

Effect of Green Nanoparticles and Some Chemical Insecticides Against Thrips Insects

Heba Farrage^{1,2}, Hamza Elsharkawy¹, Essam Abou- Elsalehein¹, Elsayed Shereif² Ahmed Abd – Elwahab¹ Eman Elgohary², Hatem Fouad^{2,3}, and Mohamed Abdelkader²

¹Plant Production Department, Faculty of Technology and Development, Zagazig University, Sharkia 44519, Egypt

² Department of Field Crop Pests, Plant Protection Research Institute, Agricultural Research Centre, Cairo 12622, Egypt

³ Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insect Pests, Key Laboratory of Biology of Crop Pathogens and Insects of Zhejiang Province, Institute of Insect Sciences, College of Agricultural and Biotechnology, Zhejiang University, Hangzhou 310058, PR China

Corresponding author: eelsalehein@yahoo.co.uk

ABSTRACT:

Thrips tabaci (lind.) is one of the most economically damaging pests of onions, because of its high rate of reproduction, short generation period, and capacity to harm onion plants during their growth season. The green synthesis of metal nanoparticles offer answers to problems related to the environment and human health. This work aimed to create nanoparticles by plant extract to study their effects on onion thrips, which have not been studied before. UV-Vis, dynamic light scattering, zeta potential, transmission electron microscopy, scanning electron microscopy, and energy dispersive spectroscopy were used to confirm the analysis of NPs. Furthermore, the primary components of NPs were revealed through chemical analysis using gas chromatography coupled with a mass spectrometer (GC-MS). Also, nanoparticles and insecticidal action were assessed after 24-hour treatment at a dosage of 140mg/mL, where the mortality rates of nanoparticles were 99.53% as opposed to 43.55% for the plant extract treatment. Moreover, the effects of NPs, plant extract, and chemical insecticides on total protein concentrations and detoxifying enzymes including α - and β -esterase, acid, and alkaline phosphatase were examined. Additionally, histological changes discovered after exposure to NPs and chemical insecticides were demonstrated. Overall, the study's results unmistakably showed that the nanoparticles may have great potential for application as cutting-edge, ecologically friendly pesticides to reduce *T. tabaci* in onion fields.

Keywords: Nanopesticides; Onion thrips; Gas Chromatography; Biochemical studies; Histological studies.

INTRODUCTION

One of the most significant crops in the agricultural sector for human consumption, processing, and exportation is the onion, *Allium cepa* L. (Alliaceae). The world's intake of onions has greatly expanded due to their health benefits (1). Onions thrips insects are the most economically damaging pest to onion crops globally (2). In Egypt, during the seasonal growth of onion plants, thrips are the most harmful bugs (3). Thrips caused feeding damage to plants by punching the leaf surface and removing sap from the cells. They then attacked the plant's interior and ate the mesophyll cells, which resulted in a loss of chlorophyll and a decrease in photosynthetic efficiency (4). One study found that the yield losses brought on by onion thrips ranged from 26 to 57%, while another study found that the losses were between 10 and 85%. Many nations, including Egypt, have recorded reports of *Thrips tabaci* (Lind.) (Thysanoptera: Thripidae) transmission of the iris yellow spot virus (IYSV) (5). Chemical pesticide treatments are one method of *T. tabaci* control (6). All kinds of insecticides are involve an increase in resistance due to the widespread and careless use of chemical pesticides in insect pest management. Conversely, it is recommended to use natural plant oils to reduce the amount of chemical pesticides and their residues (7). The secondary metabolites of allelopathic organisms are called allelochemicals. In place of chemical pesticides, plant extracts have been utilized in pest management recently (8). However, nanotechnology is a new field that deals

with materials at the nanoscale that has grown astronomically in the last few years. Nanomaterials offer answers to problems related to the environment and human health. Due to its ease of use, low energy requirements, environmental friendliness, and lack of hazardous ingredients, the green synthesis of metal nanoparticles is regarded as a significant facet of nanotechnology. The adulticidal toxicity of chemical insecticides and plant extract against Onion thrips, was investigated in this work, as well as the potential for utilizing plant extract as a reducing and stabilizing agent in the synthesis of silver nanoparticles. UV-Vis spectrophotometry, which includes energy-dispersive X-ray (EDX), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR), were used to verify the production and properties of the manufactured AgNPs.

2. MATERIALS AND METHODS

2.1. Chemical and plant materials

We acquired silver nitrate (AgNO_3) from the Chinese Company Sigma Aldrich Chemical Corporation. The dry leaves of *Hibiscus rose Sinensis* were collected from Zagazig University, Zagazig city, Sharkia Government, Egypt. Acid phosphatases, Alkaline phosphatases, α -esterases, β -esterases, and Kits for the Bradford protein test were acquired from the Chinese Nanjing Jiancheng Bioengineering Institute. The plant materials were stored in a well-ventilated area for pending additional processing after being verified and validated at Zagazig University's Faculty of Science, Department of Botany, Egypt. Chemical insecticides were received from Institute for Plant Protection Research, Egypt. The Egyptian Plant Protection Research Institute provided chemical pesticides. Every experiment used Milli-Q grade water, and all other chemicals and reagents were of the highest analytical grade, acquired from nearby business companies.

2.2. Preparation of plant leaves extract

The leaves were collected and dried at room temperature before being manually milled. After combining the 10g of powdered leaves with 200ml of distilled water, and the combination was left for an hour at 70°C in a water bath. The extract was filtered by Whatman No. 1 filter paper, and the resulting aqueous extract was utilized as a precursor for the manufacture of silver nanoparticles, as per the protocol outlined in (9).

2.4. Preliminary phytochemical testing

Using previously published methodologies, a preliminary phytochemical test was conducted on the leaf extract to identify various chemical groups of chemicals. Using the procedure outlined by (11), the contents of air-dried and powdered leaves were examined for the presence of carbohydrates, coumarins, anthraquinones, glycosides, alkaloids, and flavonoids.

2.5. Synthesis of silver nanoparticles

In order to create AgNPs, 200 mL of an aqueous AgNO_3 (3mmol/L) solution and 50mL of leaf extract were combined, and the mixture was agitated for one hour at 60°C. When the sample turned reddish brown after 24 hours, it was clear that the reduction had happened quickly and that AgNPs had formed. The combination combining AgNPs with flavonoids, proteins, and carbohydrates, was acquired, and it was whirled about twice at 10,000 rpm for 30 minutes to remove contaminants. The pellet was then collected and dried using the procedure outlined in (10).

2.6. Characterization of synthesized *Hibiscus rose*-Ag

2.6.1. UV-Vis spectral analysis

According to a procedure previously published, using a UV-2550 spectrophotometer (UV-Visible spectrophotometer Shimadzu, Japan) with a resolution of 1 nm and a range of 190 nm to 700 nm, the analysis samples were diluted with 2 mL of double distilled water.

2.6.2. Zeta potential distribution

Zeta potential at 25°C (Malvern Instruments, Z-sizer Nano) was used to determine the stability of AgNPs in an electric field.

2.6.3. FT-IR analysis

Following the given method, using a Fourier transform infrared spectrometer, Fourier transform infrared spectra were obtained (VECTORE 22, BRUKER, Germany) under identical conditions of the 4000–400 cm^{-1} range at a resolution of 4 cm^{-1} . In other places, AgNPs (1mg) and KBr (300mg) were combined. After being formed using a hydraulic pellet press, the thin AgNPs pellet was examined by FT-IR.

2.6.4. SEM analysis and EDX

With the use of an electron scanner microscope (SEM) (Hitachi, Japan, TM-1000), the size of the produced AgNPs was examined. On a copper grid covered in carbon with a mesh size of 40–400 nm, thin film samples were created by removing the example onto the grid and using blotting paper to remove excess solution. Subsequently, the SEM grid was placed under a mercury lamp for five minutes to allow the film to dry. An Energy Dispersive X-ray (EDX) was fitted to the apparatus to verify the existence of silver metal.

2.6.5. TEM analysis

Through the use of Transmission Japan Electron Microscopy (JEOL-JEM-1230), AgNPs' structural characteristics were determined. Utilizing a blotting paper cone, the excess sample was taken out of the copper grid covered in carbon. Next, the sample placed on the grid to form a slender layer.

2.6.6. X-ray diffraction analysis of AgNPs

Siemens X-ray diffractometer used to perform XRD analysis to investigate the crystalline structure of AgNPs. The CuK α radiation served as the X-ray origin, and the operation parameters, which included Current of 20 mA and 45 kV, were adhered to as documented by (11).

2.7. Chemical characterization of Hibiscus rose

Gas chromatographs (7890B, Agilent, USA) combined with mass spectrometer detectors (7000C, Agilent, USA) were used to perform GC/MS studies of E-NE and N-NE. A DB-5MS column (30 m by 0.32 mm i.d., 0.25 μ m film thickness) used as the GC-MS apparatus. The following temperature program was used to achieve the split ratio of 1:10 and helium as the carrier gas at a flow rate of 1 mL/min and injection of 0.2 μ L (1:10% hexane solution) under the analytical conditions: 60°C for 4 minutes, then 10°C/min rise to 300°C, which is held for 5 minutes. At 250°C, the injector and detector were maintained. In every case, 1 microliter of the mixes injected with diluted samples (1:10 hexane, v/v). Chemical identification was accomplished via computer matching against commercial (NIST 05, Wiley FFNSC, and ADAMS) and home-made libraries, as well as by comparing the pure chemicals of the compounds' retention durations (RTs) and their linear retention indices (LRIs) (12). Normalizing peak areas allowed for the estimation of the relative percentage of chemicals.

2.8 Toxicological studies

2.8.1. Rearing technique of *Thrips tabaci*

Thrips tabaci used in experiments were collected from *Allium cepa* (onion) fields from Sharkia Governorate and transferred to laboratory. *Thrips tabaci* were kept on each rearing plants for several generation. Every week (5-7 days) plants were replaced with new ones in order to keep *Thrips tabaci* alive. *Thrips tabaci* were prevented from external contamination by placing infested plants in cages covered with a muslin cloth, these cages were proved to be advantageous to permit good ventilation and light penetration and avoid *Thrips tabaci* attack with parasites and predators as shown in plate (2). Apterous adults from these colonies were used in this study (13).



Cultivated onion sapling



infested onion in cage

Application technique



Dipping for 10 sec



Air-drying



Replicates

Plate (1): Rearing of *Thrips tabaci* and application techniques of tested treatments

5.2 Preliminary insecticidal tests.

Four insecticides (Chlorpyrifos, Methoxy fenozide, Imidacloprid and Pymetrozine 20 % + Thiamethoxam 15%) , nanoparticles and extract which represented in (Table 1) were tested against adult of *Thrips tabaci* to determine the maximum concentration of the tested materials.

2.82. Bioassay tests

The serial concentrations of aqueous solutions for each of the tested compounds were prepared. Discs of onion leaves were dipped in the above mentioned solutions for 10 seconds then left to dry at room temperature, treated discs were put separately in Petri dishes each one consider as one replicate every 30 individuals of *thrips tabaci* were transferred to one treated leaf disc. *Thrips tabaci* treated with different compounds were counted after 24 hr. Mortality data were corrected according to (14). LC-P lines were established and LC50 values were determined.

Abbott's formula :

$$\text{Corrected mortality \%} = \frac{\text{observed mortality} - \text{control mortality}}{100 - \text{control mortality}} \times 100$$

The toxicity lines were statistically analyzed according to the method described by (15) . The relative efficiency of the tested pesticide was determined according to (16) as follow:

$$\text{Toxicity index} = \frac{\text{Lc50 or Lc90 of the compound (A)}}{\text{Lc50 or Lc90 of the compound (B)}} \times 100$$

Where: (A) = is the most effective compound.

(B) = is the other effective compound.

Relative potency values were measured according to the method describe by (17)

$$\text{Relative potency} = \frac{\text{Lc50 or Lc90 of the compound (A)}}{\text{Lc50 or Lc90 of the compound (B)}}$$

2.9. Biochemical studies

2.9.1. Preparation of samples for biochemical assays

The adult of *Thrips tabaci* collected from infested plants of onion fields, and then transferred to the same plants grown in plastic pots in the laboratory and it lets to colonies on these plants for many generations. The infested plants were sprayed with LC₅₀ of AgNPs, plant extract, and four insecticides. All compounds replicated 3 times, in addition to control samples. The *Thrips tabaci* samples (treated adults) (in 1g weight) were collected from the six groups by using fine camel hair brush after 24h. After treatment and control, all treatments placed in little bottles and frozen until the time of the analytical procedure. Using a Teflon homogenizer, the frozen *Thrips*

tabaci samples were homogenized in 5 milliliters of distilled water per sample. The homogenates underwent a 10-minute, 5000 rpm centrifugation at 5°C. The total soluble protein, α & β esterase, and acid & alkaline phosphatases were measured right away in the supernatants.

2.9.1. Determination of total soluble protein

The total soluble protein in the supernatants of homogenate *Thrips tabaci* adult was determined colorimetrically using the procedure as described by (18). The method's principle was based on the presence of an alkaline cupric sulfate, which causes the protein to produce a violet purple color whose intensity is proportionate to its concentration. In a nutshell, 5 ml of Biuret reagent and 0.2 ml of Thrips homogenate were combined, and the mixture was incubated for 30 minutes at 20–25°C. At a wavelength of 546 nm, the absorbance of the sample was measured in comparison to a blank Biuret reagent.

2.9.2. Acid and alkaline phosphatases

The technique described in (19) was used to calculate the activity of acid and alkaline phosphatase. Under specific time, temperature, and pH conditions, the phenol produced from the enzymatic hydrolysis of the substrate, disodium phenyl phosphate, combines with 4-amino antipyrine and potassium ferricyanide to produce a reddish brown color which was estimated at 510 nm. The reaction mixture has one milliliter in it of citric buffer for acid phosphatase (PH 4.9) or 1ml of sodium carbonate and bicarbonate buffer for alkaline phosphatase (pH 10:14), 1ml Disodium phenyl phosphate (substrate) along with 0.2ml of homogenate thrips. The reaction was mixed gently and incubated at 37°C for 30 minutes. 0.8ml of 0.5N NaHCO₃, 1ml of 4-amino antiphrine solution, and 1ml of potassium ferricyanide were added at the conclusion of the incubation time to the reaction mixture. In the control experiment, Homogenate (0.2ml) was added, while in blank test 0.2ml distilled water was used. The produced brown hue was assessed right away by spectrophotometer at 510 nm against blank.

2.9.3. α -estrases and β -estrases enzymes

As non-specific α - estrases and β -estrases were identified colorimetrically using α - and β -naphthyl acetate as substrates, respectively, in accordance with the procedure outlined by(20). When diazoblue sodium lauryl sulphate solution is added to thrips homogenate as an enzyme resource, naphthol produced as a result of substrate hydrolysis can be identified. This produces a strong blue color for α -naphthol or a strong red color for β -naphthol, which are measured at absorbencies of 600 and 555 nm for α -naphthol and β -naphthol, respectively.

2.10. Histological observations post-exposure to AgNPs

The histological observations in the adult *Thrips tabaci* after exposure to AgNPs for 24h at LC₅₀ and LC₉₀ were conducted with some modifications in accordance with the protocol of(21). In summary, the adult insects were dehydrated with a graded sequence of ethanol, cleaned with xylene solution, and preserved in 4% paraformaldehyde solution for 48 hours. Then, in the embedding station, they were embedded using melted paraffin in paraffin blocks. Using a rotary microtome, the paraffin blocks were divided into 5-mm- thick sections, followed by hematoxylin and eosin staining. The glass slides were inspected with a Leica DM500 China light microscope to look for anomalies.

2.11. Morphological observations post-exposure to nanoparticles

With minor adjustments, the technique outlined by(22) was used to examine the impact of AgNPs' LC₅₀ on *Thrips tabaci*'s morphological alterations. In a nutshell, the treated and untreated insects with an LC₅₀ of AgNPs for a whole day were carefully gathered and given two more washes using water. Following an overnight soak in 2.5% glutaraldehyde, samples were twice rinsed in phosphate buffer saline (PBS) and subsequently treated for one hour in osmium tetroxide. The samples were put on stubs, spurted with 45 nm gold powder, dried using a critical point drier, and then given one more PBS wash before being examined using a scanning electron microscope (TM-1000, Hitachi, Japan).

2.12. Statistical analysis

Probit analysis was used to determine the percentage of mortality data, and statistics with lower and higher 95% confidence intervals were computed for LC₅₀ and LC₉₀. Using the software SPSS version 21 (SPSS Inc., Chicago, IL, USA), one-way analysis of variance (ANOVA) was used to examine the data, and then Tukey's HSD and Duncan's test for analyzing the biological variables observed in the experiments. In all data analysis, a P-value of less than 0.05 was considered statistically significant, conducted to evaluate the variations between the control and treatment groups.

3. RESULTS

3.1. Preliminary phytochemical testing

The primary components of the *Hibiscus rosa-sinensis* plant extract were identified after an initial phytochemical investigation. **Table 1,2** demonstrates the presence of flavonoids, glycosides, and alkaloids /carbohydrates in Chinese hibiscus extract phenols, saponins, steroids, tannins and terpenes in a comparatively large quantity. The reduction of Ag^+ to Ag^0 is thought to be caused by the presence of phenolic chemicals, carbohydrates, and flavone glycosides in plants (7, 11). Previous research on *C. fistula* leaves has found the existence of reducing sugars, starch, protein, amino acids, steroids, glycosides/carbohydrates, flavonoids, tannins, alkaloids, and triterpenoids (18). It appears that anthraquinones, flavone glycosides, and carbohydrates may be found in the Chinese hibiscus plant (11). These chemicals are anticipated Plant proteins are assumed to be active molecules for the synthesis of nanoparticles, and to be in charge of the reduction of AgNO_3 to Ag^0 (19).

Table 1. Preliminary phytochemical screening of crude methanolic extract of Chinese hibiscus.

Compounds	Alkaloids	Flavonoids	Glycosides	Phenols	Saponins	Steroids	Tannins	Terpenes
Methanol extract								
Hibiscus	+	++	+++	++	-	-	+	+

Table 2. Total phenolic content (TPC), Total flavonoid content (TFC) and Antioxidant Activity of Chinese hibiscus.

Methanol extract	Total phenolic content (TPC) mg GAE/gdw	Total flavonoid content (TFC) mg QE/gdw	Antioxidant Activity (DPPH %)
Hibiscus	67.45	49.28	72.46

3.2. Characterization of nanoparticles

Recognition of what happens at the nanobiointerface requires a thorough description of nanoscale materials. AgNPs have been characterized through the use of UV-Vis, FTIR, zeta potential, SEM, EDS, and TEM. The first step in examining how silver nanoparticles form in aqueous solution is UV-vis spectroscopy. The plant extract and AgNO_3 underwent bio-reduction in the current investigation, and the modification in color to reddish brown suggested the synthesis of AgNPs. Additional researchers have also reported on the synthesis of AgNPs derived from plants (20). At about 400–600 nm, Surface plasmon resonance (SPR) bands made of silver were observed (21). Nevertheless, a thorough understanding of the actual mechanism is lacking. The AgNPs' free electrons, as previously reported, produce a surface plasmon resonance at 448 nanometers in wavelength. This could be the result of the metal nanoparticles' combined electrons vibrating in resonance (22). The silver particle's characteristic, the SPR, was detected in our investigation at 410 nm (**Figure 1A**). Consistent with our results, the Aloe vera-based investigation on the biosynthesis of AgNPs similarly showed a broad peak with a maximum wavelength of 410 nm (23). The NP exhibited a conductivity of 1.07mS/cm and a mean zeta potential of -7.78mV (**Figures 1B**), demonstrating the high stability of nanoparticles. To assess the stability of the nanoparticles and subsequently the composition of the formulation, their electrical conductivity was assessed. It offers details on the phase inversion and continuous phase phenomena of AgNPs. Any molecule's capacity to transmit electricity between two places is known as conductivity (24). (25) found that the Z-averages of the nanoemulsions of *Citrus limon* and *Melaleuca alternifolia* were -2.30mV and -2.02mV, respectively. AgNPs' form and size features can be better understood by looking at images captured by scanning electron microscopy. The following primary bands and matching assignments were shared by all of the AgNPs produced using Chinese hibiscus plant extract (*Hibiscus rosa-sinensis*). The broad bands of the O-H stretching vibration are 3852cm^{-1} , 3734cm^{-1} , 3560cm^{-1} , and 3249cm^{-1} ; the CH_3 stretching vibration is 2905cm^{-1} ; the C=O stretching vibration is 1634cm^{-1} and 1528cm^{-1} .

(caused by the amide I group); the C–O–H bending vibration is 1378cm^{-1} and 1224cm^{-1} ; and the C–N stretching vibration is 1015cm^{-1} . While 579cm^{-1} were characteristic bands of AgNO_3 solution (**Figure 1C**). It is evident that proteins' free amine groups allow them to bind to the silver nanoparticle (12). C–OH bond stretching vibration was demonstrated by the greater absorption peak at 1069cm^{-1} (9). As can be seen, the peak at 1260cm^{-1} was likely primarily caused by the hydroxyl avones' polyphenol C–O group (18). The majority of AgNPs SEM images that were seen on the micrograph were spherical. SEM research showed that the aggregate of silver particles may have created the different grain AgNPs sizes (**Figure 2A**). *Emblica officinalis* AgNPs were primarily spherical in SEM images, with a range of 7.5 to 25 nm with an average size of 16.8 nm (25). The EDX profile observed that a distinctive strong peak at 3keV, which denotes the conversion from Ag^+ to Ag^0 , was likewise given by the AgNPs. *Hibiscus rosa-sinensis* plant extract may contain phytochemical components, as evidenced by the other observed peaks of O, Cl, Al, Si, and a little peak of Fe (**Figure 2B**). Other researchers have reported the same bank peak (3keV) (11, 25). TEM micrographs revealed a spherical shape in the produced NP. (**Figure 2C**) with a size ranging from 17.03 to 29.46 nm (**Figure 2D**), which was consistent with the results of SEM images (**Figure 2**). NPs' dimensions and forms are significant in a variety of biological applications, particularly those involving vector control. Electron microscopy has verified that the majority of AgNPs and AuNPs are spherical (22). Apart from *Azadirachta indica*, which yielded morphology resembling both a spherical and a flat plate most of the AgNPs mediated by plants had a spherical form (27). AgNPs from *Emblica officinalis* ranged in size from 7.5 to 25 nm, with an average size of 16.8 nm and a spherical appearance in SEM images (29). The prepared NP remained stable even after centrifuging it for 20 minutes at ambient temperature and 10,000 rpm. After being stored at different temperatures for 60 days, there was no evidence of phase separation or creaming.

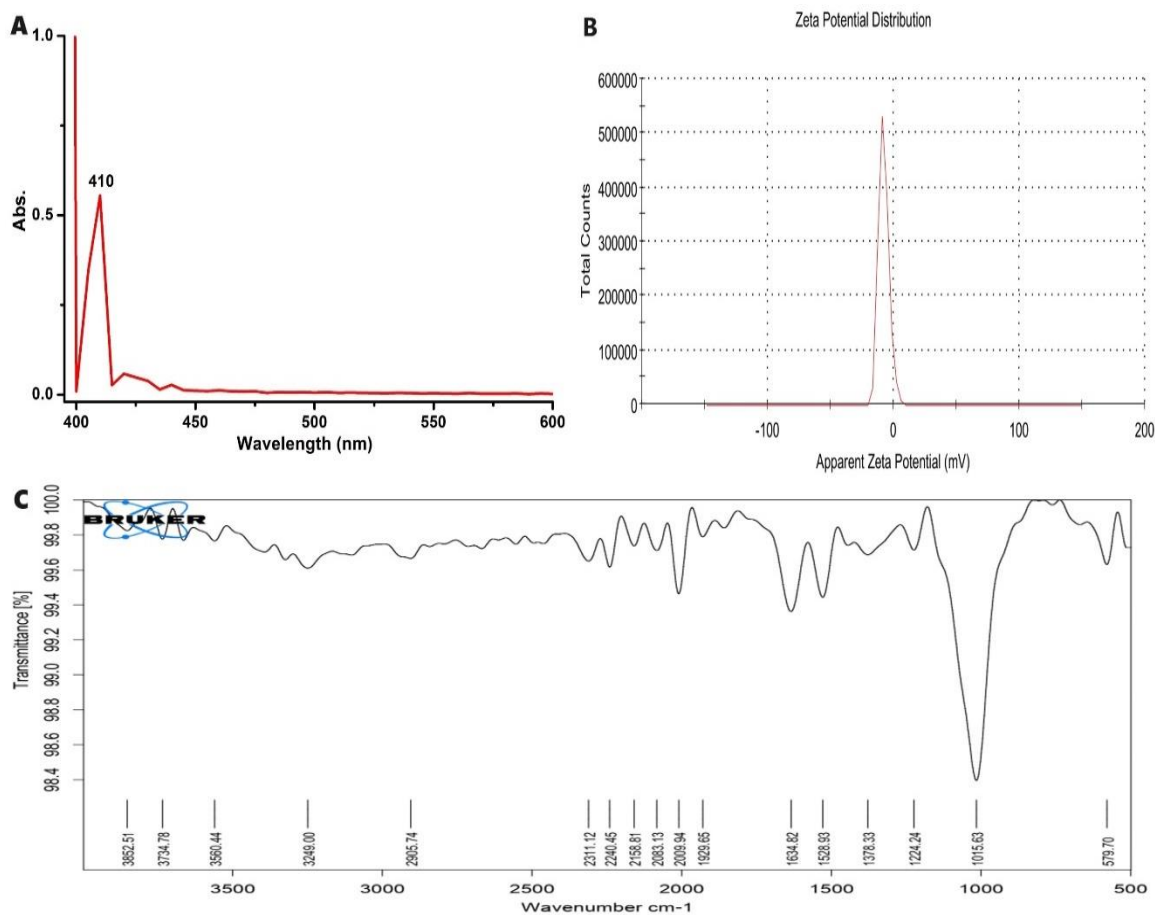


Figure 1. Characterization of nanoparticles. (A) UV–Vis absorption spectra of AgNPs synthesized using aqueous plant extract of Chinese hibiscus; (B) zeta potential distribution of AgNPs; (C) The FTIR spectra of AgNPs biosynthesized by Chinese hibiscus aqueous plant extract.

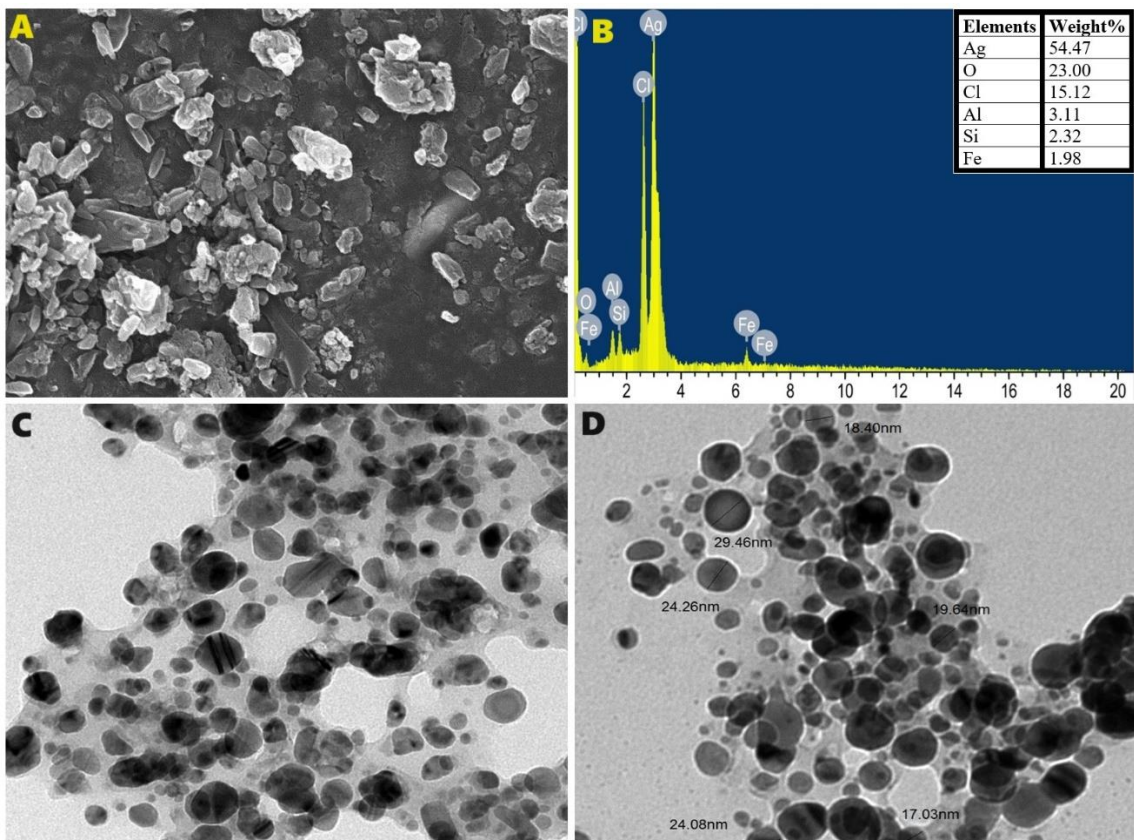


Figure 2. Characterization of AgNPs. (A) Scanning electron microscopy; (B) Energy dispersive X-ray; (C) Transmission Electron Microscopy; (D) Transmission Electron Microscopy with different sizes.

3.3. Chemical Characterization of Chinese hibiscus

The bioactive chemical components of hibiscus rose were determined using mass spectrometry and gas chromatography. NP GC-MS analyses indicated the existence of 26 chemicals. The primary chemical components of plant extract were Thiazoline (27.99%), Acetal (10.92%), Isopropyl acetate (9.63%), Ethyl propionate (6.26%), 1-Octene (5.81%), and Palmitic acid (2.26%) as displayed in **Table3**. Conversely, less than 3% of the chemical components from compounds 25 and 24 remained in the plant extract, revealed that 17.73% of the oil's main ingredient, eucalyptus oil, is composed of eucalyptol. (29) found that γ -pinene (11.17%), β -pinene (8.19%), γ -terpinene (5.91%), eucalyptol (64.80%), and α -phellandrene (3.88%) were the main components of eucalyptus oil. Nutmeg oil's main constituents were γ -terpinen (8.51%), β -pinene (26%), α -pinene (10.51%), and sabinene (9.16%) (30). According to a different study, α -pinene (7.17%), γ -terpinen (19.08%), terpinolene (2.06%), and myristicin (7.33%) are the primary constituents of nutmeg oil (21). Species, season, and crop-growing conditions can all affect the composition.

Table 3. GC -MS analysis of *Hibiscus rosa-sinensis*

Compounds	Other Names	RT (min)	Molecular Formula	Relative Peak Area (%)a
2,2-dimethyl-3-Pentanol	Neopentyl Alcohol	4.55	C7H16O	0.17
Propan-2-yl acetate	Isopropyl acetate	5.10	C5H10O2	9.63
Butane, 1-ethoxy-	Butyl ethyl ether	5.61	C6H14O	1.29
Propanoic acid, ethyl ester	Ethyl propionate	6.38	C5H10O2	6.26
1,1-diethoxy- Ethane	Acetal	6.98	C6H14O2	10.92
2-Hexanol, 2,5-dimethyl-, (S)-	N-octanol	7.70	C8H18O	0.39
Toluene	Methylbenzene	8.07	C6H5CH3	0.27

Thiocyanic acid, ethyl ester	Thiazoline	8.41	C3H5NS	27.99
Cyclopentane, 1-ethyl-3-methyl-, cis-	1-Octene	8.81	C8H16	5.81
Hexanamide	n-Caproamide	9.23	C6H13NO	1.02
3-Penten-2-one, 4-methyl-	Mesityl oxide	9.32	C6H10O	0.52
3-Ethoxyacrylonitrile	Furfurylamine	9.67	C5H7NO	0.65
2-Nonene, (E)-	Cyclononane	10.85	C9H18	0.23
Ethyl dodecyl ether	1-Tetradecanol	11.18	C14H30O	0.21
Benzene, 1,3-dimethyl-	m-Xylene	11.78	C8H10	0.23
(S)-(+)-6-Methyl-1-octanol	1-Nonanol	12.19	C9H20O	0.60
Formamide, N-(cyanomethyl)-	Cyanoacetamide	12.37	C3H4N2O	0.62
Isopropylamine, N-acetyl-	Prolinol	13.24	C5H11NO	0.23
Cyclohexane, propyl-	Cyclononene	14.55	C9H16	0.10
5-Ethylcyclopent-1-ene-1-carboxylic acid	Dimedone	43.34	C8H12O2	0.17
Neophytadiene	Labdane	44.90	C20H38	0.16
Phytol, acetate	Erucic acid	44.96	C22H42O2	0.16
2-Pentadecanone, 6,10,14-trimethyl-	Octadecanal	45.07	C18H36O	0.07
n-Hexadecanoic acid	Palmitic acid	47.98	C16H32O2	2.26
Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-	Cembratrienol	48.91	C20H34O	0.11
Linoelaidic acid	Linoelaidic acid	51.78	C18H32O2	0.60

3.4. Insecticidal activity

The compound activity of various quantities of aqueous plant extract of Chinese hibiscus, synthetic silver nanoparticles and chemical insecticides were tested against adult *Thrips tabaci* up to ten days in the current study. The aqueous extract's LC₅₀ and LC₉₀ values against adult thrips where LC₅₀ values of 96.11mg/L and the LC₉₀ values of 523.58mg/L after 72h, respectively (**Table 4**). Many plant crude extracts, including *Cannabis sativa* and *Humulus lupulus*, have previously been described as possible larvicidal agents against target mosquitoes (13). According to the current findings, synthetic AgNPs have demonstrated outstanding efficacy against *T. tabaci* with low levels of LC₅₀ and LC₉₀ of 4.89 and 84.44mg/L respectively, after 24h. While, chemical insecticides Chlorpyrifos, Imidacloprid, Pymetrozine 20%+Thiamethoxam15% and, Methoxyfenozide were the LC₅₀ values against adult thrips where 24.24, 28.82, 25.24, 25.03 mg/L, respectively (**Table 4**). Even so, the aqueous extract of the plant effects on adult thrips In contrast to AgNPs treatments, which showed a deadly effect on adult thrips at relatively low concentrations, it requires high doses to reduce adult mortality. The intrinsic harmful consequences of microscopic particles inside the cuticle may be the reason why silver nanoparticles induce larval and pupal death. There have been previous descriptions of the cross-movement of tiny particles that target specific cells inside and inhibit other physiological processes, such as melting (6). In another research, LC₅₀ values of artificial AgNPs made with leaf extract from *Rhizophora mucronata* were found to be 0.585, 2.615mg/L, and the LC₉₀ values to be 0.891 and 6.291mg/L when tested against *A. aegypti* and *C. quinquefasciatus* larvae (3). Additionally, the plant *Pergular iadaemia*, which produces latex in opposition to the larvae's first, second, third, and fourth instars, AgNPs exhibited outstanding larvicidal efficacy. Nevertheless, the *A. aegypti* and *A. stephensi* larvae showed no discernible mortality effects after either 24 or 48 hours of exposure (31).

Table 4. Toxicity of plant extract, AgNPs and chemical insecticides against adult *Thrips tabaci*.

Treatments	LC ₅₀ (mg/L)	Confidence limits		LC ₉₀ (mg/L)	Confidence limits		Slope
		lower	upper		lower	upper	
Plant extract	96.11	75.37	114.37	523.58	383.74	876.413	1.740
AgNPs	4.89	1.0053	8.928	84.44	54.44	248.02	1.036
Chlorpyrifos	24.24	15.915	31.23	112.78	90.083	167.32	1.934
Imidacloprid	28.82	18.612	36.563	173.91	125.49	330.98	1.641
Pymetrozine20%+Thiamethoxam15%	25.24	14.616	33.261	166.09	119.232	328.126	1.566
Methoxyfenozide	25.03	15.105	32.610	146.03	109.007	258.45	1.673

3.5. Biochemical studies

3.5.1. Effect of NP on enzymatic activities in the onion thrips

The biochemical response of the adult of *Thrips tabaci* was assessed after 24h of treatments with the LC₅₀ of tested compounds. The biochemical parameters investigated were determined by the total soluble protein, acid & alkaline phosphatases and α and β esterase enzymes. Nonetheless, the impact of examined substances on these enzymes as well as potential connections between enzyme inhibition and growth suppression were explored.

3.5.1.1. The total soluble protein.

One of the most significant components of insects that binds to external substances is protein. The overall protein content of the treated insect may have decreased due to a decrease in different enzyme activities (30). Furthermore, protein is one of the primary biochemical elements required for an organism's growth, development, and ability to carry out its essential functions (31). Data in **Figure 4A** the concentration of total soluble protein and the changes as a percentage from the control in adult supernatants of, *Thrips tabaci* treated with plant extract, nanoparticles, and chemical insecticides at their LC₅₀ after 24h. Generally, all treatments decrease and increase protein levels. The nanoparticles and pymetrozine 20% + thiamethoxam 15% exhibited very high reduction in protein levels (12.17 and 5.03 mg protein/ml) with decreasing percent (99 and 80%) followed by the imidacloprid, methoxyfenozide, and chlorpyrifos (78 ,73 and 66%) in protein level (4.13, 3.17, and 2.58 mg protein/ml) while the treatment of extract recorded the lowest percentage (58%) in protein levels (2.4 mg protein/ml) respectively, in comparable with soluble protein level in control treatment (0.13 mg protein/ml). The purpose of researching proteins is that they are the main building blocks of cells and are crucial to all biological activities, including reproduction (19). Plant extracts were reported to lower protein levels (25). The suppression of DNA and RNA synthesis could be the cause of the decrease in protein content. On the other hand, a drop in the enzymatic activity of different enzymes may be the cause of the total protein decrease in the treated larval instar's fifth instar. These results are in concordance with that obtained by (20) where he observed that the aqueous extract of cabbage seeds, extract of onion bulbs and cotton seed oil caused a decrease in the general mean of total soluble protein as compared to the control. As well as (32) showed that when compared to a control, neemix and basil oil significantly reduced the amount of protein of *A. craccivora*. The obtained results are also, comparable with the findings of (33) they mentioned that the reduction in protein content was more pronounced in nymphs of *Aphis craccivora* than in adults treated with mineral oils (super-misrona, shokrona, and KZ oil), neem extract, and pirimicarb. Likewise, azadirachtin significantly influenced protein level of *S.litura*. Conclusively, the above studies went side by side with the present results in revealing that, the treatment of biozoid, *T. ulbum* and uniconazole, recorded the lowest reduction percentage of cabbage aphid, *B. brassicae*. For example, the total protein content of the aphid individuals treated with *M. anisopliae* and KZ oil showed a significant drop. Moreover, (34) demonstrated that the treatments with biovar, entomopathogenic fungus and extratone, PGR and their mixtures caused decreased in total soluble protein in adult of *A. craccivora* after 48 hr. Also, (35) tested the effectiveness of two insect growth regulators (IGRs), diflubenzuron and chlorfluazuron against the freshly developed *Pectinophora gossypiella* pink bollworm larvae. The IGRs decreased total soluble proteins. In addition,

(36) stated that, treatment of pink and spiny bollworms (*P. gossypiella* and *Earias insulana*) with chinmix, spintor and biorepel compounds caused decrease in total soluble protein in both insects. (30) mentioned the biological effects of the organophosphate pesticide chlorpyrifos on the cotton leafworm, *S. littoralis*, in its fourth larval instar. Biochemical analysis showed the total protein content of the cotton leafworm larval instar was decreased by using chlorpyrifos. Finally, (30) concluded that some biochemical responses of 4th instar larvae of *S. littoralis* after 24, 48, and 72 hours, spinosad and triflumuron, either alone or in combinations with two surfactants, Triton X-100 and Tween-20, caused a high decrease in total soluble protein.

3.5.1.2. α -esteras, β -esteras, Acid and Alkaline phosphatases

Acid phosphatase (ACP) and alkaline phosphatase (ALP) are hydrolytic enzymes, which hydrolyze phosphomonoesters under acid or alkaline conditions, respectively. These enzymes are found in lepidopteran insects' midguts, muscles, nerve fibers, and Malpighian tubes. The mid gut has higher ALP and ACP activity than the other tissues. The ALP and ACP activities were found to be low during the larval molting stage and to increase gradually after molting. It is likely that the control insects' higher midgut enzyme activity results from their extensive food ingestion and use. Inhibition of the gut's peristaltic movement and an imbalance in the enzyme-substrate complex may have contributed to the treated flies' decreased enzyme activity. (37) revealed that there is a definite correlation between the amount of food that enters the alimentary canal and the synthesis of enzymes. The aphid's physiological state and the uptake, digestion, and positive transport of nutrients in the midgut are reflected in the activity of these enzymes. Animal intestinal epithelium is home to ALP, which is primarily involved in supplying phosphate ions from mononucleotide and ribonucleoproteins for a range of metabolic activities. In the present study, the activity of these enzymes of adult *Thrips tabaci* were concluded in **Figure 4B, 4C, 4D and 4E** treated with plant extract, Nanoparticles and chemical insecticides after 24h. A pronounced elevation of the specific activity of ALP and ACP enzymes were observed in the treated *Thrips tabaci*. The activity of ACP highly increased in nanoparticles treatment by 8.62%, at LC₅₀ after 24h. The exception was recorded with extract LC₅₀ which caused a reduction in the activity of ACP (18.34) relative to control. In the same trend, the alkaline phosphatase ALP activity revealed highest significance increase by 22.46% in treatment with the Pymetrozine 20% +Thiamethoxam 15%, and then recorded slightly increase with Methoxyfenozide and Chlorpyrifos (11.47 and 10.93%), respectively. On the contrary, the nanoparticles, plant extract and Imidacloprid exhibited inhibition in the relative activity of ALP enzyme with reduction percent 3.85, 50.7 and 63.09%. It is noticeable that, the decline in these enzymes' activity following a few days of treatment suggested that this material affect gut physiological events (i.e., ion transport) that might influence these enzymes. After two days of therapy, there may have been a significant release of acid and alkaline phosphatases due to the loss of the mid-gut epithelium, which may have contributed to the rise in gut enzymatic activity. The insect may attempt to compensate for these abnormal characteristics by producing an excessive amount of these enzymes in order to grow and develop more quickly as it loses weight and stops feeding. Indeed, post-treatment ALP and ACP activity suggested that altering the midgut's physiological balance could have an impact on these enzymes. It appears that a lower level of ACP indicated a decrease in the rate of metabolism, phosphorus release for energy metabolism, and metabolite transport rate. This could be because the therapies had a direct impact on the control of enzymes. These results are in accordance with that obtained by (11) recorded a negligible change in the alkaline and acid phosphatases activities in treated larvae of *S. littoralis* with Eucalyptus sp. plant extracts at LC₅₀. Also, (20) recorded an increase in both ACP and ALP activity in larvae of *Parasarcophaga argyrostoma* treated with the juvenile hormone, pyriproxyfen. In addition, (38) stated that, the alkaline phosphatase activity of the integument under the effect of the chitin inhibitor (chlrofluzuron). Surprisingly, the treatment of biozed caused slightly elevation in alphaesterases activity but it caused significance inhibition in beta- esterases activity in adult of cabbage aphid, *B. brassicae* (L.). Although each of the studied insects showed a considerable increase in alpha-esterase activity, *B. tabaci* and *A. gossypii*. Subsequently, all plant (oils and extract) treatments caused irregular action on esterase's activities. While alpha esterase activity significantly decreased in *B. tabaci* and *A. gossypii* upon azadirachtin treatment, beta esterase activity dramatically increased. Only alpha-esterase activity in *B. tabaci* and *A. gossypii* significantly enhanced after treatment with damascus extract, respectively. Furthermore, the only tested substance that did not significantly affect the activity of acid enzymes in either of the two examined insects was damaseia extract. While, (39) revealed that because of their capacity to detoxify defensive chemicals in order to adapt to these host plants, esterase increase in generalists, with the highest level found on *V. fabae* and the same rise in specialist *A. fabae*, with the highest level found on *Cynanchum acutum*. Finally, the increase of total EST activity after 48h on cabbage aphid observed in our experiments could be related with a time lag which is necessary for the sugar esters to be modified and induce the increase of enzyme activity inside the aphid body (40).

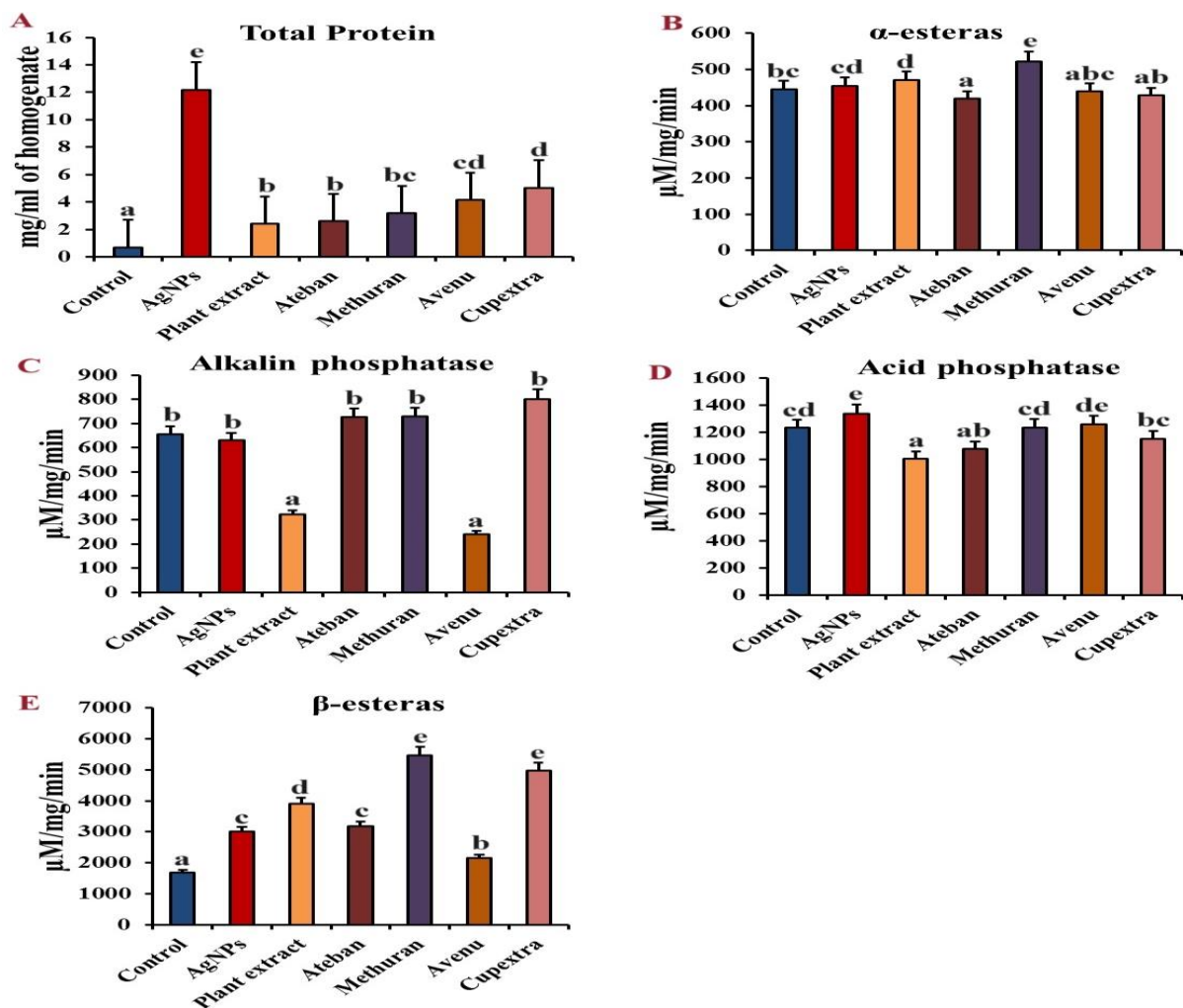


Figure 4. Effects of Nanoparticles, plant extract, and chemical insecticides on the total protein level and enzyme activities in adult of Thrips insects. (A) Total protein concentration; (B) α -esteras; (C) Alkaline phosphatase; (D) Acid phosphatase; (E) β -esteras. Different letters above the bars of each figure indicate significant differences based on Tukey's test at $P < 0.05$ between control and other treatments. Each bar represents the mean $\pm$ SE of three replicates using different preparations of insect homogenates.

3.6. Histological observations postexposure to AgNPs

The histological results in adult Thrips treated with Nanoparticles, plant extract, and Ateban for 24h at LC_{50} and LC_{90} are shown in **Figure 5**. When thrips insects were subjected to AgNPs, their histological structures in the muscles, cuticles, and digestive tract-particularly the hind gut were altered compared to the control group, particularly at LC_{90} . At LC_{50} , these same regions showed very minor abnormalities. In contrast to control insects, which showed normal appears for the abdomen, thorax, and head in addition to normal cells, the digestive tract's epithelial cells showed decreased cellular interactions with surrounding cells, separation of cells from the basal lamina, and post-treatment cell deterioration, according to these studies. Thrips muscles, epithelial cells, and cuticles are the tissues most damaged by AgNPs, according to histological examinations conducted on adult insects. After being exposed to eucalyptus oil nanoemulsion larvae of *C. quinquefasciatus* show evidence of midgut leakage, damaged midgut epithelial cells, and damaged peritrophic membranes according to (19, 29). Enzyme release during digestion and absorption of nutrients are both related to the midgut region of insects, which is a crucial area. Furthermore, as a sensitive measure of toxicity directed at some dangerous compounds, the integrity of the midgut can be investigated.

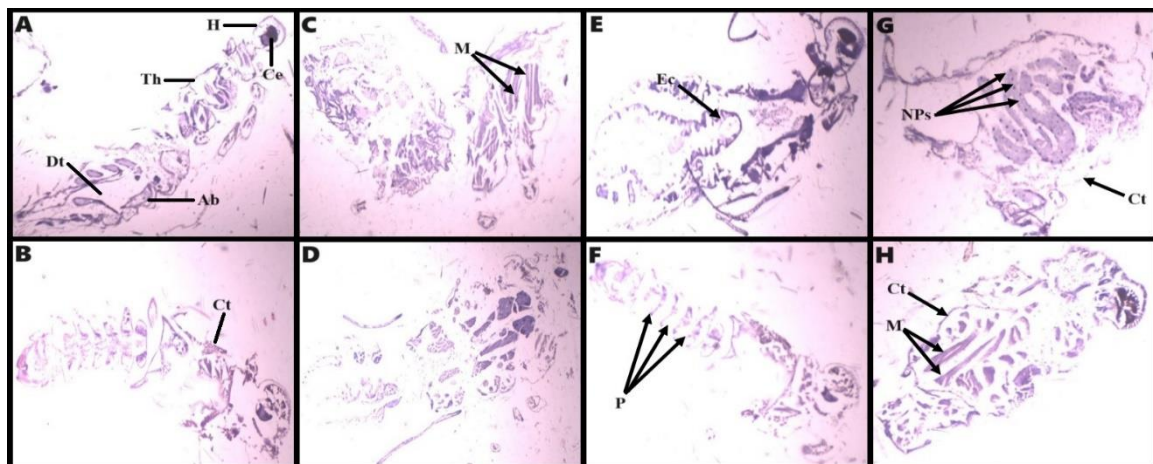


Figure 5. Histological study of Thrips insect's post-exposure to Nanoparticles, plant extract, insecticides. (A, B) Control; (C, D) plant extract; (E, F) insecticides; (G, H) Nanoparticles. H, Head; Th, Thorax; Ab, Abdomen; Ct, Cuticle; Ce, Compound eye; M, Muscles; Dt, Digestive tract; Ec, Epithelial cells; P, Pleurotergite; NPs, Nanoparticles. Arrow indicates damages in Thrips insects.

3.7. Morphological observations postexposure to nanoparticles

Figure 6 illustrates how AgNPs at LC₅₀ and LC₉₀ affect thrips' morphological alterations. AgNPs of LC₅₀ and LC₉₀ significantly altered the morphology of various thrips body parts, as demonstrated by the SEM photos. Treatment causes malformations in the pleura, sternum, and tergum cuticles as well as damage to the clypeus, mandible, maxillary palp, labium, glossa, paraglossa, and labial palp, among other mouthparts. Conversely, the untreated insects showed no morphological abnormalities and all of their parts seemed to be intact. Consequently, these findings suggest that AgNPs were highly important in decreasing thrips. The SEM studies of morphological alterations in thrips insects provide evidence that AgNPs may impact motility, development, and lethality through their action in the cuticle. Because there is less information in the literature about the effects of nanoparticles on thrips. There was no comparison made between the SEM data and the findings of the previous studies. However, earlier research on the structural anomalies caused by *Tagetes minuta* oil against *A. craccivora* and *Plutella xylostella* were few and provided strong support for the results of this investigation (34, 46). Additionally, the *Planococcus lilacinus* treated with *ocimene* displayed several anomalies on the cuticle (body fluid encrustations and accumulation), thoracic leg, and setae (11). Other authors' findings (47) indicate that when treated with different nanoparticles, where the cuticles of *A. egypti* and *C. quinquefasciatus* larvae were altered in the head, thorax, abdomen, and siphon.

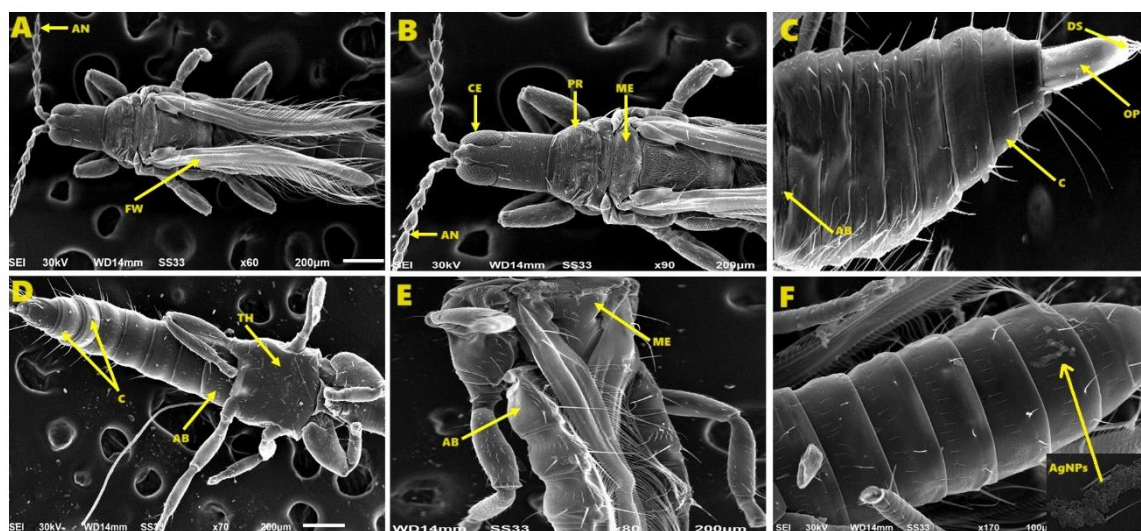


Figure 6. Morphological study of Thrips insect's post-exposure to AgNPs. (A, B, C) Control; (D, E, F) AgNPs at LC₅₀ and LC₉₀. Th, Thorax; Fw, Forewing; Ce, Compound eye; Ab, Abdomen; An, Antennae; C, Ctenidia; Pr, Pronotum; Me, Mesonotum; Op, Ovipositor; Ds, Dorsal split. Arrow indicates damages in Thrips insects

4. Conclusions

Hibiscus rose plant extract nanoparticles have been created to manage *T. tabaci* for the first time. When compared to its bulk plant extract, the NPs formulation made from the extract of the hibiscus rose plant ensures better efficacy as an insecticidal agent against *T. tabaci*. Our findings also shed light on the mechanism of action of plant extract-NPs on *T. tabaci*, which both affect morpho-histological structures and enzymatic pathways. Since they are made of plant material, plant extracts are naturally pesticidal and eco-friendly. The results of this study suggest that nanoparticles can be used as a secure and reliable replacement for dangerous chemical pesticides in the treatment of *T. tabaci*.

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