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RESEARCH ARTICLE

Impact of *Capsicum annuum* aqueous extract and green synthesized silver nanoparticles on the biology, physiology, and histology of the *Periplaneta americana*

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Abstract

Investigating environmentally suitable substitutes is necessary due to the growing dependence on synthetic pesticides. This research examined the efficacy of chili pepper (*Capsicum annuum*) fruit extract and its synthesized silver nanoparticles against *Periplaneta americana*, applying five concentration levels 20%, 40%, 60%, 80%, and 100%. Experimental groups, which included control groups, were composed of ten adult American cockroaches each with three replicates treated to a 10-second spray application through exposure times of 24 to 96 hours with observations made during that time. The result displayed that chili pepper silver nanoparticles demonstrated greater toxicity compared to the aqueous fruit extract, particularly in female American cockroaches compared to adult males. Furthermore, both compounds caused biochemical alterations in the midgut, enhanced lipase and amylase levels, reduced total protein and protease levels. In certain regions, our study revealed histological abnormalities of American cockroach midguts, that were treated with *C. annuum* ecofriendly nanoparticles, columnar cells, and regenerating nidi that were completely deformed and degraded in males *P. americana*, but longitudinal muscles were absent in females and the regenerative nidi were slightly affected by revealing proliferation of the cells. It is concluded that the research contributes to ecologically friendly pest management techniques by paving the way for the investigation of chili pepper silver nanoparticles as a safer and more sustainable cockroach management tool.

***Keywords*:** *Periplaneta americana*; *Capsicum annuum*; Eco-Friendly alternative; histological; physiological studies

1. *Introduction*

The American cockroach, *P. americana* L., is a common and annoying home pest that is especially common in tropical areas of the world. Cockroaches are regarded as a high-priority urban pest due to their unattractive appearance, damage to storage products and home items, and capacity to transmit disease Ebeling (1978). Cockroaches are an unusual and ancient species of hemimetabolous insect with a life cycle consisting of three stages: egg, nymph, and adult Mullins (2015), belonging to the Blattidae family, order Blattodea, and class Insecta. According to Tinker and Ottesen (2021), their ancestors appeared during the Carboniferous epoch, about 300–350 million years ago. Although there are now about 4700 species of cockroaches known to exist, at least twice that many are believed to be undiscovered Beccaloni (2014). The high survival, low nutritional requirements, and simplicity of creating gnotobiotic and germ-free animals make the American cockroach, *P. americana*, a popular lab animal Dukes et al. (2021) and León et al. (2021). Utilizing plants and plant-based products (essential oil) to control cockroaches is safe, economical, and environmentally friendly, according to several researches conducted by Isman (2000), Koul et al. (2008), and Regnault-Roger et al. (2012). The ability of various plant parts, such as leaf, stem, seed, flower, and rhizome extract, to repel cockroaches has been studied by Aldo et al. (2021). The GC-MS is a widely used technique for detecting volatile organic chemicals in materials Ieri et al. (2017) and Wong et al. (2017). Gas chromatography is used in many different applications. Its main use, nevertheless, is in the analysis and separation of mixtures that contain several different

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substances, such as hydrocarbons, solvents, and essential oils Mohammed *et al*. (2016). For the creation of metallic nanoparticles, plant extracts containing proteins and other phytochemicals are preferred over conventional physical and chemical processes because they act as a capping ligand and reductant, preventing particle aggregation Yilmaz *et al*. (2011). Particles with at least one dimension smaller than 100 nm are referred to as nanoparticles (NPs). They have been identified as crucial constituents of nanomaterials, which can be further modified for application in various fields connected to nanotechnology Keck and Müller (2013). Utilizing nanoparticles of different sizes, nanotechnology applications are currently being used to develop new nanopesticides Soppimath et al. (2001). Additionally, using chemicals like polymer compounds of which polyethylene glycol is the most promising as materials tankers or carriers of nanoparticles is essential to the creation of nano-pesticides Liu (2006). Green synthesis of nanoparticles utilizing plant extracts created very pure and monodispersed nanoparticles that were safe for the environment and cost-effective Jafarizad *et al*. (2017). In addition, several attempts have been made to produce green silver nanoparticles (Ag NPs) using plant extracts such as ginger and lantana camara, which help in the development of new pesticides and insect-repellant ingredients Chinnamuthu and Boopathi (2009). Nanoparticles are more effective at controlling pests because their constituents are more invasive than those in their normal form, which has a stronger effect on the pest's body Athanassiou et al. (2018). Because the extract from some plant parts, such as fruits, roots, leaves, seeds, and stems, contains phytochemicals that act as reducing and stabilizing agents, these portions have been used to make various types of nanoparticles Narayanan and Sakthivel (2010). A related study that used chili extract on flies found that raising the concentration content had positive effects on cockroaches and flies as pesticides and repellents. Because organic pesticides can naturally break down, the results additionally revealed that utilizing them is safer than using commercial pesticides Septiati et al. (2022). One of the most prevalent nanoparticles, silver nanoparticles are non-antibiotic, antibacterial, and regarded as a precious metal. They have been revealed to have harmful and other perhaps adverse impacts on mammals. Nevertheless, it is still unclear if silver nanoparticles harm insects. The impact of nanoparticles of silver on the model invertebrate creature *P. americana*, was investigated in this study. Malathion is an organophosphate pesticide commonly used to control pest insects Dales *et al*. (1989). It was demonstrated that *P. americana* populations that performed all tests were susceptible to higher levels of monooxygenases and the prescribed dosage of malathion Tahir et al. (2017). The ejection of enzymes from the midgut has been revealed through multiple studies to have a defensive impact against foreign and damaging substances Li *et al*. (2017). When American cockroaches were treated with azadirachtin (made from neem seeds), their midgut enzyme activity was reduced to 50% and their development was inhibited Paranagama et al. (2001). However, according to a study by Majumdar *et al*. (2016), deltamethrin seriously harmed the peritrophic membrane and striated border in the midgut of *P. americana*. In insects, the gut epithelium contains many cells that are specialized to produce digestive enzymes, absorb tiny organic nutrients, and exchange water and inorganic ions between the gut lumen and body fluids Billingsley and Lehane (1996). According to Cnubben et al. (2001), reactive oxygen species are extremely reactive molecules because they contain unpaired valence shell electrons that interact randomly with vital macromolecules as proteins, DNA, and lipids, particularly those found in cell membranes, disrupting physiological processes. The study's primary focus is to evaluate the mortality of chili pepper extract and its green silver synthesized nanoparticles on adult *P. americana* and on how American cockroach, *P. americana* L., respond to various amounts of silver nanoparticles and eco-friendly chili pepper nanoparticles applied to their physiological and histological midgut.

2. Materials and Methods

2.1. Collection of Cockroaches

The present study was carried out at the laboratory of Entomology, Faculty of Science laboratory building (B), South Valley University (26° 19' 11" N 32° 74' 80" E). Samples of *P. americana*, collected from El-Gabalaw village (26° 13' 28" N and 32° 77' 31" E), were classified according to morphological and taxonomic keys conducted by Linnaeus (1758) and Gurney and Fisk (1991).

2.2. Insect rearing

American cockroaches were reared at the Animal House of Laboratory building (A), Faculty of Science, South Valley University, under laboratory conditions in clear plastic containers (40 × 30 × 20 cm) covered with wire mesh at room temperature (27 ± 2 ^{o}C), 12 h light/dark period, and humidity. The insects were fed with dry cat food and cotton soaked with water. Cages had cupboards placed inside of them for protection. Feces were removed from the cages after they were thoroughly cleaned.

2.3. Plant materials

Fruits of *C. annuum* (Chili pepper fruits), Family: Solanaceae classified according to Herbarium of the Faculty of Science, South Valley University, were commercially purchased from Qena city, (26° 16' 10" N and 32° 72' 17" E), table (1).

Table 1. Insecticide, aqueous extract, and silver nanoparticles for the *C. annuum* against American cockroach

Trade name	Active ingredient	Rate
Malathion	Malathion	100-80-60-40 and 20% of the crude materials
Silver nanoparticles	Nanoparticles	
Chili pepper fruit aqueous extract	*Capsicum annuum* (Crude extract)	
Chili Ag NPs	Nanoparticles	

2.4. Insecticide

Malathion acts as positive control (+ve), [Diethyl (dimethoxythiophosphorylthio) succinate] was commercially purchased from a local insecticidal store in Qena City.

2.5. Chemicals and reagents used in physiological study in table (2).

Table 2. Illustrated the chemicals and reagents used in the study

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No.	Chemicals	Suppliers
1	Commasie brilliant blue G-250	Sigma Chemical Company
2	Bovine albumin standard	Stanbio Laboratory (Texas, USA).
3	Dinitrosalicylic acid (DNS) reagent	Sigma Chemical Company
4	1 % Starch (Soluble potato starch, Lintner grade)	Sigma Chemical Company
5	lipase determination kit	bio-diagnostic company, in Giza, Egypt.
6	Ninhydrin reagent	Sigma Chemical Company

2.6. Preparation of aqueous extract of C. annuum

The chili pepper fruits were ground into a fine powder by using a tissue grinder (IKA A10, Germany) and stored in a dry airtight container. 100 gm of powder was dissolved in 1000 ml sterile distilled water and shaken using a shaker at 180 rpm for 24 hrs. Whatman (No. 1) filter paper was used to filter the obtained extract, and the filtrate was collected, placed in a 1000 Erlen-

Meyer flask, and kept cold (4°C) until used and later changed from Verástegui et al. (1996).

2.7. Synthesis of silver nanoparticles

2.7.1. From chili pepper extract C. annuum

We prepared a 2 mM solution of silver nitrate, 0.339 g of silver nitrate powder added to 1000 ml distilled water, 100 ml of freshly prepared $AgNO_3$ solution was added dropwise to 100 ml of the stored aqueous chili pepper extract at (50-60°C) while stirring continuously for reducing Ag^+ ions. Before being used, the produced solutions were incubated at 37 °C in a dark room Allam *et al*. (2022).

2.7.2. From $AgNO_3$

We prepared 1% of Tri-sodium citrate as a reducing agent, and 0.1 g of Tri-sodium citrate powder was added to 10 ml distilled water. 100 ml of freshly prepared $AgNO_3$ solution was heated at **(**100-130 °C**)** until boiling then we added dropwise from Tri-sodium citrate Chicea et al. (2023).

2.8. Gas chromatography-mass spectrometry (GC-MS) analysis of aqueous extracts of C. annuum

The biological composition of the chili pepper extract was examined using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The temperature of the column oven was first kept at 60°C, then increased by 5°C per min to 250°C, which was held for two min, and then increased by 30°C per min to 300°C. It kept the injector temperature at 270°C. Helium was used at a constant flow rate of 1 ml per min as the carrier gas. After a 4 min solvent delay, 1 µl of diluted samples were automatically injected using an Autosampler AS3000 in the split mode with GC. In full scan mode, EI mass spectra covering the m/z 50-650 range were acquired at 70 eV ionization voltages. Both the ion source and the transfer line were set to 280°C and 200°C, respectively. The components were identified by comparing their mass spectra with those of the WILEY 09 and NIST14 mass spectral databases Abdelkhalek et al. (2022).

2.9. Characterization of nano-scale silver nanoparticles

The zeta potential of the generated nanoparticles was measured to assess their stability against aggregation, and the particle size distribution was verified using dynamic light scattering (DLS). It uses incident light from a He Ne laser at 632 nm with an external angle of 90 degrees for DLS and 18.9 degrees for Zeta potential (PSS, Santa Barbara, CA, USA). At the Nanomaterial Investigation Lab of the Central Laboratory Network (CLN) of the National Research Centre (NRC), Cairo, Egypt, zeta potential and DLS were measured. Five min were spent sonicating the samples with 0.2g/5 ml of Millipore water El-Monairy *et al*. (2023), Atomic Force Microscopy (AFM) was performed in ambient air using commercial AFM equipment (Solver P47, NT-MDT). Typically, images were taken using a rectangular silicon nitride cantilever in the tapping mode (NSC_{35}/Si_3N_4/AlBS, MikroMasch) Grobelny *et al*. (2011), Fourier Transform Infra-Red Spectroscopy (FTIR) spectra of Ag NPs were recorded at room temperature in the 4000–400 cm^{-1} range using a Perkin-Elmer spectrophotometer. Following the recording of diffuse reflectance spectra in the 200–800 nm wavelength range using the UV140404B spectrophotometer, numerical data was plotted in the 'Origin 7' program Slman et al. (2018), and Following X-Ray Diffraction (XRD) examination, the mixture of silver nanoparticles was centrifuged for 30 min at 10,000 rpm. Ag NP residue was twice washed with double-distilled water at 80°C to produce Ag NPs for X-ray powder diffraction investigations. On a Shimadzu XRD-6000, powder XRD patterns were collected using copper radiation (Cu Ka, 1.5406) at 40 kV and 30 mA Slman et al. (2018).

2.10. Bioassays

The cockroaches were randomly divided into five groups, 10 cockroaches for each, treated with different concentrations sprayed for 10 sec using a sprayer (High-pressure Air Pump Bottle Spray) with freshly prepared concentrations of (100, 80, 60, 40, and 20 ml/L) of chili pepper extract, chili pepper Ag NPs, Ag NPs acted as a negative control (-ve), malathion of (1.5 µl/500 ml) recommended field dose, and compared to

control group which received nothing, each treatment was replicated three times.

2.11. Mortality evaluation

The mortality rate was recorded at 24 to 96 hrs and any insect that didn't respond to three probes with a blunt probe at five min recovery period was considered dead. For the toxicity index, all the mortality data were statistically analyzed according to Finney (1971). The toxicity index (T.I.) was calculated for each insecticide according to the equation of Sun (1950).

2.12. Effect of chili pepper extract, silver nanoparticles, and malathion at LC_{50} on biological activities of treated adult male and female P. americana

Selected biological activities were determined for adults treated with the LC_{50} of treated compounds. The following biological parameters were determined for adult male and female *P. americana*:

1. Fecundity (number of oothecae).
2. Fertility (percentage of malformed oothecae and hatched nymphs).
3. Weight, length**,** and width of the *P. americana* oothecae**.**

2.13. Effect of chili pepper extract, silver nanoparticles, and malathion at LC_{50} on the physiological activities of treated P. americana

Midguts of adult males and females were treated with LC_{50} of insect groups (10 cockroaches/group) and each treatment was replicated three times.

2.13.1. Dissection of the adult P. americana

Before dissection, *P. americana* in the control group was killed by freezing in a refrigerator at (-4°C) then fixed onto the dissection board with pins, then removed all legs and wings. The dissection was performed by longitudinally cutting from the tergum using medical scissors, and the midgut was gradually removed from the alimentary canal using fine forceps and placed in a petri dish of saline. The electrophoretic separation of total protein and digestive enzymes (amylase, protease, and lipase) was conducted on the midgut of adult American cockroaches which were subjected to LC_{50} of M, Ag NPs, Ch EX, and Ch NPs, as well as an untreated control group.

2.13.2. Preparation of crude gut extract

Utilizing a modified version of the de Oliveira et al. (2014) procedure, guts were processed for biochemical examination. In a cup with ice water and 1 milliliter of cooled buffer (0.1 M phosphate buffer, pH 7.0), four dissected guts were combined and stirred until they were incorporated. The homogenate was centrifuged at 14,000 g for 20 min at (4°C). Following collection and measurement, the supernatant was kept at (-8°C) until it was used in the subsequent days.

2.13.2.1. Determination of total protein

The total protein was calculated using the Bradford (1976) procedure. The protein reagent was made by dissolving 100 mg of Coomassie Brilliant blue G-250 in 50 ml of 95% ethanol. 100 milliliters of 85% (W/V) phosphoric acid were added to this mixture. The resulting solution was diluted to a final volume of 1 L of reagent. 50 µl of serial concentrations of bovine serum albumin, ranging from 10 to 100 µg, were pipetted into test tubes together with 50 µl of sample solution to generate a standard curve. Adjusting the test tubes to 1 ml required the application of phosphate buffer (0.1M, pH 6.6). After adding 5 ml of protein reagent to the test tube, the contents were combined by vortexing. A blank produced with 5 ml of protein reagent and 1 ml of phosphate buffer was used to test the absorbance at 595 nm after two min and an hour had passed.

2.13.2.2. Determination of amylase activity

The digestive enzymes were determined using Amin (1998) modifications of the Ishaaya and Swirski (1976) method. 20 µl of diluted enzyme solution was incubated with 250 µl of 1% starch in 50 mM acetate buffer pH 5.0, which contained 20 mM NaCl and 0.1 mM $CaCl_2$, for 10 min at 30°C. For termination of the reaction, 250 µl of the Dinitrosalicylic Acid (DNS) reagent was added to each tube after the water had been boiling for five min. The samples were refrigerated and diluted with 2.5 ml H_2O before being scanned at 550 nm using the Spectronic 1201 Spectrophotometer (Beckman, USA). Glucose was the standard. To produce glucose equivalents in a series of steps, the enzyme

supernatant was suitably diluted. Three seedling groups were analyzed in triplicate for each test's amylase activity. To indicate the enzyme activity, the value of µg glucose released/min/gm fresh weight was utilized.

2.13.2.3. Determination of protease activity

2.13.2.3.1. Apparatus

The midguts were homogenized in a cold glass Teflon homogenizer (ST-Mechanic-Preczyina, Poland) for biochemical analysis. The centrifugation procedure was carried out in a chilled centrifuge (6 MR, USA). A double-beam ultraviolet/visible spectrophotometer (Sectronic 1201, Milton Roy Co., USA) was used to measure the absorbance of colored materials**.**

2.13.2.3.2. Preparation of midgut insects for analysis

Distilled water containing 50 mg/ml was used to homogenize the midguts. Centrifugal homogenization was performed for 15 min at 5°C and 8000 rpm in a refrigerated centrifuge. The deposits were disposed of while the supernatant was kept in a deep freezer until it was required.

2.13.2.3.3. Procedure

Following an hour of incubation at 30°C, the rise in free amino acids extracted from the substrate protein (albumin) was used to measure proteolytic activity, as Tatchell et al. (1972) had demonstrated. One milliliter of 0.1 M phosphate buffer (PH 8), 100 µl of midgut homogenate, and 100 µl of 0.5% bovine serum albumin comprised the reaction mixture. To stop the reaction, 1.2 ml of 20% TCA (trichloroacetic acid) was added. The mixture was allowed to stand for 15 min before being centrifuged for 20 min at 3000 rpm. The quantity of amino acids that were produced was then quantified using the supernatant. The method presented by Lee and Takahashi (1966) was utilized to colorimetrically assess amino acids utilizing the ninhydrin reagent. The reaction mixture consisted of 100 µl of supernatant, 1.9 ml of ninhydrin-citrate (PH 5.5), 0.2 ml of 0.5 M citrate buffer (PH 5.5), and 1.2 ml of glycerol. The mixture's acquired color was measured at 570 nm after it had been heated for 12 min in a boiling water bath and cooled with tap water. The reagent blank was not changed in any way. Gathering everything and substituting 100 µl of distilled water for the supernatant. D, L alanine was used as the standard, and the amino acids were expressed as µg alanine /min/g.b.wt.

2.13.2.4. Determination of lipase activity

50 µl of the sample solution and 500 µl of R1 (3.4 mmol/L of Taurodesoxycholate, 2.6 mmol/L of Desoxycholate, and 12 mmol/L of Calcium chloride) were pipetted into test tubes to create a standard curve. The tubes were then carefully mixed and incubated for 5 min at 37°C. To start the reaction, 125 µl of R2 (3.4 mmol/L of Taurodesoxycholate and 0.13 mmol/L of DGMRE) was added, and the tubes were once more thoroughly mixed. The reaction mixture was made up of two buffers: 40 mmol/L of Goods Buffer (pH 8.0) and 1.5 mmol/L of Tartrate Buffer (pH 4.0). Two min later, the absorbance of red methyl resorufin at 578 nm was measured in air, as illustrated by Tahoun and Abdel-Ghaffar (1986).

2.14. Effect of chili pepper extract, silver nanoparticles, and malathion at LC_{50} on the histological changes of treated adults

American cockroaches' midguts were preserved in 10% neutral buffered formalin (pH 7.2), dehydrated in a sequence of increasing alcohols, cleaned in xylene replacements, and embedded in paraffin wax for histological analysis. Six micrometer-thick paraffin slices were made, and Harris hematoxylin and eosin were used to stain Gabe (1976). The Leica Microsystem (LEICA Application Suite (LAS) EZ, User Manuals, Version 3.4, March 2017) was used to investigate and analyze photomicrographs in the Faculty of Science, Botany and Microbiology Department, South Valley University.

2.15. Statistical analysis

2.15.1. The median lethal concentration (LC_{50}) and slope values calculated by the computerized probit analysis program LdP Line Program, Bakr (2005).

2.15.2. The results were expressed as mean ±SE. They carried out GraphPad Prism (version 9) by one-way analysis of variance (ANOVA) followed by the student Newman-Keuls T-test, prism, and image analyzer. Software. Values of $P<0.05$ are statistically significant Manzoor et al. (2012).

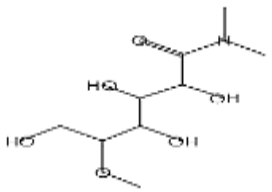
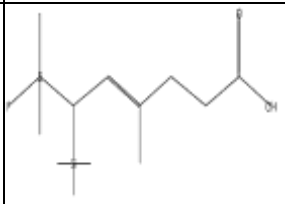
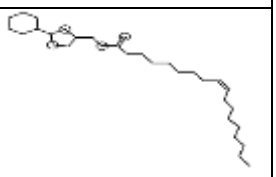
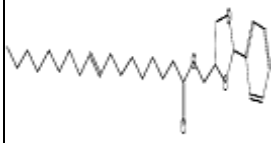
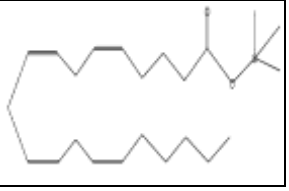
2.15.3. The length and width of oothecae of American cockroaches were measured by Software ImageJ (version 2006).

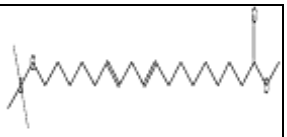
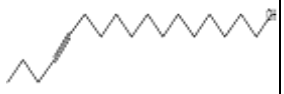
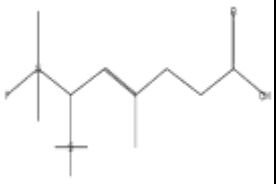
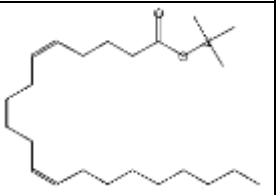
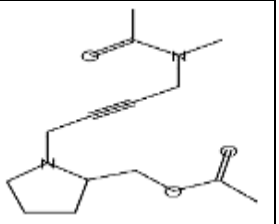
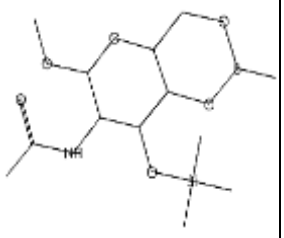
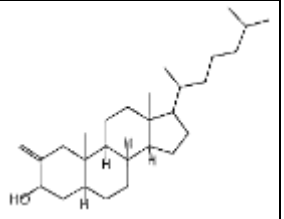
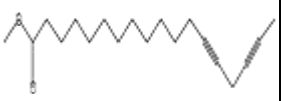
3. Results

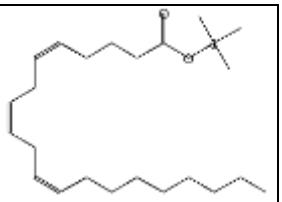
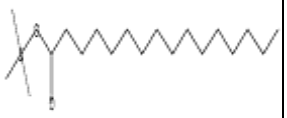
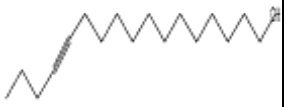
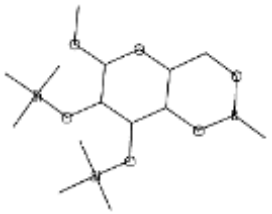
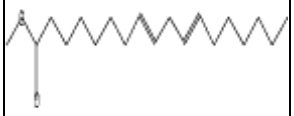
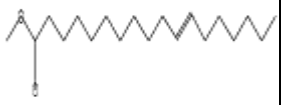
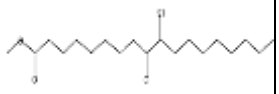
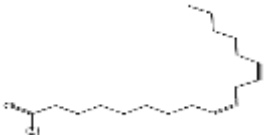
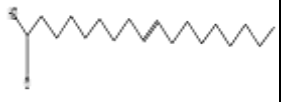
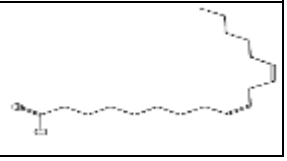
3.1. GC-MS Analysis of the C. annuum fruit extract

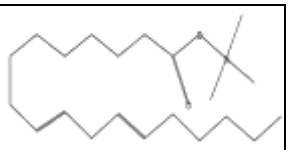
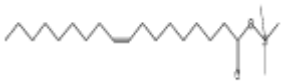
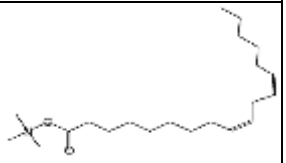
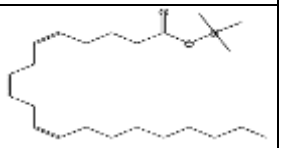
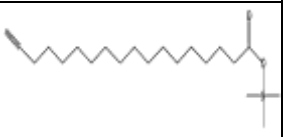
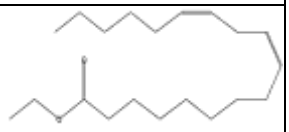
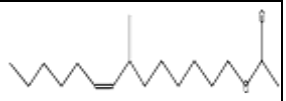
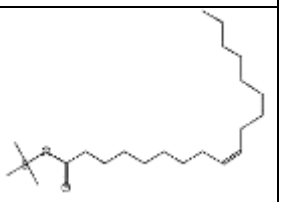
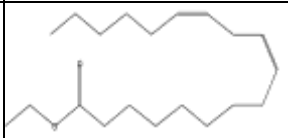
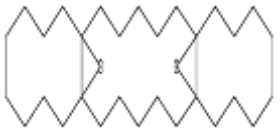
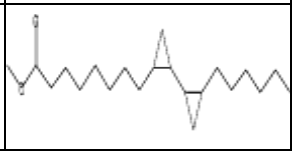
Using GC-MS analysis, 37 chemical compounds, listed in table (3) along with their retention time, molecular formula, molecular weight, area (w/w), and chemical structure, from various classes were found, including sugars, fatty acids, alcohols, esters, hydrocarbons, and amino compounds. In contrast to the fatty acid chemical class, which had the highest amount of acid components (oleic acid, detected at an RT of 30.48 min), the esters chemical class was the most dominant, with trimethylsilyl ester, the major component of linoelaidic acid, being revealed at an RT of 31.75 min. For these chemical classes, the following was the order of occurrence in terms of the number of components: 15 esters > 11 acids > 4 alcohols > 3 others > 2 sugars > 1 hydrocarbon > 1 amino compound. The GC-MS analysis revealed thirty-seven chemical compounds from various chemical classes in the *C. annuum* extract. These chemical classes percentage average occurrence can be arranged as: 67.54 (acids) > 23.26 (esters) > 4.49 (others) > 2.59 (alcohols) > 1.47 (hydrocarbons) > 0.34 (sugar) > 0.28 (amino) compounds.

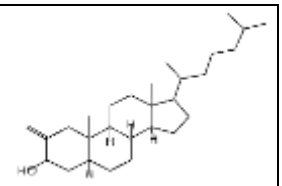
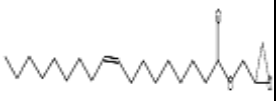
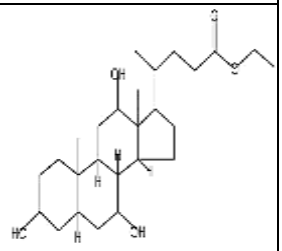
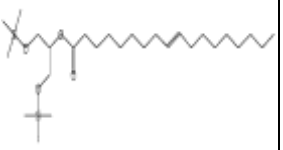
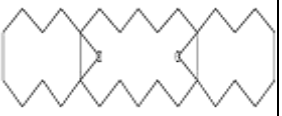
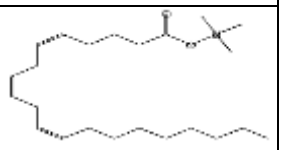
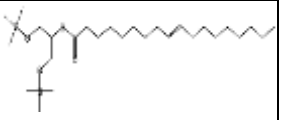
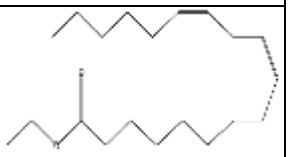
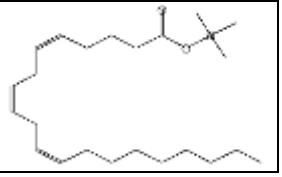
Table 3. Chemical composition of chili pepper, *C. annuum* fruits extract analyzed by GC-MS.

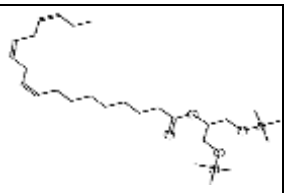
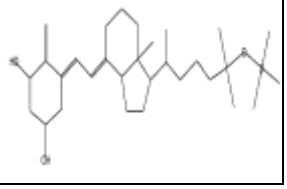
Peak	Compound Name	Retention Time	Area %	Molecular Formula	Molecular Weight	Structure
1	5-O-Methyl-d-gluconic acid dimethylamid	7.09	0.13	$C_9H_{19}NO_6$	237	
2	6-Dimethyl(trimethylsilyl)silyloxyt et radecane	7.17	0.10	$C_{19}H_{44}OSi_2$	344	
3	4-Hexenoic acid, 4-methyl-6-(fluorodimethylsilyl)-6-tr imethylsilyl	9.82	0.49	$C_{12}H_{25}FO_2Si_2$	276	
4	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester	11.28	0.10	$C_{28}H_{44}O_4$	444	
5	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans	11.33	0.14	$C_{28}H_{44}O_4$	444	
6	Arachidonic acid	12.44	0.19	$C_{23}H_{40}O_2Si$	376	

7	**Methyl 18-oxidanyloctadeca-9,12-dienoate**	**16.05**	**0.14**	**$C_{22}H_{42}O_3Si$**	**382**	
8	**13-Heptadecyn-1-ol**	**16.27**	**0.51**	**$C_{17}H_{32}O$**	**252**	
9	**4-Hexenoic acid, 4-methyl-6-(fluorodimethylsilyl)-6-tr imethylsilyl**	**19.49**	**0.14**	**$C_{12}H_{25}FO_2Si_2$**	**276**	
10	**5,8,11-Eicosatrienoic acid, (Z)-TMS derivative**	**21.02**	**0.29**	**$C_{23}H_{42}O_2Si$**	**378**	
11	**Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-p yrrolidyl]-2-butynyl]**	**23.13**	**0.28**	**$C_{14}H_{22}N_2O_3$**	**266**	
12	**à-d-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3 o-(trimethylsilyl)-, cyclic methylboronate**	**25.12**	**0.12**	**$C_{13}H_{26}BNO_6Si$**	**331**	
13	**17-Octadecynoic acid**	**26.06**	**0.63**	**$C_{18}H_{32}O_2$**	**280**	
14	**Cholestan-3-ol, 2-methylene**	**26.25**	**1.51**	**$C_{28}H_{48}O$**	**400**	
15	**13,16-Octadecadiynoic acid, methyl ester**	**26.41**	**0.55**	**$C_{19}H_{30}O_2$**	**290**	
16	**Pentadecanoic acid**	**27.28**	**2.84**	**$C_{15}H_{30}O_2$**	**242**	

17	**5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative**	**28.08**	**0.32**	**$C_{23}H_{42}O_2Si$**	**378**	
18	**Palmitic Acid, TMS derivative**	**28.92**	**4.77**	**$C_{19}H_{40}O_2Si$**	**328**	
19	**13-Heptadecyn-1-ol**	**29.17**	**0.17**	**$C_{17}H_{32}O$**	**252**	
20	**á-d-Glucopyranoside, methyl 2,3-bis-o-(trimethylsilyl)-, cyclic methylboronate**	**29.27**	**0.09**	**$C_{14}H_{31}BO_6Si_2$**	**362**	
21	**8,11-Octadecadienoic acid, methyl ester**	**29.43**	**1.75**	**$C_{19}H_{34}O_2$**	**294**	
22	**11-Octadecenoic acid, methyl ester**	**29.61**	**3.84**	**$C_{19}H_{36}O_2$**	**296**	
23	**Octadecanoic acid, 9,10-dichloro-, methyl ester**	**30.17**	**0.10**	**$C_{19}H_{36}C_{l2}O_2$**	**366**	
24	**Linoelaidic acid**	**30.30**	**1.95**	**$C_{18}H_{32}O_2$**	**280**	
25	**Oleic Acid**	**30.48**	**32.41**	**$C_{18}H_{34}O_2$**	**282**	
26	**9,12-Octadecadienoyl chloride, (Z,Z)**	**30.79**	**0.41**	**$C_{18}H_{31}ClO$**	**298**	
27	**9-Octadecenoic acid (Z)**	**30.93**	**3.14**	**$C_{18}H_{34}O_2$**	**282**	
28	**9,12-Octadecadienoyl chloride, (Z,Z)**	**31.09**	**0.34**	**$C_{18}H_{31}ClO$**	**298**	

29	**Linoelaidic acid, trimethylsilyl ester**	**31.75**	**7.00**	**$C_{21}H_{40}O_2Si$**	**352**	
30	**Oleic Acid, (Z)-, TMS derivative**	**31.89**	**14.83**	**$C_{21}H_{42}O_2Si$**	**354**	
31	**9,12-Octadecadienoic acid (Z,Z)-, TMS derivative**	**32.02**	**0.24**	**$C_{21}H_{40}O_2Si$**	**352**	
32	**5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative**	**32.23**	**0.29**	**$C_{23}H_{42}O_2Si$**	**378**	
33	**17-Octadecynoic acid, TMS derivative**	**32.42**	**2.72**	**$C_{21}H_{40}O_2Si$**	**352**	
34	**Linoleic acid ethyl ester**	**32.75**	**0.15**	**$C_{20}H_{36}O_2$**	**308**	
35	**7-Methyl-Z-tetradecen-1-ol acetate**	**32.86**	**0.75**	**$C_{17}H_{32}O_2$**	**268**	
36	**9,12-Octadecadienoic acid (Z,Z)-, TMS derivative**	**33.21**	**1.24**	**$C_{21}H_{40}O_2Si$**	**352**	
37	**1-Heptatriacotanol**	**33.33**	**1.53**	**$C_{37}H_{76}O$**	**536**	
38	**Linoleic acid ethyl ester**	**33.60**	**0.39**	**$C_{20}H_{36}O_2$**	**308**	
39	**Tricyclo[20.8.0.0(7,16)]triaconta ne, 1(22),7(16)-diepoxy**	**33.88**	**1.31**	**$C_{30}H_{52}O_2$**	**444**	
40	**[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester**	**34.80**	**0.38**	**$C_{21}H_{38}O_2$**	**322**	

41	**Cholestan-3-ol, 2-methylene-, (3á,5à)**	**34.92**	**1.50**	**$C_{28}H_{48}O$**	**400**	
42	**6,9,12,15-Docosatetraenoic acid, methyl ester**	**35.55**	**2.18**	**$C_{23}H_{38}O_2$**	**346**	
43	**Glycidyl oleate**	**35.68**	**4.02**	**$C_{18}H_{34}O_2$**	**282**	
44	**Ethyl iso-allocholate**	**36.16**	**0.65**	**$C_{26}H_{44}O_5$**	**436**	
45	**2-Oleoylglycerol, 2TMS derivative**	**36.27**	**0.82**	**$C_{27}H_{56}O_4Si_2$**	**500**	
46	**Tricyclo[20.8.0.0e7,16]triacontan, 1(22),7(16)-diepoxy**	**36.65**	**0.16**	**$C_{30}H_{52}O_2$**	**444**	
47	**5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative**	**37.00**	**0.15**	**$C_{23}H_{42}O_2Si$**	**378**	
48	**2-Oleoylglycerol, 2TMS derivative**	**37.11**	**0.10**	**$C_{27}H_{56}O_4Si_2$**	**500**	
49	**Linoleic acid ethyl ester**	**37.23**	**0.20**	**$C_{20}H_{36}O_2$**	**308**	
50	**1-Heptatriacotanol**	**37.37**	**0.38**	**$C_{37}H_{76}O$**	**536**	
51	**5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative**	**38.42**	**0.15**	**$C_{23}H_{42}O_2Si$**	**378**	

52	**1,25-Dihydroxyvitamin D3, TMS derivative**	**38.55**	**0.40**	**$C_{30}H_{52}O_3Si$**	**488**	
53	**1,25-Dihydroxyvitamin D3, TMS derivative**	**38.87**	**0.98**	**$C_{30}H_{52}O_3Si$**	**488**	

3.2. Tests proving the formation of Ag NPs

The synthesis of $AgNO_3$ and chili pepper (*C. annuum*) silver nanoparticles were carefully examined. Silver nanoparticles were created when the aqueous silver ions were added to the plant extract that was being tested. It was observed that the solution for Ch NPs turned from red to orange as silver nanoparticles developed. Furthermore, when the color became dark brown, the $AgNO_3$ and tri-sodium citrate dihydrate mixing was halted Figs. (1 & 2, a). The Ch NPs had an average particle diameter of 50 nm when the particle size distribution was evaluated using a DLS approach, Fig. (1, b), whereas the average diameter of Ag NPs was 30 nm, Fig. (2, b). The plant extract's dual functions as a capping and reducing agent were confirmed by the FTIR analysis of silver nanoparticles, which also confirmed the presence of certain functional groups, Figs. (1 & 2, d). The AFM has been used to determine the shape, size, and morphology of nanoparticles (AFM). As seen in Figs. (1 & 2, c), it reveals that the silver nanoparticles are mostly spherical and widely dispersed, though some of them have asymmetrical shapes.

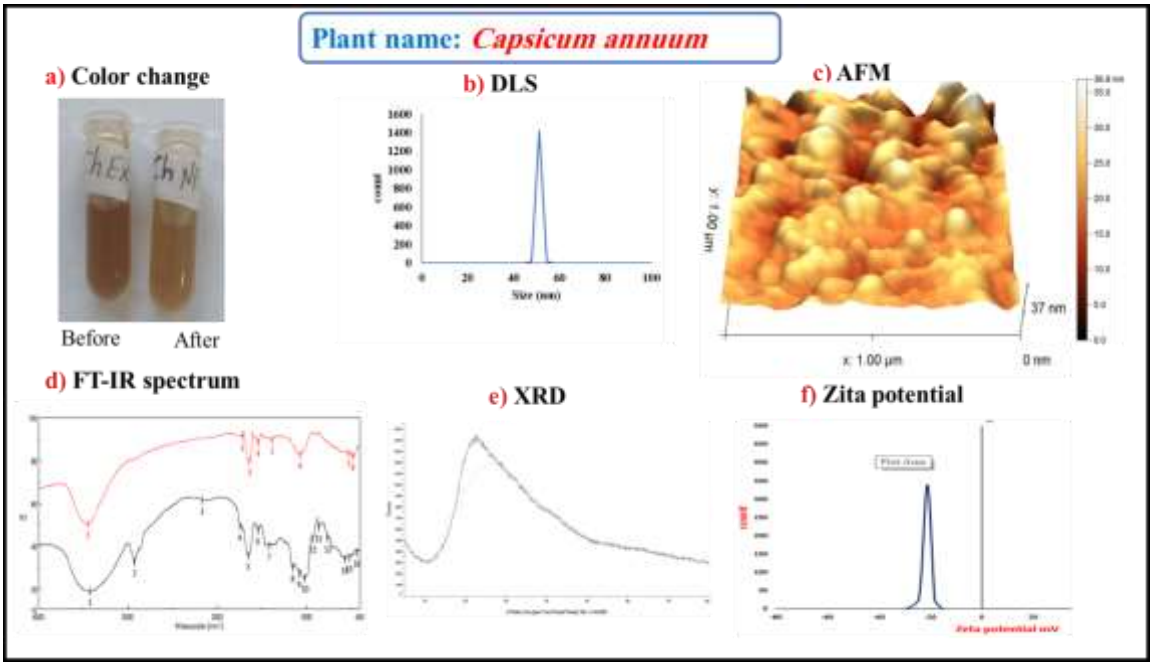


Figure (1): Tests of silver nanoparticles formation by aqueous fruits extract of chili pepper (*C. annuum*)

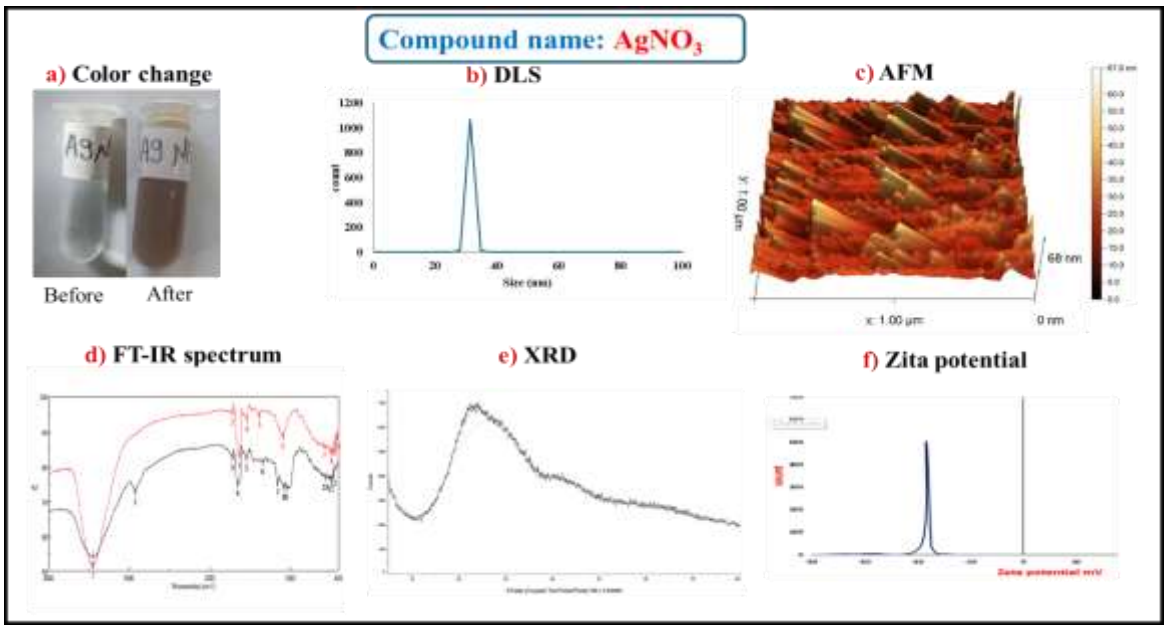


Figure (2): Tests of silver nanoparticles formation by silver nitrate compound (Ag NO_3)

3.3. Toxicity study

The M, Ag NPs, Ch EX, and Ch NPs were tested and evaluated to determine their impact on adult males and females at the laboratory conditions.

3.3.1. Toxicity of the tested compounds on the male

Each type of compound resulted in different mortality percentages. Data in table (4) indicated that the order of efficiency of the tested compounds at LC_{50} levels could be descending arranged as follows: M > Ag NPs > Ch NPs > Ch EX. The corresponding LC_{50} values were 18.55 > 38.79 > 47.95 > 49.20%, respectively. All adult males in the control group (0% of corrected mortality) survived and didn't reveal any signs of toxicity. The Ag NPs, Ch NPs, and Ch EX were 47.81, 38.68, and 37.7 T.I. as effective as M at the LC_{50} level, respectively.

Table 4. Toxicity of tested compounds against male *P. americana***.**

Compounds	LC_{50} %	Confidence limits of LC_{50}		Slope	Index (T.I.)
		Lower limit %	Upper limit %		
M (+ve)	18.55	10.68	24.41	2.97±0.64	100
Ag NPs (-ve)	38.79	21.77	53.25	1.51±0.43	47.81
Ch NPs	47.95	41.37	54.38	4.47±0.63	38.68
Ch EX	49.20	41.91	56.59	3.84±0.57	37.7

T.I. = Index compared with M

3.3.2. Toxicity of the tested compounds on the adult female

Data in table (5) represented that the order of efficiency of the tested compounds at LC_{50} levels against adult females *P. americana* could be descending arranged as follows: M > Ch NPs > Ag NPs > Ch EX. The corresponding LC_{50} values were 13.66 > 25.50 > 28.27 > 40.37%, respectively. All adult females in the control group (0% of corrected mortality) survived and did not demonstrate any signs of toxicity. The Ch NPs, Ag NPs, and Ch EX were 53.55, 48.31, and 33.83 T.I. as effective as M at the LC_{50} level, respectively.

Table 5. Toxicity of tested compounds against female American cockroach, *P. americana*

Compounds	LC_{50} %	Confidence limits of LC_{50}		Slope	Index (T.I.)
		Lower limit %	Upper limit %		
M (+ve)	13.66	4.53	20.53	2.39±0.63	100
Ch NPs	25.50	-	-	0.77±0.42	53.55
Ag NPs (-ve)	28.27	17.93	36.42	2.24±0.46	48.31
Ch EX	40.37	30.04	49.94	2.33±0.46	33.83

T.I. = Index compared with M

3.4. Biological activities of P. americana after exposure to the LC_{50} of the tested compounds

3.4.1. The effect of the viability of P. americana oothecae

After the treatments in which adult males and females were exposed to LC_{50} of tested compounds (M, Ag NPs, Ch NPs, and Ch EX), data in table (6) detected the viability of mated American cockroaches. Female American cockroaches deposit the ootheca, dark brown colored (bean-shaped), after carrying it for a few hrs to a few days. There were 27 ootheca in the control group overall, but Ch NPs was thought to be the most viable group in terms of oothecae oviposition at LC_{50} (13 ootheca). Additionally, M, Ag NPs, and Ch EX groups oviposited (4, 7, and 10 ootheca, respectively). After oviposition, the highest malformed number of oothecae was Ag NPs, Ch EX, and Ch NPs groups had 5, 5, and 4 malformed oothecae and the least number in M group (2 oothecae). On the other hand, the control group hadn't any malformed ootheca. Data in table (6) illustrated that there were twenty-six hatched oothecae in the control group overall, but Ch NPs had the most hatched 9 oothecae, while hatched oothecae were in descending order as Ch EX= 5, M and Ag NPs= 2 oothecae, respectively. Each ootheca produced an average of 16 nymphs; in the control and Ag NPs groups however, M, Ch NPs, and Ch EX groups produced 14 nymphs demonstrated in table (6) and Fig. (3).

Table 6. Viability of oothecae of *P. americana* after the exposure to the LC_{50} of the tested compounds.

Compounds	Total oothecae	No. of malformed oothecae	Malformed oothecae rate (%)	Hatched oothecae	Hatchability (%)	No. of Nymphs per ootheca
Control	27	0	0	26	96.30	16
M	4	2	50	2	50	14
Ag NPs	7	5	71.43	2	28.57	16
Ch NPs	13	4	30.77	9	69.23	14
Ch EX	10	5	50	5	50	14

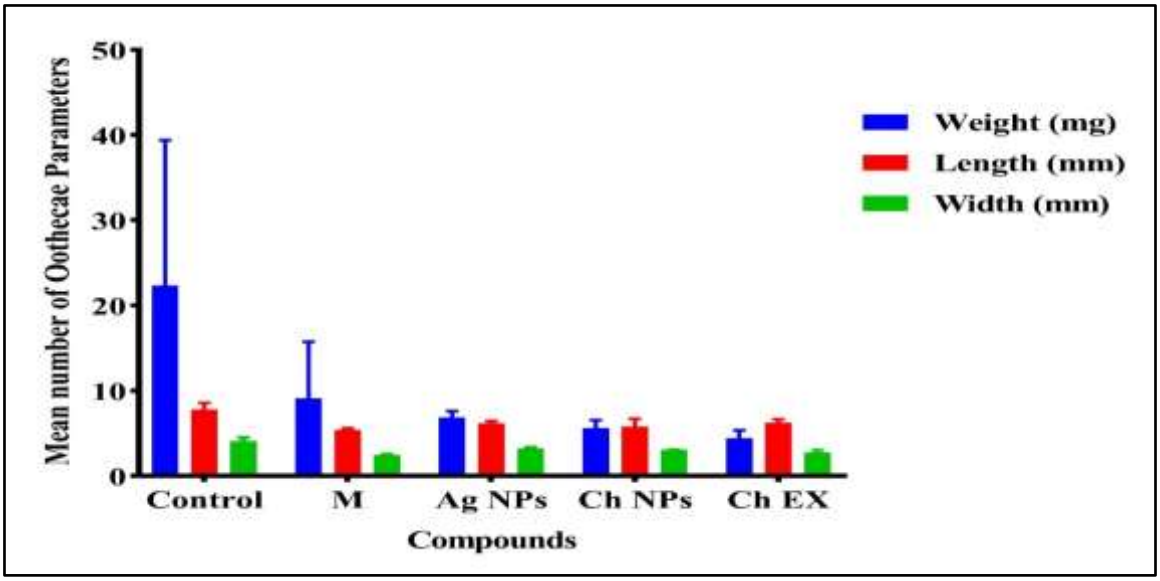


Figure (3): Mean values ±SE of oothecae parameters of *P. americana* due to the exposure with the LC_{50} of the tested compounds

3.4.2. Effect of LC_{50} of tested compounds on the weight of the oothecae of P. americana

Data in Fig. (4) represented the oothecae average weights of the control group (22.34±17.06 mg) compared with other tested compounds. The largest average weight of ootheca was M group (9.11±6.64 mg) but the smallest was Ch EX (4.43±0.95 mg).

3.4.3. Effect of LC_{50} of tested compounds on length of the oothecae of P. americana

The Fig. (4) demonstrated that the oothecae mean length of a control group (7.79±0.80 mm) compared with other tested compounds. The longest length of ootheca was Ch EX and Ag NPs groups (6.24±0.4 mm and 6.20±0.26 mm) but the shortest was M (5.40±0.20 mm).

3.4.4. Effect of LC_{50} of tested compounds on the width of the oothecae of P. americana

Data in Fig. (4) illustrated that the oothecae average width of a control group (4.11±0.42 mm) compared with other tested compounds. The maximum width of ootheca was Ag NPs (3.23±0.16 mm) but the minimum was M (2.45±0.10 mm).

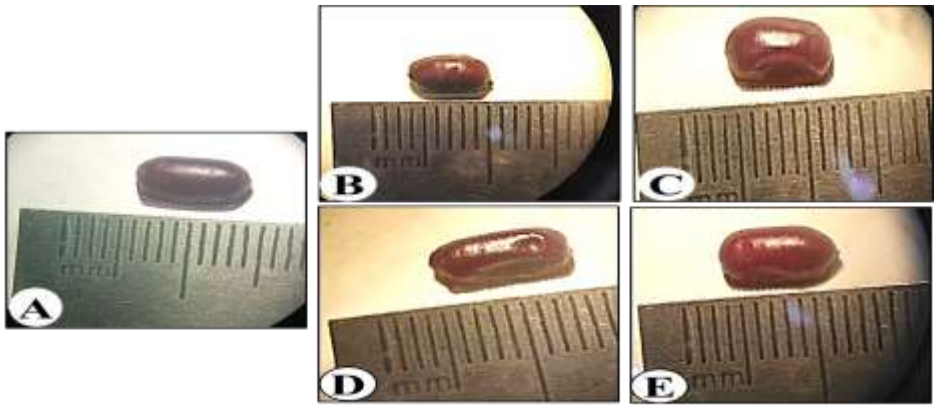


Figure (4): Oothecae of *P. americana* due to the exposure of the LC_{50} of the tested compounds (A) Control; (B) M; (C) Ag NPs; (D) Ch NPs; and (E) Ch EX, respectively

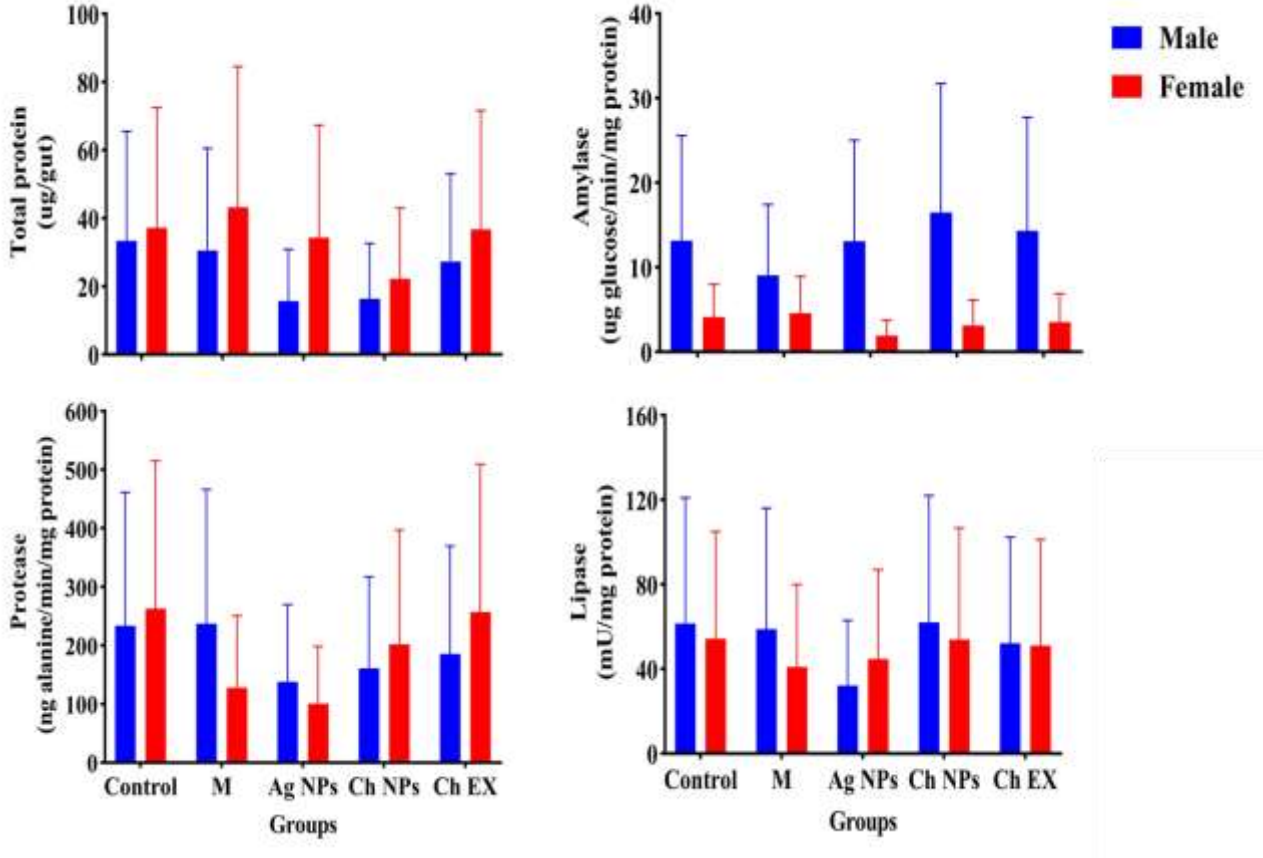


Figure (5): Illustrated changes in the mean values ±SE of the total protein, amylase, protease, and lipase in control and different compounds in male and female American cockroaches, *P. americana* were observed

3.5. Effect of crude plant extracts, their silver nanoparticles, and malathion at LC_{50} on physiological activities of treated midgut adult male P. americana

3.5.1 Determination of total protein

In Fig. (5), the quantitative results of total protein for each treatment are illustrated. Different sample sets were analyzed to find recognizable patterns of relevance. The average control group (65.5 ug/gut) and those treated with M (60.57 ug/gut) and Ch EX (53.1 ug/gut) revealed a slight but noticeable difference. The Ch NPs group significantly reduced total protein levels compared to the control group (32.63 ug/gut). Lastly, it was found that the Ag NPs group had a significant impact on the reduction of total protein (30.87 ug/gut).

3.5.2. Determination of amylase

The Fig. (5) illustrated the quantitative amylase results for each treatment. Significant trends were found in the analysis between the various sample sets. Those treated with average Ch NPs, Ag NPs, and Ch EX groups (31.7, 25, and 27.73 ug glucose/min/mg protein, respectively) and the control group (25.57 ug glucose/min/mg protein) revealed a slight but noticeable difference. Finally, compared to the average control group, the M (17.4 ug glucose/min/mg protein) demonstrated significant benefits in lowering average amylase.

3.5.3. Determination of protease

The protease quantitative data for each treatment is represented in Fig. (5). Significant trends were found in the analysis between the various sample sets. Those treated with average M (466.33 ng

alanine/min/mg protein) and the control group (461.33 ng alanine/min/mg protein) represented a slight but noticeable difference. The average protease was 370 ng alanine/min/mg protein in the Ch EX group. When compared to the control group, the Ch NPs and Ag NPs groups significantly reduced protease (317.33 and 270 ng alanine/min/mg protein).

3.5.4. Determination of lipase

The Fig. (5) displayed the quantitative lipase data for each treatment. The study revealed noteworthy patterns among the different sample groups. There was a small but discernible difference between the groups treated with average Ch NPs (122 mU/mg protein) and the control group (121 mU/mg protein). The M and Ch EX groups drastically decreased lipase levels (116 and 102.33 mU/mg protein), respectively, in comparison to the control group. Finally, lipase was significantly reduced in the Ag NPs group (63 mU/mg protein).

3.6. Effect of crude plant extracts, their silver nanoparticles, and malathion at LC_{50} on physiological activities of treated midgut adult female P. americana

3.6.1. Determination of total protein

The quantitative results of total protein for each treatment are illustrated. Different sample sets were analyzed to find recognizable patterns of relevance. The average control group (72.43 ug/gut) and those treated with Ch EX and M (71.6 and 84.5 ug/gut) revealed a slight but noticeable difference. The Ag NPs group significantly reduced total protein levels compared to the control group (67.4 ug/gut). Lastly, it was found that the Ch NPs group had highly significant impacts on the reduction of total protein (43.07 ug/gut) revealed in Fig. (5).

3.6.2. Determination of amylase

The quantitative results for amylases for each treatment were demonstrated in Fig. (5). The study revealed notable patterns among the different sample groups. A small but discernible difference was seen between the average M treatment group (8.93 ug glucose/min/mg protein) and the control group (7.97 ug glucose/min/mg protein). Amylase was considerably lower in the Ch EX and Ch NPs groups than in the control groups (6.87 and 6.1 ug glucose/min/mg protein). Finally, the groups treated with Ag NPs illustrated a considerable reduction in amylase (3.77 ug glucose/min/mg protein).

3.6.3. Determination of protease

Each treatment's protease quantitative data is represented in Fig. (5). Analysis of the several sample sets revealed important tendencies. Those that received the average Ch EX (509.67 ng alanine/min/mg protein) and the control group (515.3 ng alanine/min/mg protein) illustrated a minor but discernible difference. The protein levels of protease in the Ch NPs and M groups were 397.33 and 250.67 ng alanine/min/mg, respectively. The Ag NPs group, at last, found a very significant decrease in protease (198.3 ng alanine/min/mg protein).

3.6.4. Determination of lipase

The quantitative lipase data for each treatment is illustrated in **Fig. (5)**. The study found significant differences between the various sample groups, with the groups treated with average both Ch NPs (106.67 mU/mg protein) and the control group (105 mU/mg protein) representing a slight but noticeable difference. The levels of lipase in the Ch NPs were 101.33 mU/mg protein, while the Ag NPs and M groups significantly reduced the lipase levels (87 and 80 mU/mg protein) compared to the control group.

3.7. Histological studies

3.7.1. Normal histology of the midgut

The normal midgut architecture was illustrated in Figs. (6, A, a). The midgut is made up of an outside layer of longitudinal muscles that follow the stratum of the enteric epithelium lining the inside of the gut and an inner layer of circular muscles. These cells' outer ends were resting on a basement membrane. The peritoneal muscles are the thinnest layer of the midgut. The structure of enteric epithelium can be divided into two main cell types: regenerative nidi, which are cells that rebuild other epithelial cells after they are killed, and columnar cells, which have distinct boundaries and almost usually have a striated border.

3.7.2. Histopathological observation in the treated groups of adult males

Transverse midgut sections of American cockroaches treated with M exhibited longitudinal and circular muscle layers that were roughly typical in shape when stained with hematoxylin and eosin. The typical architecture of the columnar cells of the regenerating nidi and intestinal epithelium has been lost. Columnar cells have a deformed and degraded shape.

Additionally, striated edges are warped. The cytoplasm is quite weak and vacuolated, Fig. (6, B). During the American cockroach treated with Ag NPs, sections revealed longitudinal muscles are modified. The columnar cells' shape has eroded and warped. Cell growth results in regenerative nidi that are concentrated in the lumen's center and significantly deteriorate, Fig. (6, C).

The midgut sections of American cockroaches treated with Ch NPs illustrated circular and longitudinal muscle layers are normal in shape. Columnar cells and regenerating nidi have completely deformed and degraded, Fig. (6, D).

When American cockroaches treated with Ch EX transverse midgut sections stained with hematoxylin and eosin illustrated circular and longitudinal muscle layers are normal in shape. The form of columnar cells is distorted and deteriorated. Regenerative nidi are formed proliferation of the cells and concentrated in the center of the intestinal cavity and severely degenerated, Fig. (6, E).

3.7.3. Histopathological observations in the treated groups of adult females

When American cockroaches treated with M, transverse midgut sections stained with hematoxylin and eosin indicated that longitudinal muscles were distorted and deteriorated, whereas circular muscles were detached from layers. The typical architecture of the columnar cells of the regenerating nidi and intestinal epithelium has been lost. Columnar cells have a deformed and degraded shape. Additionally, the striated border is warped, Fig. (6, b). Transverse midgut sections of the American cockroach treated with Ag NPs revealed thicker circular muscle layers and deformed longitudinal muscles. As a result of cell proliferation, columnar cells extended toward the lumen. The regenerative nidi have a less typical form, Fig. (6, c).

The transverse midgut sections of American cockroaches treated with Ch NPs illustrated longitudinal muscles are deformed and degraded, whereas circular muscular layers are less normal in shape. Regenerative nidi and columnar cells were slightly affected by revealing proliferation of the cells, Fig. (6, d).

When American cockroaches treated with Ch EX stained with hematoxylin and eosin illustrated circular, longitudinal muscle layers and columnar cells are normal in shape. Regenerative nidi are formed proliferation of the cells and are concentrated in the center of the intestinal cavity, Fig. (6, e).

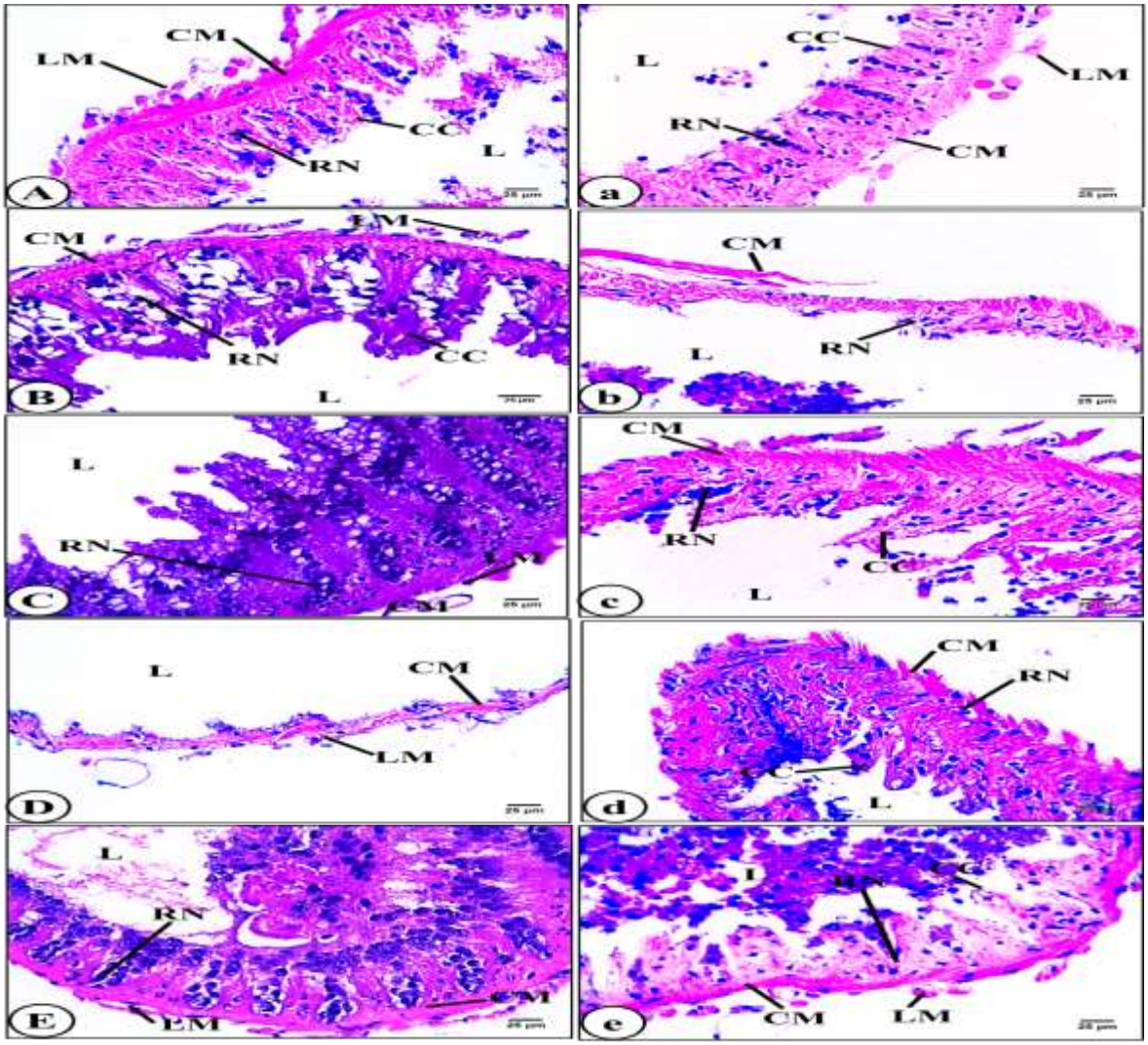


Figure (6): Photomicrographs of transverse sections in midgut of an adult males (capital letters) and females (small letters) *P. americana* illustrated the histological changes in layers stained with hematoxylin and eosin due to the exposure to LC_{50} of (A, a) Control groups. (B, b) M. (C, c) Ag NPs. (D, d) Ch NPs. (E, e) Ch EX groups revealed (LM) longitudinal muscles, (CM) circular muscles, (RN) regenerative nidi, (CC) columnar cells, and (L) lumen.

(H&E, bar= 25 µm)

4. Discussion

Economically, American cockroaches are considered a serious pest that endangers people's health. The continuous application of chemical pesticides has forced us to consider environmental safety, which has led to the need to find plant-based alternatives for cockroach control. Our research indicates that the aqueous extract of chili peppers and its silver nanoparticle can biocontrol both adult males and females *P. americana*. According to a higher percentage of chili pepper extract (*C. annuum*) increases the extract's efficacy as a pesticide. Nanoparticles were demonstrated to have an entomotoxic effect on both adult male and female *P. americana* in the current investigation. When compared to the crude aqueous extract of chili peppers, the LC_{50} values of the silver nanoparticles were more hazardous to adults. Nanoparticle technology is one of the new methods being researched and tested in the field of pest control. It has been used to increase the effectiveness of applied pesticides, whether in medical and veterinary pests like flies, cockroaches, and mosquitoes Campos et al. (2020) or agricultural pests like those reported by Abd El-Zaher (2017).

Additionally, nanoparticles can be used as herbicides and insecticides Bhattacharyya et al. (2010). Furthermore, the biological traits of *P. americana* were affected. Numerous authors' earlier research studies backed up this finding. The death percentages of German cockroach nymphs and adults increased as the quantities of silver nanoparticles underfeeding and contact methods increased, as demonstrated by Said (2017). When *P. americana* was fumigated with a German chamomile nanoemulsion at a concentration of 10 mg/liter, El-Khodary et al. (2020) illustrated that the mortality rate was 44.44% after 72 hours of application. In agricultural and medicinal insects, the nanoparticle compound may have a pesticide and insect-repelling action Magro et al. (2019) and Campos et al. (2020). The use of plant extracts in pest management is increased by this research, which confirms the biological activity of a crude extract from chili pepper plants against *P. americana*. Plant extracts are environmentally benign and have high bioactivity against a range of pests. Guo et al. (2025) investigating plant extracts that have a potent repellent or toxic effect on pests and their bioactivities against pests can promote the development of novel ecologically friendly and environmentally benign pesticides. Nanoparticles have insect-repellent properties because of their physiological changes that alter the body Nel et al. (2006), and their application in insect pest control is novel Bhattacharyya et al. (2010). NPs made from *Nelumbo nucifera* aqueous leaf extract have been investigated for their ability to kill insects Santhoshkumar et al. (2011). The present study revealed a reduction in weight, length, and width of oothecae when American cockroaches were sprayed with Ag NPs and chili pepper silver nanoparticles. Additionally, the present investigation discovered that the sprayed application of chili pepper silver nanoparticles was more successful in cockroach management, perhaps as a result of the nanoparticles' ability to swell and enter the alimentary canal or respiratory spiracles. The nanoparticle's toxic action may have resulted from their interaction with different chemical compounds within the insect's body, which inhibited vital processes such as the inhibition of the insect's enzyme mechanism, DNA changes or damage, oxidative stress-induced defects in biological organs, protein degradation, and a decrease in membrane permeability, all of which ultimately led to cell death. These results concur with those of Benelli (2016), and Azarudeen et al. (2017). The presence of several bioactive and vital nutrients is linked to the quality of chili pepper fruit extract Keck and Müller (2013), and these components are influenced by several parameters, including the maturity stage and genotype, these investigations agreed with *C. annuum* that contained thirty-seven chemical compounds. Because GC-MS analysis revealed that the majority of the chemicals in the investigated aqueous extract were fatty acids and esters, such as palmitic acid, octadecatrienoic acid, oleoylglycerol, and oleic acid, which have anti-inflammatory, anti-allergic, anti-nociceptive, antioxidant, antibacterial, and antifungal properties, *C. annuum* has been indicated as a potential bioinsecticide as in Santos et al. (2013). As a result, green synthesized silver nanoparticles more effectively than crude extract in toxicity, biochemical, and histological examinations. According to Ohtani et al. (1990) and Yff et al. (2002), palmitic acid has antimicrobial and cholesterol-lowering qualities. It also has been illustrated to have hepatoprotection against galactosamine and significant cytotoxicity against cancer cell lines. Proteins in the extract could bind to silver nanoparticles through free amino or carboxyl groups, according to Gole et al. (2001). The amine (-NH), carboxyl (-C=O), and hydroxyl (-OH) groups of leaf extracts reported by *Prasad et al. (2011)* are the main ingredients used in the synthesis of silver nanoparticles; the results of GC-MS analysis of *C. annuum* fruit extract proved the presence of these synthetic substances. The American cockroach, according to Bell and Adiyodi (1982), is an omnivorous insect that feeds deliberately. There may be traces of the crude extract in the antenna, wing, foreleg, midleg, hindleg, abdomen, corpse, or exuviae that it could feed. Therefore, Ag NPs

were also of interest; nevertheless, it is crucial to determine the right concentration, stability, and shelf life of the insecticidal phytochemicals in the crude extract. Furthermore, through glands, pore canals, and a soft intersegmental membrane, all the phytochemicals in chili pepper crude extracted with molecular weights under 500 could penetrate the integument. Once digested, it was scattered to the desired spot. Except for 2-Oleoylglycerol, which has a molecular mass of 500, and 1-Heptatriacotanol, which has 536, the results were consistent with the accumulation of these substances in the lipid tissue of *P. americana*. According to the study, the dark brown (bean-shaped) ootheca *P. americana* measured 7.79±0.80 mm in length, 4.11±0.42 mm in width, and 0.22±0.17 gm in weight. We found that the oothecae lengths reported by Cornwell (1968) and Borah and Hazarika (2019**)** were 8-10 mm. Earlier studies found that the total number of eggs per ootheca varied. For example, Barbara (2014) found 16 and 14–16 eggs per ootheca, these outcomes aligned with what we had observed, while Whitworth and Ahmad (2007) reported 12–40 eggs per ootheca. In the midgut of insects, columnar cells primarily perform the synthesis and secretion of digestive enzymes. It is expected that the midgut endocrine cells and digests in the lumen will contribute to the synthesis and secretion of digestive enzymes and/or the absorption of nutrients Lehane et al. (1995), as the midgut epithelial cells are not neuronally controlled Lehane (1998). The midgut of a typical cockroach is the most crucial component of the digestive tract since it is mostly used for food preparation and nutrient absorption and contributes significantly to metabolic processes Ballan-Dufrançais (2002). Most of the epithelial cells in the midgut are tall, uniform columnar cells. One characteristic of the generative cell zone is the distinct nidus, which is located in the basal part of the epithelium and never comes into touch with the gut lumen. The nidus lacks a junctional apparatus that connects cells to the basal lamina or nearby cells. Endo et al. (1983**)** agreed with these results. When metals (Ag) interfere with the digestive system, they build up in the gut cells and prevent them from entering the hemolymph Pigino et al. (2005). It is evident from this study that adult *P. americana* displayed different responses in terms of energy reserves and enzyme activity after being treated with the investigated chili pepper green synthesized nanoparticles and silver nanoparticles. Due to the distinct physico-chemical characteristics of nanosilver and the free ions it releases, Eisler (1996) suggested that the buildup of silver has had a negative impact on growth. According to the study, adult male and female American cockroaches' total protein content decreased because of the biochemical impacts of *Capsicum* sp. green silver nanoparticles and silver nanoparticles, which is confirmed **by** Eldefrawy et al. (2022). The substantial reduction in protein content observed following exposure of adult male and female *P. americana* to the LC_{50} of silver and chili pepper nanoparticles is comparable to this. According to Abou El Ela et al. (2023), *Culex pipiens* larvae demonstrated a notable reduction in total protein content following a 24 hr exposure to the garlic essential oil's LC_{50}. To maintain cellular metabolism under insecticidal stress, amino acids may be diverted into the Krebs cycle as keto acids, which could explain the drop in total protein content Khosravi and Sendi (2013). The decrease in total protein metabolism should ultimately result in physiological abnormalities that lead to larval mortality Parthiban et al. (2020). According to Felix *et al*. (2021), proteases in insects can strengthen larval resistance to pesticides by rupturing the peptide bonds of dietary proteins in the stomach. They revealed how the protease levels in *Aedes aegypti* and *Aedes albopictus* larvae were slightly reduced by L. grata essential oils. This result is in line with the slight decrease in protease activity seen in the adult male and female *P. americana* in the current investigation after they were exposed to malathion, silver nanoparticles, and chili pepper silver nanoparticles. The amylase activity of *Aedes aegypti* and *Aedes albopictus* larvae exposed to *L. grata* essential oils proved to significantly decrease by Felix et al. (2021), which is consistent with these findings. This is probably caused by the disintegration of cells that

produce enzymes, which kills the larvae Gupta *et al*. (2011). According to Felix *et al*. (2021), amylase hydrolyzes glycogen to create glucose. Several investigations have been conducted on this cockroach's digestive lipase, including those by Eisner (1955) and Treherne (1958). For smaller lipid molecules to be absorbed from the digestive tract and transferred to other tissues, this lipase helps break down fats. The fatty acids that are thus made available have a high energy equivalence per unit weight, which makes them ideal for use as an energy source. These studies confirmed our findings, which are highly significant as a moiety of complex lipids, including phospholipids, which are structural components of cellular and subcellular organelles. The neuropeptide Crustacean Cardioactive Peptide (CCAP) was extracted from the cockroach's midgut and has been found to enhance the activity of the midgut amylase. This indicates that columnar cells are closely associated with cells that express CCAP. These results are in agreement with the discovery that the midgut epithelium of *P. americana* had amylase-containing columnar cells Lima et al. (2003), and that the midguts of cockroaches *D. punctata* Fuse et al. (1999) and *Nauphoeta cinerea* Elpidina et al. (2001) also demonstrated notable amounts of amylase activity. As demonstrated by Bodláková et al. (2018), the stimulatory effect of Adipokinetic Hormone on *P. americana* amylases has been verified. Additionally, it has been demonstrated that hormones also affect lipases and proteases, two additional digesting enzymes in the cockroach gut. As illustrated by Bodláková et al. (2017), in vitro studies demonstrate that hormones directly affect lipase and protease in the same way that they affected amylase. In the present investigation, we deliberately targeted the midgut. The midgut of control and treated adult cockroaches exhibited histological evidence of major cytological abnormalities following the spraying of *C. annuum* extract and nanoparticles. Singh (2006) examined the effects of benzidine and 1-nitroso-2-napthol on the midgut of *P. americana* and discovered an additional layer of epithelium oriented toward the lumen. There were some outgrowths because of the nidi's mitotic activity. The thickness of the muscle layers changed in the midgut of American cockroaches treated with malathion, nanoparticles, and chili pepper fruit extract. These findings were corroborated by Sutherland et al. (2002), who reported that treated insects displayed a significant increase in epithelial thickness along with a retraction of the peritrophic membrane. The maximal boric acid concentration (20%) appears to have eliminated the midgut epithelial cells. Furthermore, alterations in the arrangement of midgut epithelial cells were seen following treatment with plant lectins or protease inhibitors. After ingesting potato protease inhibitor II, *Teleogryllus commodus* decreases the thickness of the midgut wall, vacuolates the epithelial cells, enlarges the microvilli, creates cellular protrusions into the midgut, and finally ruptures individual or small groups of epithelial cells. Additionally, the midgut epithelial cells of *Lygus hesperus* indicated notable damage upon exposure to phytohemagglutinin, a lectin derived from *Phaseolus vulgaris* Habibi et al. (2000). When American cockroaches were treated with LC_{50} of *C. annuum*, its silver nanoparticles, malathion, and silver nanoparticles, significant changes were observed in the current study, including disruption of the peritrophic membrane, striated border, regenerative cells, and longitudinal muscles. Our findings indicated that malathion had a major adverse effect on adult American cockroach populations. These findings concurred with Tahir et al. (2017) that *P. americana* revealed resistance to malathion, as indicated by a significantly higher insecticide-detoxifying enzyme activity compared to the susceptible control group. This was due to an increase in the enzymatic activity of nonspecific esterases, monooxygenases, and glutathione S-transferases, which led to the development of resistance. Additionally, several studies demonstrated the beneficial effects of malathion on the physiology and histology of the adult midgut. For example, *P. americana* has developed resistance to organophosphates Sogorb and Vilanova (2002) and pyrethroids Azizi et al.

(2014) through behavioral adaptation or physiologically enhanced metabolic detoxification processes Liu and Yue (2000). It is believed that the development of insecticidal resistance is mostly caused by these metabolic processes Soderlund (2005). The onset of a deltamethrin-mediated degenerative process may be indicated by vacuolization in the midgut. Cytoplasmic vacuolization, which triggered autophagy and death, was connected to deltamethrin-mediated early changes Gutiérrez et al. (2016). Deltamethrin exposure may result in an imbalance in the homeostasis of the midgut, which can hinder the body's ability to digest food for nutrients Huang et al. (2015), dysregulate breathing Unkiewicz-Winiarczyk and Gromysz-Kałkowska (2012), and restrict the production of signaling molecules that regulate its own physiology Caccia et al. (2019). However, direct toxicity may not always be the cause of deltamethrin's toxic effects. Ahmed (1995) claims that the peritrophic membrane, the development of vacuoles in the apical portion of the cell, and the proliferation of epithelial cells were all destroyed by the histopathological action of chamomile plant oil extract. These results are in line with the severe histological alterations observed in *Culex pipiens* larvae's midgut. Similar histological changes were reported by Sharma and Chattoraji (1964) for *P. americana* treated with lindane, Lal et al. (1970) for *Spodoptera litura* treated with endosulfan, diazinon, and dichlorvos, and Balakrishnan (1987) for *Plebiogryllus guttiventris* treated with fenitrothion. Changes in the insect's alimentary canal have been reported by Mukherji and Hardas (1954), Lal et al. (1970), and Misra (1981) in response to insecticidal stress. Under severe conditions, including treatment with malathion, phosphamidon, parathion, carbaryl, and endrin, *Hieroglyphus nigrorepletus*, Misra (1981), and *Chrotogonus trachypterus*, Singh (1990) exhibit serious necrosis of the alimentary canal's epithelial lining cells. In insects, the breakdown of the epithelial layer of the alimentary canal appears to be a common stress response caused by exposure to various toxins as well as physiological challenges such as malnutrition Rosaiah and Mukherjee (1985).

5. Conclusion

The present study is a contribution to further understanding of the mechanism underlying the action of chili pepper nanoparticles (Ch NPs) and its aqueous extract on insect digestive physiology leading to the deteriorated life parameters of the pest insects, *P. americana*. It can also be concluded that silver suspension-induced stress alters enzymatic levels in the midgut tissues and causes major cytological midgut disruptions. These compounds can therefore efficiently combat a range of medical insects, such as the American cockroach. The present study is a contribution to further understanding of the mechanism underlying the action of chili pepper nanoparticles (Ch NPs) and its aqueous extract on insect digestive physiology leading to the deteriorated life parameters of the pest insects, *P. americana*. It can also be concluded that silver suspension-induced stress alters enzymatic levels in the midgut tissues and causes major cytological midgut disruptions. These compounds can therefore efficiently combat a range of medical insects, such as the American cockroach.

Declarations

Ethical approval
All experiments in this study were accepted by the Research Ethics Committee of the Faculty of Science, South Valley University, Qena governorate, Egypt. (Code No. 008/02/25).

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Conflicts of Interest
The authors disclosed no conflict of interest.

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