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Novel synthesis of silver nanoparticles using melon aqueous extract and evaluation of their feeding deterrent activity against housefly *Musca domestica*

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ABSTRACT

Objective: To synthesize silver nanoparticles (AgNPs) adopting a green approach and evaluate their toxicological potential against housefly *Musca domestica* which is responsible for spreading a number of fatal diseases.
Methods: In the present work, AgNPs were synthesized by consuming secondary metabolites present in the fresh aqueous extract of melon. While the toxicity of the synthesized materials were recorded against house fly via feeding deterrence protocol.
Results: Surface plasmon resonance was observed at 430 nm and the average crystallite size calculated from powder X ray diffraction was (18 ± 6) nm. Significant results were observed at different concentrations in the range of 2–10 mg/mL.
Conclusions: Fresh melon extract resulted in a simple one-step formation of AgNPs with better control over the size of the material and the noteworthy insecticidal activity was displayed. This study proved the insecticidal potential of AgNPs against *Musca domestica* and its candidacy for replacing commercially available insecticides (organic/inorganic) which are synthesized in nature.

1. Introduction

Musca domestica (*M. domestica*), a kind of housefly, is one of the most common insects commonly found around human food, utensils and settlements and is the mechanical carrier for more than a hundred diseases in humans and animals in addition to other bacterial, protozoal, viral and helminthic infections via the transmission of a range of pathogens[1].

According to the Food and Drug Administration, the housefly is also responsible for food contamination and is categorized as a chief causative agent in the spreading of a range of infections such as salmonellosis, shigellosis and cholera. In the literature, insecticidal studies have been performed on the adults of houseflies while other stages of the life cycle of the housefly, *i.e.* larvae and pupa remained neglected, even though only about 15% of the total *M. domestica* housefly population exist as adult[2].

Houseflies fed on human wastes, foods and decaying matter are the vectors of pathogenic protozoan, metazoan parasites, bacteria and viruses. Housefly is one of the major contributors in a variety of food-borne human diseases including shigellosis, typhoid, cholera, tuberculosis, infantile diarrhea, bacillary dysentery, anthrax and ophthalmia[3].

Nanobiotechnology is a rapidly growing field of scientific research which incorporates material science and biology[4]. A variety of nanoscale materials with interesting structures have been applied in the areas of catalysis, solar cells, medicine, water treatment, and

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their anti-bacterial, anti-fungal and antiprotozoal activities provide solutions to scientific, ecological and biological challenges[5].

Biosynthesis of nanoparticles highlights the intersection of the nanotechnology and biotechnology. Besides, green synthesis methods of nanoparticles are cost-efficient alternatives to conventional physical and chemical ones of metallic nanoparticles synthesis[6].

Extracts from plants containing proteins and other phytochemicals used for synthesizing metallic nanoparticles are advantageous over conventional physical and chemical methods, as they act as a reductant and a capping ligand and prevent the aggregation of particles[7]. Plants such as *Stevia rebaudiana*, *Citrus sinensis*, *Artemisia nilagirica* and *Mangifera indica* were used to synthesize nanoparticles[8-11]. The biological synthesis methods of nanoparticles tender certain advantages as these methods can be carried out more easily and cost-effectively than conventional methods[12].

Silver nanoparticles (AgNPs), amongst different metallic nanoparticles are efficient materials widely used in nanomedicine because of their distinctive properties. AgNPs possess anti-bacterial properties and could be used as an additive instead of antibiotics. Silver in colloidal state is zerovalent and contains particles with 1 nm to 1 μm. AgNPs are more effective then ionic silver due to the increasing surface area and better porosity[13].

The present report is in continuation of the recent work to investigate an unreported green synthesis route for AgNPs using extracts derived from melon and their characterizations[14]. The aim of executing this project was to probe the insecticidal efficacy of nanometric silver against houseflies *M. domestica*.

2. Materials and methods

2.1. Preparation of aqueous fruit extract

Unripened melons (*Cucumis melo*) were collected from a local agriculture farm and used for aqueous extract preparation. Briefly, 25 g melon was thoroughly washed with distilled water, dried and chopped into small pieces and blended with 100 mL sterile water. The blend obtained thus was filtered through Whatman No.1 filter paper with a pore size of 25 μm and the filtrate was preserved in the refrigerator (-4 ℃) for further use.

2.2. Synthesis of silver nanoparticles

Ninety milliliters of 2 mmol/L silver nitrate solution in water was added to 10 mL of melon extract at room temperature and kept for 30 min to reduce Ag^+ ions. Dark brown colored reaction mixture of biologically synthesized AgNPs was centrifuged at 8 000 r/min for 15 min and resulting pellet was redispersed in sterile water followed by filtration through millipore filter. The percentage yield was calculated by quantitative estimation of unreacted Ag^+ obtained from the supernatant by atomic absorbance spectroscopy and compared with the stock solution.

2.3. Instrumentation

UV-visible spectra was recorded in the range of 200–800 nm with UV-visible spectrophotometer SP-1103. Samples were analyzed by atomic absorbance spectrophotometer (FAAS, Perkin Elmer 400). Fourier transform infrared spectra (FTIR) was recorded on Brüker Spectrum-100 FTIR spectrophotometer using KBr pellet method in the range of 4 000–400 cm^{-1}. Thermo gravimetric (TG) analysis was conducted on Q500 V20.13 Build 39 thermal analyzer in a platinum pan at the rate of 10 ˚C/min under flowing nitrogen environment from ambient to 1 000 ˚C. Field emission scanning electron microscope (FE-SEM) was recorded on Japan Electron Optics Laboratory (JEOL, JSM-7600F, Japan). Elemental analyses data were obtained using oxford-EDS system from JEOL (JSM-7600F, Japan). Powder X-ray diffraction analysis was carried out on JDX-3532 JEOL Japan X-ray diffractometer (40 kv, 30 mA, monochromic) using a Cu K (alpha) source (105418 Å). The scanning range used for the sample analysis was $20^\circ \leqslant 2\theta \geqslant 70^\circ$ with a scanning rate of 0.5 s per step and a step size of 0.05˚.

2.4. Housefly collection and maintenance

Adult populations of *M. domestica* houseflies were collected with a sweep net from a fruit juice shop at a local market. Flies were then moved to entomological cages (30 cm × 30 cm × 30 cm) at (26 ± 1) ˚C under 70% humidity and a 12:12 light/dark cycle. Water and food were provided to adult flies with 1:1 v/v mixture of milk and table sugar, approximately. Bran-milk mixture was prepared at a weight ratio of 1:3, and 100 g of Bran-milk mixture was placed on a plastic plate used for oviposition site.

2.5. Bioassay

The bioassay test against *M. domestica* flies was conducted as reported earlier[2]. For the bioassay test against *M. domestica*, serial different aqueous concentrations of AgNPs and 2,2-dichlorovinyl dimethyl phosphate (DDVP) were prepared in sterilized double distilled water. These concentrations were 2, 4, 8 and 10 mg/mL. DDVP, a volatile organophosphate, was used as a positive control. To evaluate their toxicological effects against *M. domestica*, 12 g of the feeding mixture was weighed and stirred well for few minutes with 100 mL of sterile water in a 250 mL beaker till homogeneity with each of the above prepared concentrations (2, 4, 8, and 10 mg/mL). Such 12 g media containing varying concentrations of AgNPs, aqueous plant extract and DDVP were distributed on 4 glass tubes (3 g media/tube). All tubes were held uncapped for 2 h to allow drying. Fifteen 4–5 days old adult *M. domestica* houseflies were placed into each glass tube and capped by cotton wool (60 adult *M. domestica* were used per each concentration, *i.e.* each test was replicated four times). Water was stirred with untreated diet to serve as the control. A solution silver nitrate (10 mg/mL) was used in all experiments under identical conditions in four replicates. Each group was assessed for mortality after 1, 2, 3, and 4 h of exposure by gently

stimulating the insects with a needle and non-responsive insects were considered dead. Observed mortality percentage was corrected according to Abbott's formula[15].

3. Results

3.1. UV-visible spectroscopy

The intensity of the absorbance band increased with time due to a continuous process of silver ions reduction to AgNPs and the increase in the number of AgNPs. Surface plasmon resonance of colloidal silver was observed at 430 nm which remained closed to it throughout the course of the reaction (Figure 1). Silver SPR stability in the solution remained unaffected when the particles were dispersed into the aqueous solution without getting agglomerated.

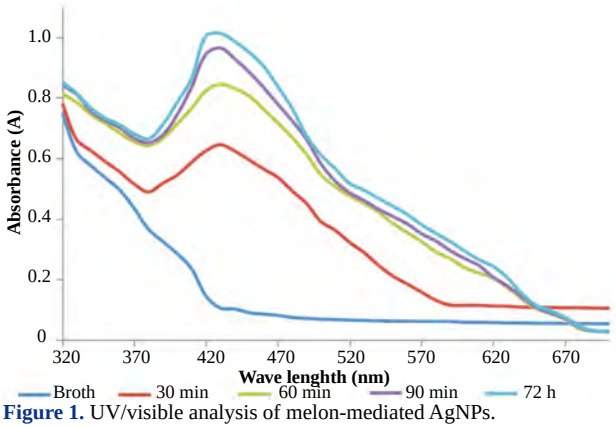


Figure 1. UV/visible analysis of melon-mediated AgNPs.

3.2. FTIR spectra

For the identification of capping/stabilizing agents as well as reducing agents, FTIR spectra of the prepared AgNPs were recorded (Figure 2). Prominent peaks observed in the spectrum of melon-prepared AgNPs were at 3 320–3 275, 2 950, 1 645, 1 535, 1 410, 1 260, 1 040 and 501 cm^{-1}, respectively.

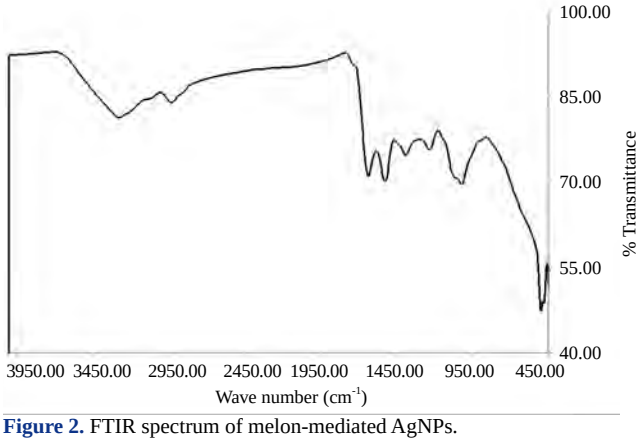


Figure 2. FTIR spectrum of melon-mediated AgNPs.

3.3. TG analysis

TG analysis of prepared AgNPs was carried out to study the thermal stability of nanoparticles at higher temperatures and the data obtained was plotted in Figure 3 where a three-step distinctive degradation pattern was observed at 125 ℃ and 318.8 ℃ and 570 ℃, respectively.

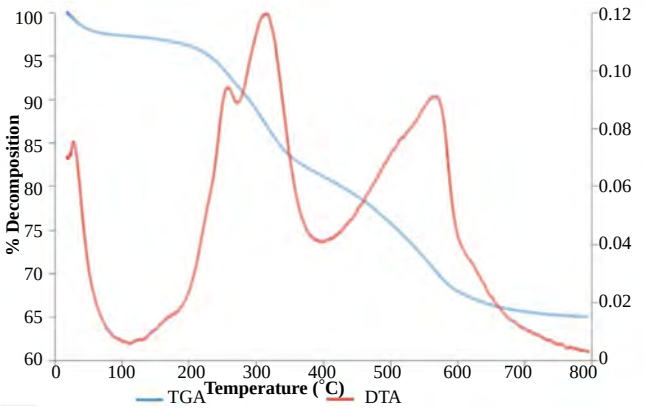


Figure 3. Thermogram of melon-mediated AgNPs.
TGA: Thermo gravimetric analysis; DTA: Differential thermal analysis.

3.4. FE-SEM and energy-dispersive X-ray (EDX)

The surface morphology of the prepared AgNPs was confirmed by FE-SEM. FE-SEM images of melon-synthesized AgNPs were seen as spheres with aggregation. The size of the prepared AgNPs was found within the range of 13–25 nm [mean size of about (18 ± 6) nm] with aggregation and lacked monodispersity. For elemental analysis confirmation, the prepared nanoparticles were subjected to EDX analysis (Figure 4).

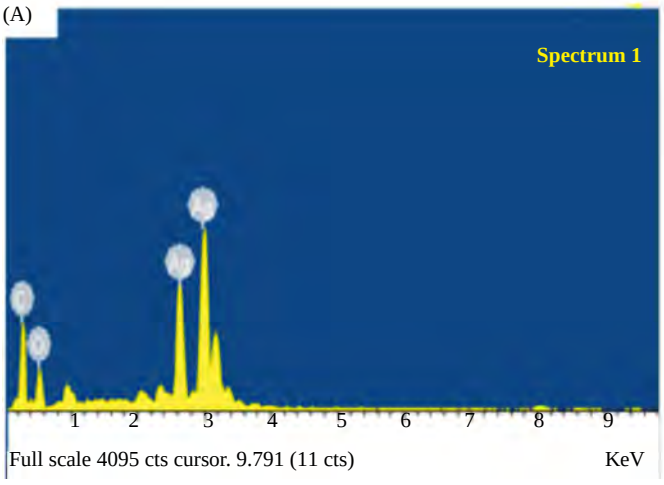
3.5. XRD

Phase composition and the distribution of the crystallite nature of the prepared AgNPs were obtained from XRD and the average crystalline size was also calculated and confirmed from Scherrer equation while three peaks were observed at 2θ values of 38.05, 46.35 and 65.35 (Figure 5).

3.6. Bioassay against houseflies

In this work, the insecticidal activity of biosynthesized AgNPs was assayed on houseflies after 1, 2, 3 and 4 h of exposure. The mortality rates were observed and the mortality results were obtained from silver nitrate solution and melon-mediated AgNPs against houseflies of *M. domestica*. The synthesized AgNPs showed significantly high mortality, *i.e.* 71.67% in 1 h, 91.66% in 2 h; 100.00% in 3 h of exposure at the concentration of 10 mg/mL; and 61.67% in 1 h, 70.00% in 2 h, 89.29% after 3 h and 100.00% mortality after the exposure of 4 h at the concentration of 8 mg/mL. Similarly, it displayed 43.33% in 1 h, 60.00% in 2 h and 62.50% and 76.92% mortality after the exposure of 3 and 4 h respectively at the concentration of 4 mg/mL. While at 2 mg/mL concentration, it showed the maximum mortality (69.23%) after the exposure of 4 h. No significant mortality was observed for silver nitrate for the

(A)



(B)

Element	Weight (%)
CK	22.94
OK	31.11
AgL	45.95
Totals	100.00

Figure 4. EDX investigation of AgNPs.
A: EDX spectrum of AgNPs; B: Calculated percent elemental analyses.

exposure of 2 h at 10 mg/mL. While a very low mortality (7.69%) was observed even after the exposure of 4 h. Positive control DDVP gave 100.00% mortality at 10 mg/mL in 1 h and 100.00% mortality at 8 mg/mL after 2 h, and also 100.00% mortality after 3 h at 4 mg/mL. At 2 mg/mL, it showed 100.00% mortality after the exposure of 4 h (Table 1). The synthesized AgNPs exhibited improved activities as compared to *Manilkara zapota* leaves extract AgNPs that demonstrates that the AgNPs are excellent insecticidal agents.

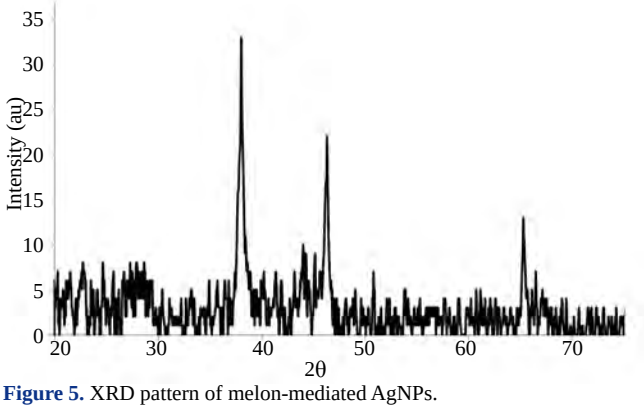


Figure 5. XRD pattern of melon-mediated AgNPs.

4. Discussion

Metal ions interact with biological entities which have the ability to reduce these to metallic nanoparticles. Plant-mediated synthesis of AgNPs has been the focus of interest owing to its eco-friendly nature. Upon mixing the melon extract and silver nitrate solution for a short time, the color of the mixture changed to dark brown from pale yellow due to SPR of formed AgNPs. The green synthesized AgNPs were characterized by several analytical methods.

Synthesis of AgNPs was confirmed by UV-visible spectral analysis; characteristic SPR absorption bands were observed due to the combined vibration of free electrons of metallic silver displaying yellowish brown color. The SPR bands got broadened due to size distribution of the particles with little aggregation. The excitations of electrons on nanoparticles surface in the conduction band are collectively called SPR and distinctive absorption spectra are shown by the metallic nanoparticles in the UV-visible region. Surface plasmon occurred at 430 nm and gradually an intensity increased with the time which confirmed the formation of nanoparticles[16]. AgNPs stability could be attributed to capping or stabilizing agents which contained proteins and other biomolecules presenting the extract into the reaction

Table 1
The insecticidal activity of synthesized AgNPs against *M. domestica*.

Test material	Concentration (mg/mL)	Number of the exposed		Number of the dead				1 h		2 h		3 h		4 h	
		Nunber of exposed/replica	Total exposed	1 h	2 h	3 h	4 h	M^a	M^c	M^a	M^c	M^a	M^c	M^a	M^c
Control	-	15	60	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 0.00	8.00 ± 0.00	0.00	0.00	0.00	0.00	8.00	8.00	14.00	14.00
Plant	40	15	60	6.00 ± 1.00	8.00 ± 0.81	15.00 ± 0.95	31.00 ± 0.96	10.00	10.00	13.33	13.33	25.00	19.64	52.00	44.23
aqueous	30	15	60	3.00 ± 0.50	5.00 ± 0.50	13.00 ± 0.96	25.00 ± 0.96	5.00	5.00	8.33	8.33	22.00	16.07	42.00	32.69
extract	10	15	60	2.00 ± 0.57	3.00 ± 0.50	11.00 ± 0.50	18.00 ± 0.57	3.33	3.33	5.00	5.00	18.00	12.50	30.00	19.23
	5	15	60	0.00 ± 0.00	2.00 ± 0.57	4.00 ± 0.81	13.00 ± 0.95	0.00	0.00	3.33	3.33	7.00	0.00	22.00	9.62
AgNPs	10	15	60	43.00 ± 0.96	55.00 ± 0.96	60.00 ± 0.00	60.00 ± 0.00	71.67	71.67	91.66	91.66	100.00	100.00	100.00	100.00
	8	15	60	37.00 ± 1.25	42.00 ± 0.57	54.00 ± 1.29	60.00 ± 0.00	61.67	61.67	70.00	70.00	90.00	89.29	100.00	100.00
	4	15	60	26.00 ± 0.57	36.00 ± 0.81	39.00 ± 0.95	48.00 ± 0.81	43.33	43.33	60.00	60.00	65.00	62.50	80.00	76.92
	2	15	60	12.00 ± 0.81	17.00 ± 0.96	35.00 ± 0.96	44.00 ± 0.81	20.00	20.00	28.33	28.33	58.33	55.36	73.33	69.23
$AgNO_3$	10	15	60	0.00 ± 0.00	4.00 ± 0.00	8.00 ± 0.95	12.00 ± 0.00	0.00	0.00	6.66	6.66	13.33	7.14	20.00	7.69
DDVP	10	15	60	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	8	15	60	49.00 ± 0.50	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00	81.66	81.66	100.00	100.00	100.00	100.00	100.00	100.00
	4	15	60	35.00 ± 0.50	51.00 ± 0.50	60.00 ± 0.00	60.00 ± 0.00	58.33	58.33	85.00	85.00	100.00	100.00	100.00	100.00
	2	15	60	18.00 ± 0.57	31.00 ± 0.50	46.00 ± 0.57	60.00 ± 0.00	30.00	30.00	52.00	52.00	77.00	75.00	100.00	100.00

M^a :Mortality (%); M^c : Corrected mortality (%).

medium[17].

FTIR spectrum proved the presence of secondary metabolites obtained from melon extract as capping agents in the characteristic range for polyfunctional hydrocarbons. FTIR spectroscopy is an important tool for the identification of the functional groups of biomolecules responsible for stabilization and reduction of nanoparticles. Hydroxyl group's characteristic broad band was observed from 3 320–3 275 cm^{-1} due to alcohols, phenolics and various carbonyl groups of the extract[18-20]. The bands at 2 950 cm^{-1} was assigned to stretching vibrations of groups containing methylene; while bands at 1 645 and 1 535 cm^{-1} were the characteristic bending vibrations of the amides I and II linkages found in proteins, respectively[21,22]. Peaks at 1 410 cm^{-1} and 1 260 cm^{-1} were assigned to stretching vibrations of C–N aromatic amines and C–O of carboxylic acids, respectively[23]. Peak at 1 040 cm^{-1} corresponded to the C–N aliphatic amines stretching vibrations. A strong peak was observed at 501 cm^{-1} corresponded to the stretching frequency of the interaction of metal with biomolecules on the surface of the nanoparticles[24]. The overall observation obtained from FTIR spectroscopic study confirmed that the amino acids residues of the proteins had stronger capability to bind with metal and acted as capping ligand of AgNPs and prevented them from aggregation. It is reported in the literature that the proteins presenting in biomass bind to metal nanoparticles through cysteine residues contain free amino functionality[25]. We conclude that the biological molecules (proteins, alcohols and phenolic compounds) could probably perform the function of both reduction and stabilization agents for AgNPs in aqueous solution.

The FE-SEM images of the nanoparticles showed that the AgNPs were polydispersed. It was confirmed from the image that the synthesized nanoparticles were capped by biomolecules. The formation of nanoclusters may be due to the presence of elevated concentrations of biologically active molecules in the colloidal solution. It has been reported that the shape of nanoparticles affected the optical and electronic properties of metallic nanoparticles considerably[26,27].

The crystalline nature of AgNPs was clearly shown from the EDX pattern, which was caused by the reduction of silver ions using melon extract. At 3 kv, a strong signal peak for silver was observed, which confirmed the formation of silver nanoparticles. Due to SPR metallic AgNPs usually show absorption peak approximately at 3 kv. The spectrum also showed weak signal for carbon and oxygen peak, which may have originated from the biomolecules capping the surface of AgNPs, a former study showed individual spherical shaped AgNPs in the range 2.5–4 kv[28].

In powder X ray diffraction patterns, a few unassigned peaks were observed which were marked with asterisk. These were due to the crystallization of biological moieties presenting in the melon extract which stabilized the AgNPs' surface[29,30]. Sharp powder X ray diffraction patterns proved the nano silver in typical range ($2\theta = 40^{\circ}$, 45° and 65°) and Scherrer formula was also successfully applied[31,32]. Characteristic peaks at 2θ values of 38.05, 46.35 and 65.35 corresponded to (111), (200) and (220) planes of silver metal, respectively. XRD pattern displayed characteristic Bragg peaks at (111), (200) and (220) corresponding to the face centered cubic AgNPs, which confirmed the crystalline nature of AgNPs[33]. The average size of the prepared AgNPs was approximately 20 nm which showed agreement with the measured values of FE-SEM.

The stability of AgNPs is attributable to the formation of silver electride which may form a thin layer on the aqueous surface of the reaction mixture and the secondary metabolites containing proteins *etc.* are believed to cap the AgNPs thereby preventing the agglomeration. It is also believed that polyphenols reduce Ag^{+} to Ag^{0} and this concept stems from the antioxidant action of phenolics. It has been proven in this paper that the green synthesis of AgNPs using fresh melon extract is an environmentally benign method and the biological reduction of silver would be very beneficial for the development of green methods.

In DTA data which peaked at 125 ˚C and 318.8 ˚C and 570 ˚C were due to the evaporation of physically adsorbed water molecules (125 ˚C), decomposition of the capping ligands that cover the surface of nanoparticles. The primary weight loss observed till 145 ˚C was attributed to the physically absorbed water molecules. Thereafter, a continuous weight loss occurring with two distinct peaks at 318.8 ˚C and 570 ˚C until 600 ˚C showed the removal of stabilizing agents that cover the fine surface of nanoparticles. From TG/DTA analysis, it was clear that the total weight loss of the analyzed AgNPs was about 34.93%, which showed that the metallic core (nanoparticles) was surrounded by biomolecules. Metallic silver residue was around 65.06%.

Several workers have demonstrated the larvicidal activity of plants extracts and plant-mediated AgNPs against *Anopheles subpictus* and *Culex quinuefasciatus* larvae. *M. domestica* has been the subject of interest using plant extracts, essential oils obtained from plants and AgNPs; while crude oil obtained from *Mentha piperita* and *Eucalyptus globulus* exhibited 100.00% mortality; coumarin compounds displayed effective insecticidal activity via topical application[34]. The present investigation proved the synthesized AgNPs to be effective against *M. domestica* at lower doses which established their candidacy as a promising alternative. During the current study, we observed that green synthesized AgNPs are a good future alternative for the control of *M. domestica*.

Finally, this is the first report on the fresh melon extract-mediated AgNPs synthesis and their feeding deterrent activity against *M. domestica*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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