 cm^{-1} and 1633.51 cm^{-1} in FeO-NPs.

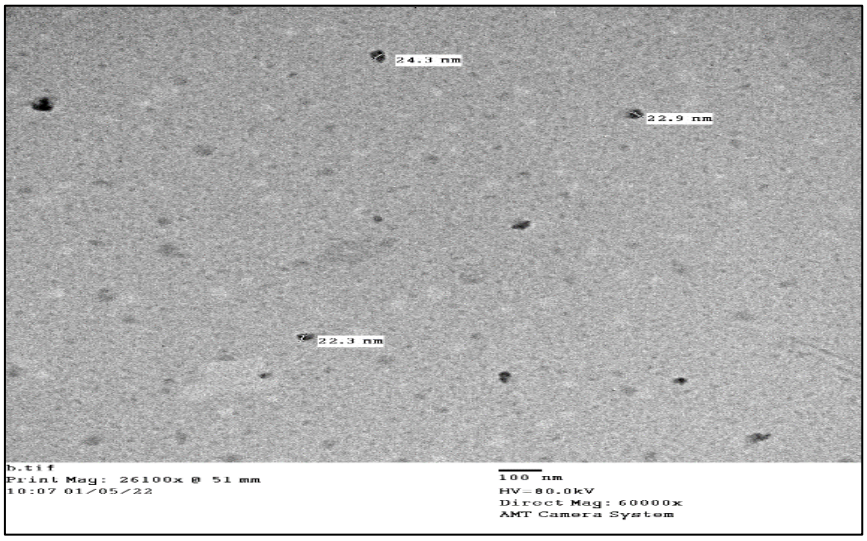

Fig. 2a. TEM Valuable for AEANP-AgNPs.

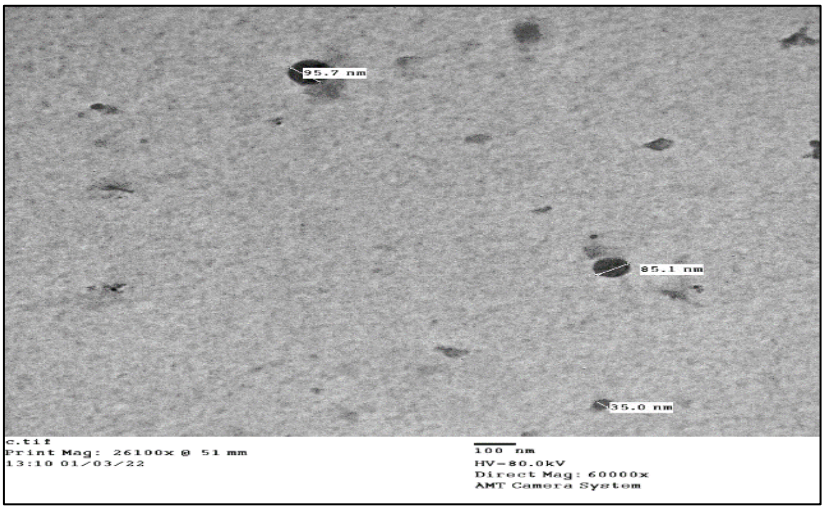

Fig. 2b. TEM Valuable for AEANP-FeONPs.

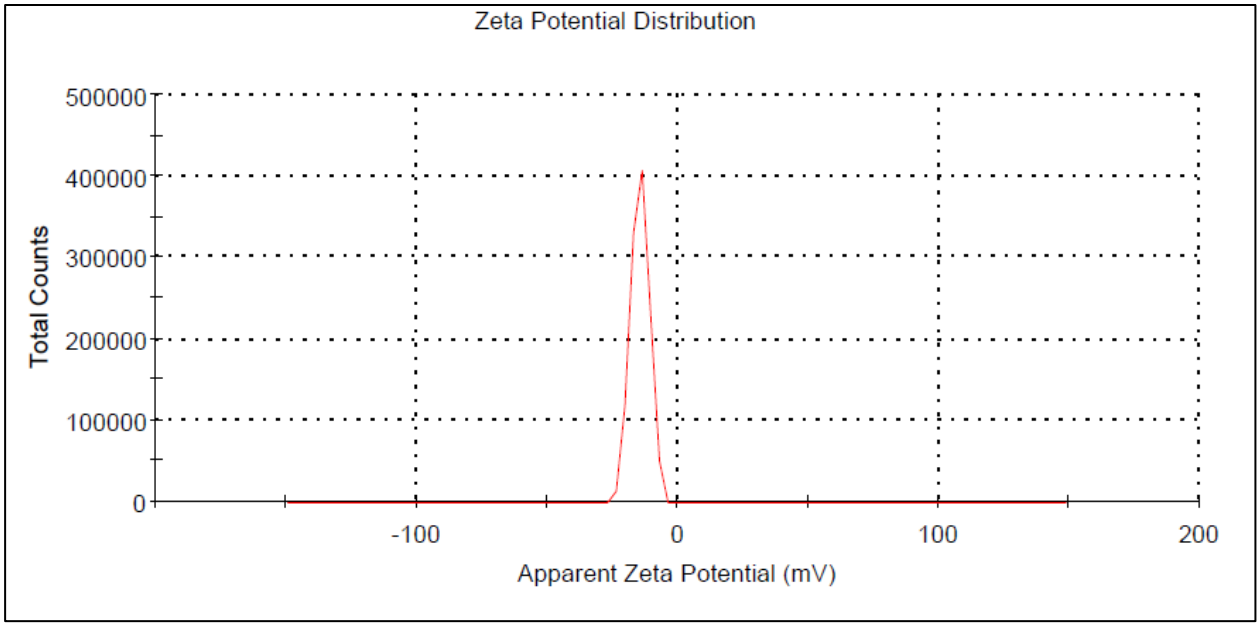

Fig. 3a. Zeta potential value for AEANP-AgNPs.

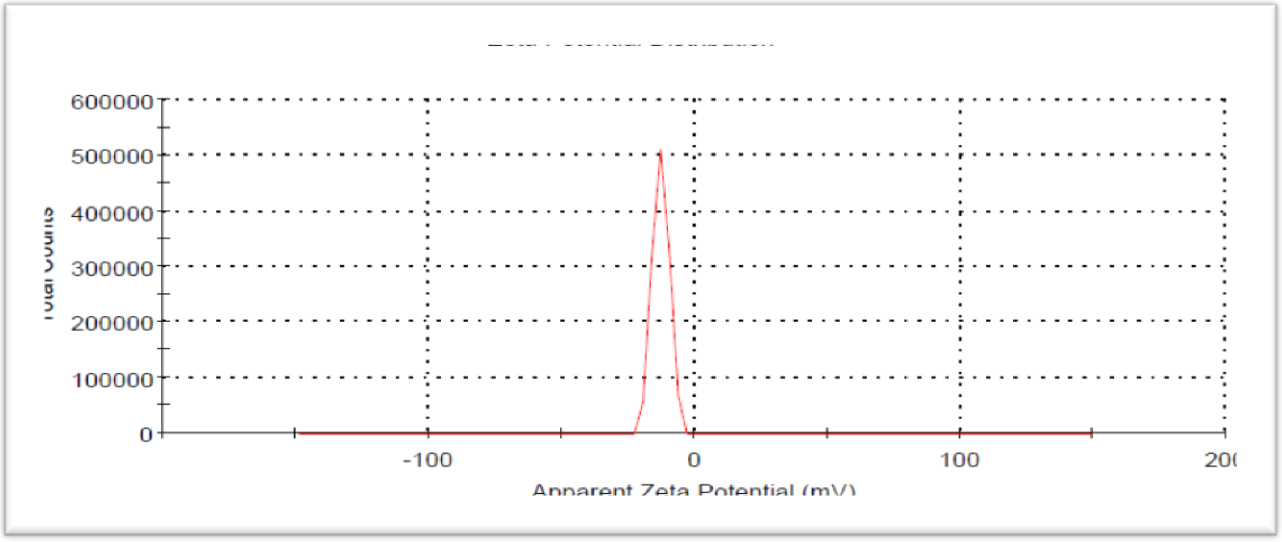

Fig. 3b. Zeta potential value for AEANP-FeONPs.

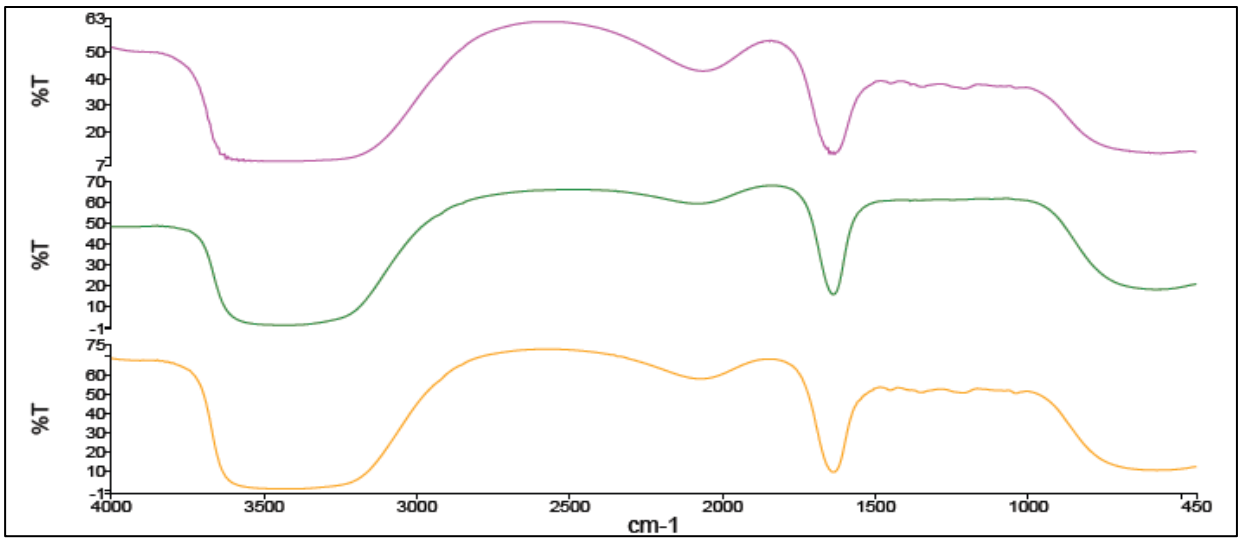

Fig. 4. IR analysis of aqueous extract of *Acacia nilotica* pods, AEANP-AgNPs and AEANP-FeONPs.

3.1.5. EDX analysis

The chemical composition of AEANP-AgNPs and AEANP-FeONPs was acquired through the utilization of energy dispersive X-ray spectroscopy (EDX) plot of SEM image (Fig. 5a, b). The EDX analysis (Fig. 5a) revealed the turnout of the essential texture of silver (Ag) at the concentration of 4.39±0.05 percent and oxygen (O) at the concentration of 44.97±0.52 percent within the examined specimen. The graphical representation further indicates the existence of carbon (C) at the level of 48.20±0.28, sulfur (S) at the level of 0.29±0.02, chlorine (Cl) at the level of 0.98±0.03, copper (Cu) at the level of 0.21±0.02, and potassium (K) at the level of 0.96±0.03 in the EDX image of Ag-NPs. Conversely, the EDX analysis (Fig. 5b) demonstrated the turnout of the requisite composition for iron (Fe) at the concentration of 1.27±0.05 percent and oxygen (O) at the concentration of 32.15±0.51 percent within the sample. The graphical representation also indicates the existence of carbon (C) at the rate of 57.56±0.57, sodium (Na) with percent 0.95±0.06, chlorine (Cl) at a rate of 5.10±0.07, sulfur (S) at a level of 0.27±0.02, calcium (Ca) with 0.30±0.0, phosphorus (P) with percent 0.66±0.04, silicon (S) with percent 0.18±0.02, magnesium (Mg) with percentage 0.66±0.04 and potassium (K) at the rate of 1.29±0.04.

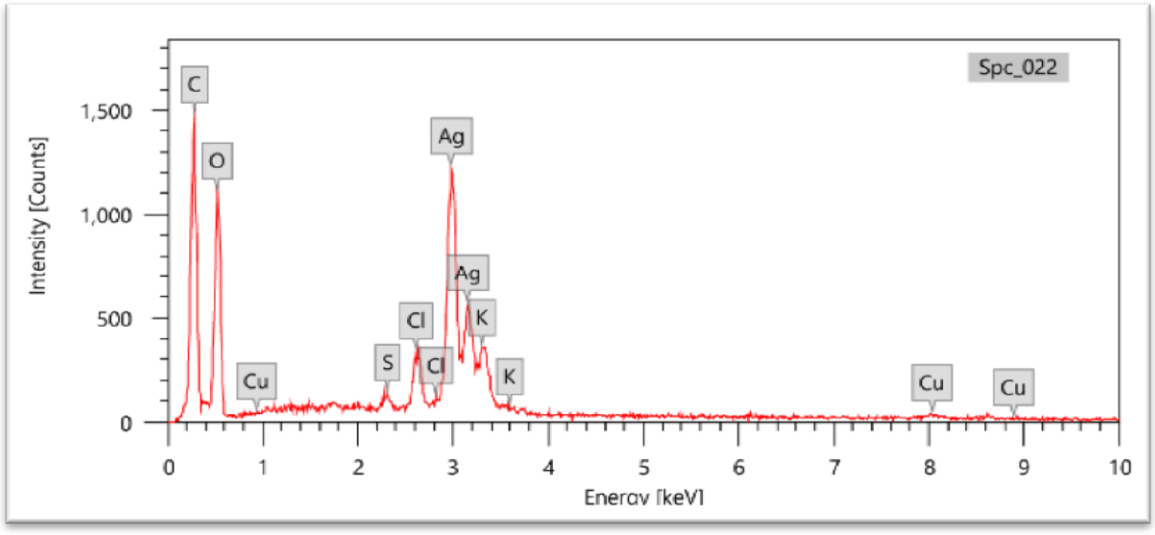

Fig. 5a. EDX analysis for AEANP-AgNPs.

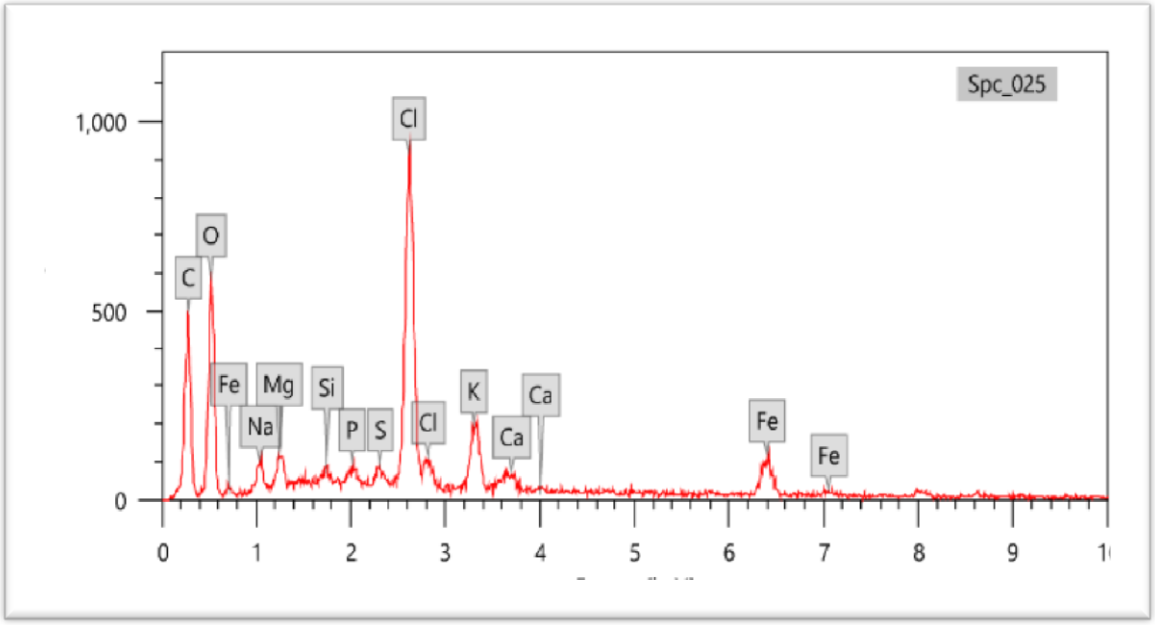

Fig. 5b. EDX analysis for AEANP-FeONPs.

3.2. The laboratory evaluation with plant pathogenic bacteria

The study illustrated that the three concentrations of AEANP-AgNPs with *X. euvesicatoria* 1X-MAS strain didn't exhibit any inhibition against the bacterial strain. On the other hand, the study showed that AEANP-AgNPs concentrations exhibited varied inhibition against *P. syringae* pv. *tomato* Pst1-MAS strain *in vitro*. The third concentration (5100 ppm) was higher in the inhibition against *P. syringae* pv. *tomato* (18 mm) with efficacy (42.8) compared to the control (Table 4a and Fig. 6a). The study exhibited that, the first concentration (2700 ppm) of AEANP-FeONPs with *X. euvesicatoria* 1X-MAS strain didn't exhibit inhibition while the second (5400 ppm) or the third (8100 ppm) of AEANP-FeONPs was equal to inhibition (7mm) with efficacy (36.8%) against *X. euvesicatoria* 1X-MAS strain compared to the control. While AEANP-FeONPs concentrations haven't exhibited any inhibition against *P. syringae* pv. *tomato* Pst1-MAS strain (Table 4b and Fig. 6b).

Table 4a. Evaluation of different concentrations of AEANP-AgNPs against plant pathogenic bacterial strains *in vitro*

Con.(ppm)	Mean±SE (1)	Efficacy %	Mean±SE (2)	Efficacy %
1700	00.0±0.0 b	00.0	10±0.56 c	23.8
3400	00.0±0.0 b	00.0	11±0.58 c	26.1
5100	00.0±0.0 b	00.0	18±0.58 b	42.8
Negative control	00.0±0.0 b	00.0	00±0.0 d	00.0
Antibiotic control	19±0.57 a	100	42±0.56 a	100
LSD (0.01)	1.15		2.31	

Mean±SE of the inhibition zone for (1) *X. euvesicatoria* and (2) *P. syringae* pv. *Tomato*, respectively. Groups denoted by the same letter are not significantly different (p<0.01) based on the results of Duncan's multiple range test.

Table 4b. Evaluation of different concentrations of AEANP-FeONPs against plant pathogenic bacterial strains *in vitro*

Con. (ppm)	Mean±SE (1)	Efficacy %	Mean±SE (2)	Efficacy %
2700	00±0.0 c	00.0	00.0±0.0 b	00.0
5400	7±0.57 b	36.8	00.0±0.0 b	00.0
8100	7±0.57 b	36.8	00.0±0.0 b	00.0
Negative control	00±0.0 c	00.0	00.0±0.0 b	00.0
Antibiotic control	19±0.33 a	100	42±0.33 a	100
LSD (0.01)	2.0		1.15	

Mean±SE of the inhibition zone for (1) *X. euvesicatoria* and (2) *P. syringae* pv. *Tomato*, respectively. Groups denoted by the same letter are not significantly different (p<0.01) based on the results of Duncan's multiple range test.

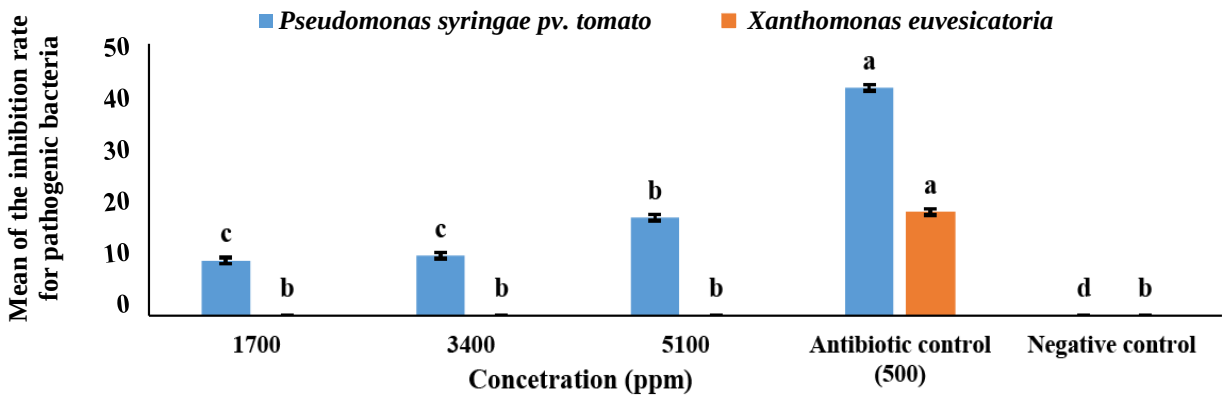

Fig. 6a. Evaluation of different concentrations of AEANP-AgNPs against plant pathogenic bacterial strains *in vitro*

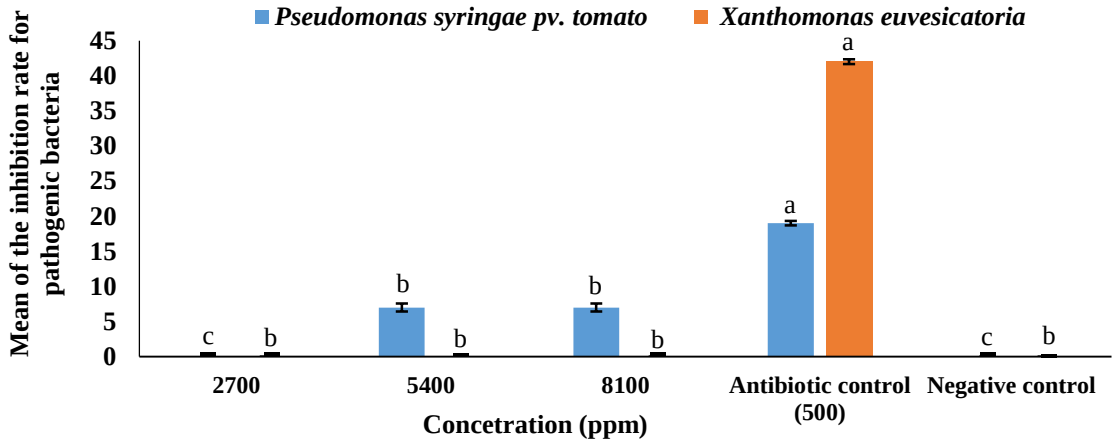

Fig. 6b. Evaluation of different concentrations of AEANP-FeONPs against plant pathogenic bacterial strains *in vitro*

3.3. Pathogenicity test for treated strains (*in planta*)

The study showed that AEANP-FeONPs 8100 ppm concentration reduced *Xanthomonas euvesicatoria* 1X-MAS ability to exhibit severe symptoms of bacterial spot disease on the pepper plant. The disease severity on plants was low relatively compared to the positive control and the disease reduction percentage was 34.5 % (Table 5 and Fig. 7). On the other hand, AEANP-AgNPs 5100 ppm concentration wasn't reduced significantly severe of the tomato bacterial speck symptoms and *P. syringae* pv. *tomato* Pst1-MAS was able to exhibit the symptoms on the tomato plant. No significant difference between the positive control and the treatment whereas the disease reduction percentage was 10.3 % (Table 5 and Fig. 7).

Table 5. Evaluation of the treated bacterial strains on disease severity on their hosts

Treatment	B.S. (1)	B.S. (2)
AEANP-FeONPs Co. 8100	3.6±0.05 b	null
AEANP-AgNPs Co. 5100	null	23.3±0.60 a
Infected control	5.5±0.05 a	26±0.58 a
Negative control (healthy)	0.0±0.0 c	0.0±0.0 b
% Disease reduction	34.5	10.3
LSD (0.05)	0.73	3.2

Groups denoted by the same letter are not significantly different (p<0.05) based on the results of Duncan's multiple range test. Bacterial strains (1) *X. euvesicatoria*, (2) *P. syringae* pv. *tomato*

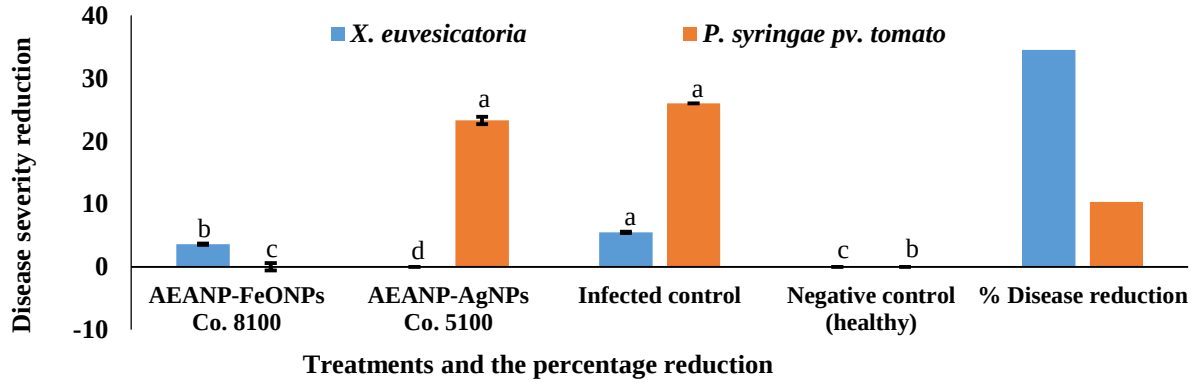

Fig. 7. Disease severity reduction on pepper plants: negative control, positive control, treated *X. euvesicatoria* , and *P. syringae* pv. *tomato*

3.4. Toxicity tests on land snails *in vitro*

3.4.1. Ag-NPs Toxicity

AEANP-AgNPs demonstrated molluscicidal activity against *M. cartusiana* and *E. vermiculata* snails. No death was detected in the untreated snails. The mortality of adult snails exposed to different concentrations of AEANP-AgNPs was significantly higher compared to the untreated snails ($P<0.05$). Table 6 and Fig. 8 showed that the death rate rose with increasing concentrations of AEANP-AgNPs. The concentration 5100 ppm caused 99.5% and 40% mortality for *M. cartusiana* and *E. vermiculata*, respectively compared with other concentrations. So, *M. cartusiana* snails were found to be more sensitive to the toxic effect of silver nanoparticles than adults of *E. vermiculata* ($P<0.005$).

Table 6. Toxicity of AEANP-AgNPs applied topically on two adult land snails after 48 h. *in vitro*

Con. (ppm)	Observed response %	
	Monacha cartusiana	*Eobania vermiculata*
1700	45±0.57 c	10±0.58 c
3400	80±0.58 b	15±0.56 b
5100	99.5±0.06 a	40±0.57 a
$LSD_{0.05}$	1.63	1.99

Groups denoted by the same letter are not significantly different (p<0.05) based on the results of Student-Newman-Keuls's test.

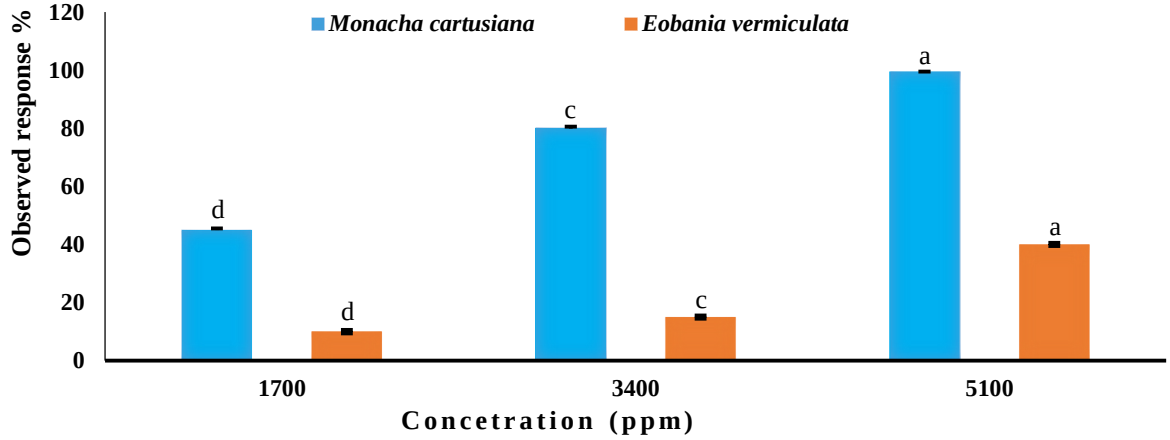

Fig. 8. Evaluation of different concentrations of AEANP-AgNPs against land snails, *Monacha cartusiana* and *Eobania vermiculata in vitro*

Probit analysis showed that the LC_{50} concentrations of AEANP-AgNPs for adult snails *M. cartusiana* and *E. vermiculata* were 1.89×10^3(1231.96-2351.76) ppm and 7.62×10^3 ppm, respectively, (Fig. 9). Table 7shows the different LC_{50} values of AEANP-AgNPs on adult snails.

Table 7. Lethal concentrations (LC_{50}) of AEANP-AgNPs (ppm) against adult *Monacha cartusiana* and *Eobania vermiculata* snails using contact techniques *in vitro*

Land snails	LC_{50}	Lower limit	Upper limit	Slope
M. cartusiana	1.89×10^3	1231.96	2351.76	4.19±1.09
E. vermiculata	7.62×10^3	null	null	2.18±1.05

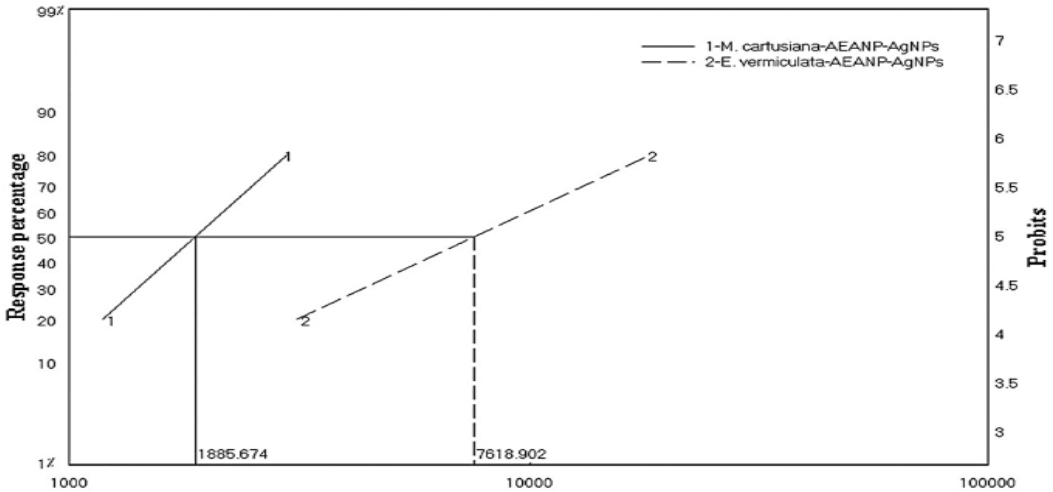

Fig. 9. Regression lines of AEANP-AgNPs against *Monacha cartusiana* and *Eobania vermiculata* after 48 h exposure times using contact techniques *in vitro*

3.4.2. FeO-NPs Toxicity

The results showed that AEANP-FeONPs exhibited effeteness toxicity against glass clover and chocolate bands snails. The mortality of adult snails exposed to different concentrations of AEANP-FeONPs was significantly higher compared to untreated snails. Table 8 and Fig. 10 display that the death rate rises with increasing concentrations of AEANP-FeONPs. The last

concentration 8100 ppm caused 75% and 40 ppm mortality for *M. cartusiana* and *E. vermiculata*, respectively compared with other concentrations. So, *M. cartusiana* snails were found to be more sensitive to the poisonous impact of AEANP-FeONPs than adult snails, *E. vermiculata* ($P<0.005$). The effect of AEANP-FeONPs on adult snails was found to be concentration-dependent (Table 8).

Table 8. Toxicity of AEANP-FeONPs applied topically on two adult land snails after 48 h. *in vitro*

Con. (ppm)	Observed response, %	
	Monacha cartusiana	***Eobania vermiculata***
2700	35±0.57 c	10±0.1 c
5400	60±0.59 b	25±0.1 b
8100	75±0.60 a	40±0.58 a
$LSD_{0.05}$	1.998	

Groups denoted by the same letter are not significantly different (p<0.05) based on the results of Student-Newman-Keuls's test.

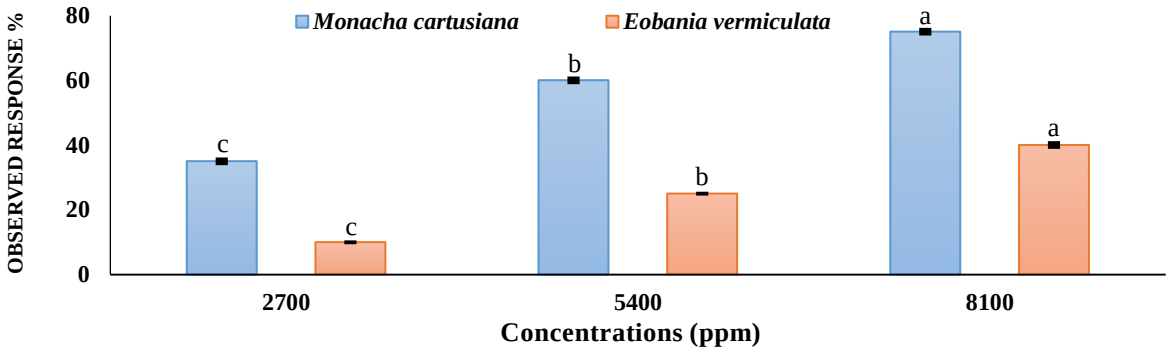

Fig. 10. Evaluation of different concentrations of AEANP-FeONPs against land snails, *Monacha cartusiana* and *Eobania vermiculata in vitro*

Probit analysis showed that the LC_{50} concentrations of AEANP-FeONPs for adult snails *M. cartusiana* and *E. vermiculata* were 4.07×10^3 ppm, 10.78×10^3 ppm, respectively, (Fig. 11) at 48 h. Table 9 shows the different LC_{50} values of AEANP-FeONPs on adult snails.

Table 9 . Lethal concentrations (LC_{50}) of AEANP-FeONPs (ppm) against adult *Monacha cartusiana* and *Eobania vermiculata* snails using contact techniques *in vitro*

Land snails	LC_{50}	Slope
Monacha cartusiana	4.07×10^3	2.21±0.862
Eobania vermiculata	10.78×10^3	2.16±1.003

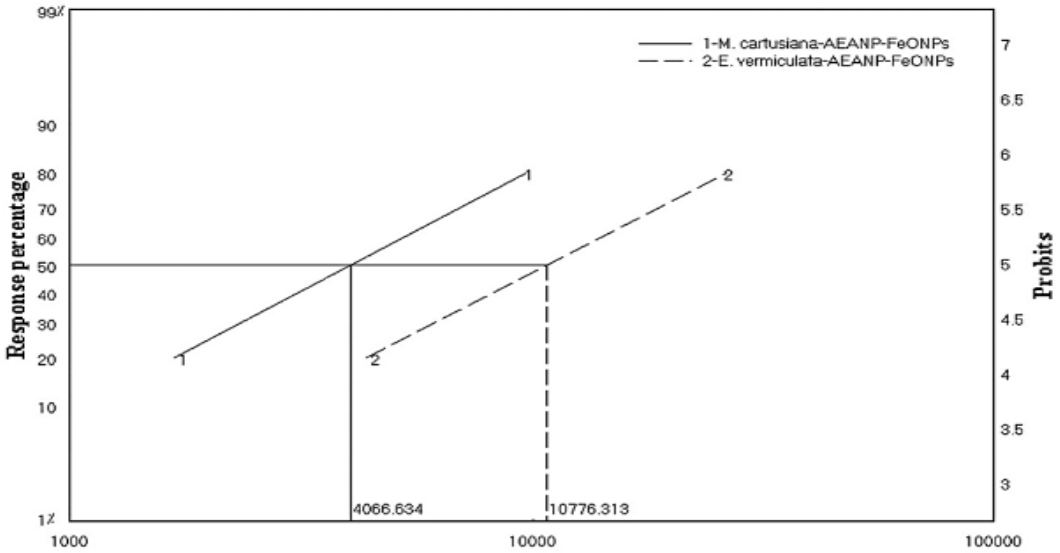

Fig. 11. Regression lines of AEANP-FeONPs against *Monacha cartusiana* and *Eobania vermiculata* after 48 h exposure times using contact techniques *in vitro.*

4. Discussion

Nanotechnology promises in the fight against agricultural pests. Green synthesis is a low-cost, effective, safe, biocompatible, and most importantly environmentally friendly alternative to harmful chemicals, as green nanoparticles have high catalytic properties, minimizing the need for harmful chemicals (Malhotra and Alghuthaymi, 2022; Britto-Hurtado and Cortez-Valadez, 2022; Patel, 2022).

The study aimed to investigate the AEANP-AgNPs and AEANP-FeONPs generated in an environmentally acceptable way utilizing an aqueous extract of *A. nilotica* pods as a molluscicide to terrestrial snail and bactericidal to pathogenic bacterial strains.

A. nilotica pods extract gas-phase chromatography mass chromatography and spectroscopy identified several bioactive components. These results indicate that the *A. nilotica* pods contain many phenolic and polyphenol compounds. The findings of this investigation have been disclosed by antecedent scholars (Abdel-Hady et al., 2018; Vivekanandhan et al., 2018; Saheed, 2021). Undoubtedly, the polyphenols and phenolic acids that are the main components of the *A. nilotica* pod extract showed interaction with $AgNO_3$ and $FeCl_3.6H_2O$ solutions and indicated the decrease of Ag^+ and Fe^{+++} ions.

Transmission electron microscopy is an exceedingly used technique in a variety of scopes including biology and nanotechnology. It has shown remarkable effectiveness in visualizing metallic nanoparticles (Mujahid et al., 2022). The abovementioned results are consistent with previous reports in the relevant literature (Purohit et al., 2022; Devadharshini et al., 2023; Liu et al., 2023).

On the other hand, the application of AEANP-AgNPs and AEANP-FeONPs through function realization or coating processes has the potential to augment stability, reactivity, and environmentally beneficial properties. These modifications ultimately influence zeta potential of the nanoparticles, as indicated by Tran et al., 2015; Liaqat et al., 2022. The observed result indications elucidate a clear correlation between the surface potential, also known as the zeta potential, and the stability of AEANP-AgNPs and AEANP-FeONPs it used. This discovery is consistent with the discoveries reported by Adak et al. (2021); Mohamed et al., (2022); Abdelaziz et al., (2023), all of which closely correspond to this numerical representation.

FTIR analysis shows the presence of absorption bands suggesting the possibility of biological reduction and stabilization processes may occurring and leading to the generation of surface plasmon resonances. The FTIR spectrum exhibited a band at 3435.87cm-1, corresponding to hydroxyl groups (O–H), which was attributed to the presence of flavonoids and phenolic compounds in the aqueous extract of *Acacia nilotica* pods (Emam and Eassa, 2021; Saheed, 2021). The bands at 2066.10 cm^{-1} were in line with symmetric stretches of CH_3, indicating the existence of long-chain alkyl compounds in the aqueous extract of *Acacia nilotica* pods (Elsayim et al., 2021). The absorption bands 1634.23 cm-1 (C=C), 1450.66 cm-1 (aromatic C–H), and 1350.87 cm-1 (aromatic C–O) were linked to the presence of flavonoid compounds. The IR spectrum profile acquired for the aqueous extract of *A. nilotica* pods investigated was highly similar to previously published data on propolis from different sources (Da'na et al., 2018; Alotibi, 2019).

Furthermore, the utilisation of energy dispersive X-ray spectroscopy (EDX) plot of SEM image showed many concentrations of silver and iron, these elements are present in the aqueous extract of *A. nilotica* pods, and they ensure the reduction of silver and iron and the formation of nanoparticles according to (Kanagasubbulakshmi and Kadirvelu, 2017; Da'na et al., 2018; Saratale et al., 2019; Zubair et al., 2022).

The study showed that even with increasing concentrations of the tested material of AEANP-AgNPs with *X. euvesicatoria* 1X-MAS strain didn't exhibit any inhibition against this bacterial strain. However Bibi et al. (2023); Mehmood et al. (2023) reported that green AgNPs with *Berberis vulgaris* extract was effective in the inhibition of the causal agent bacterial spot disease *in vitro.* Varympopi et al. (2022) found that the copper nanoparticles significantly inhibited the causal agent of bacterial spot disease (*X. euvesicatoria*) compared with copper formulations, the higher concentration of AEANP-AgNPs had higher inhibition of *P. syringae* pv. *tomato*. This result is similar to Schiavi et al. (2021); Danish et al. (2022); Schiavi et al. (2023); Parveen and Siddiqui (2023). Besides, Elsharkawy et al. (2020) found that zinc oxide nanostructures exhibited significant inhibition for *P. syringae* pv. *tomato in vitro*.

On the other hand, except for the lower concentration, with increasing concentrations of AEANP-FeONPs, the inhibition wasn't different against *X. euvesicatoria* 1X-MAS strain and this consisted with Elbasuney et al. (2022); Zeeshan et al. (2023). In contrast, all concentrations of AEANP-FeONPs haven't any inhibition effect against *P. syringae* pv. *tomato* Pst1-MAS strain. These results disagreed with Danish et al. (2022) which reported that increasing *Cassia fistula* leaf synthesized silver nanoparticle concentrations is followed by an increase in the inhibitory ability of *P. syringae* as a pathogen for tomato plants under laboratory conditions. Besides, Ferric oxide nanoparticles (Fe_2O_3NPs) can be used as inhibitors for tomato pathogenic wilt (Elbasuney et al., 2022).

On the other hand, the treated strain of *X. euvesicatoria* 1X-MAS by AEANP-FeONPs 8100 ppm concentration had low relatively severe symptoms of bacterial spot disease on the pepper plant. This result is consistent with Bytešníková et al. (2022). Gul et al. 2024, explained that nanoparticles worked role as a bactericidal through damage of the cellular membrane and degradation of deoxy nucleic acid and proteins. Besides, Elbasuney et al. (2022) reported that ferric oxide nanoparticles (Fe_2O_3NPs) had increased antioxidant enzymes in treated plants and suppressed the pathogens. In contrast, the treated strain of *P. syringae* pv. *tomato* Pst1-MAS by AEANP-AgNPs 5100 ppm concentration exhibited aggressive severe of the tomato bacterial speck symptoms on tomato plants. The result agreed with Schiavi et al. (2021). Also, Parveen and Siddiqui (2023) reported that green nanoparticles suppressed the disease severity of bacterial speck symptoms in the leaves of tomato plants.

Previously reported by Ali et al. (2015) indicated that exposure to silver nanoparticles decreased the activity and vitality of land snails, resulting in a 20% death rate among the treated snails, *in vitro*. The findings of Khidr (2018) align with these results, as they verified that nano-chitosan exhibited molluscicidal properties against *M. obstructa* and *E. vermiculata*, with LC_{50} values of 0.157% and 1.38%, respectively. Also, Moustafa et al. (2018) revealed that the mortality of freshwater snails and the infection stage increased in a dosage and time-dependent manner for exposed to AgNPs, reaching 100% mortality after one hour. Further, according to Morsy and Mohamed (2022), the LC_{50} values of nano-silver were (0.11% and 0.97%) assessed after exposure days of treatment on (*Ageotis ipsilon*) and (*M. obstructa*), respectively. Moreover, Wang and Liu (2023) demonstrated that exposure to 500 µg/L of AgNPs, caused damage to the soft tissues of snails, *Lymnaea stagnalis* and a reduction in its fecundity after 21 days.

Prior research has demonstrated that exposure to iron nanoparticles like Fe_2O_3, has a notable impact as molluscicides (Caixeta et al., 2021). Besnaci et al. (2016) observed toxicity for Fe_2O_3 affected distortion and a poor hatching rate for the embryonic stages of *Helix aspersa*, during the twelve days. Also, Dorostkar et al. (2017) discovered that nanoparticles made of iron had impacts on (*Toxocara vitulo*) in the twelve hours. Too, they discovered the death rate of the snails, *Biomphalaria alexandrina* and *Schistosoma mansoni* became 100% when exposed to varying concentrations of (iron NPs) for 48 hours (Khalil et al., 2018).

5. Conclusions

Some of the concentrations of AEANP-AgNPs did not exhibit inhibition degrees with *Xanthomonas euvesicatoria* while it exhibited inhibition degrees with *Pseudomonas syringae* pv. *tomato* with increasing nanoparticle concentrations. The concentrations of AEANP-FeONPs did not exhibit inhibition degrees with *Pseudomonas syringae* pv. *tomato* while it exhibited a low inhibition degree with *Xanthomonas euvesicatoria* until the higher tested concentrations. *In planta*, the higher concentration of AEANP-FeONPs exhibited relative suppression against the bacterial spot disease on pepper plants while the higher concentration of AEANP-AgNPs didn't exhibit a significant suppression against the bacterial speck disease in tomato plants. *Monacha cartusiana* snails were more sensitive to the toxic effect of Ag-NPs and FeO-NPs than adult snails, *Eobania vermiculata*. The mortality for adult snails was increased with increasing concentration of both nanoparticles.

List of abbreviations

AEANP-FeONPs:	Aqueous extract of *Acacia nilotica* pods with iron oxide nanoparticles.
AEANP-AgNPs:	Aqueous extract of *Acacia nilotica* pods with silver nanoparticles.
AEANP:	Aqueous extract of *Acacia nilotica* pods.
EDX:	Energy dispersive X-ray.
FT-IR:	Fourier transform infrared.
NPs:	Nanoparticles
SEM:	Scanning electron microscope
TEM:	Transmission electron microscopy.
MW:	molecular weight.
GC-MS:	Gas chromatography-mass spectrometry

Declarations

Conflict interests

No found conflict of interest

Funding

No Funding sources.

Authors contributions

All authors contributed to producing this study.

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