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Research Article

Toxicity of Nanoparticles against *Drosophila melanogaster* (Diptera: Drosophilidae)

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In recent years, nanotechnology has become one of the most promising new approaches for pest control. In our screening program, laboratory trials were conducted to determine the effectiveness of five sources of silver nanoparticles (Ag NPs) and sulfur nanoparticles (S NPs) on larval, pupal, and adults of the fruit fly *Drosophila melanogaster*. Nanoparticles of silver and sulfur were synthesized through reducing, stabilizing, and capping plant leaf extracts method and different concentrations (10, 50, 100, 200 ppm) were tested on *D. melanogaster*. Results showed that silver nanoparticles (Ag NPs) were highly effective on larvae, pupae, and adults' mortality and egg deterrence. On the contrary, none of the tested nanoparticles has a significant effect on pupae longevity. The results also showed that silver nanoparticles can be used as a valuable tool in pest management programs of *D. melanogaster*.

1. Introduction

Pests are frequently mentioned as a major constraint to improved food production and higher agricultural productivity [1]. Insects, diseases, and weeds destroy more than 40% of the potential world food production [2]. It has been estimated that up to 15% of crops worldwide are lost because of insect damage alone [3]. In 2006 and 2007, the world used approximately 5.2 billion pounds of pesticides, with insecticides constituting the second biggest part of the world pesticide use at 17%. Moreover, for the global market of crop protection products, market analysts forecast revenues of over 52 billion US dollars in 2019 [4]. Furthermore, there were more than 1055 active ingredients registered as pesticides [5]. The World Health Organization and the UN Environment Programme estimate that each year 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18000 of whom die [6]. In addition, pesticide use reduces biodiversity, reduces nitrogen fixation [7, 8], contributes to pollinator decline [9], destroys habitat [10], and threatens endangered species [6]. Pests can develop a resistance to the pesticide, necessitating a new pesticide [11]. Alternatively a greater dose of the pesticide can be used to counteract the resistance, although this will cause a worsening of the ambient pollution problem. On average, overall effectiveness of pest control is only 33% and is the lowest for insect [12]. L. A. Lacey and C. M. Lacey [13] recommended using alternatives to pesticides including the use of metal nanoparticle (NPs) [14, 15]. This method is becoming increasingly popular and is often safer than traditional chemical pesticides [14, 15]. The advantage of using plants for the synthesis of nanoparticle is that they are easily available, safe to handle, and possess a broad variability of metabolites that may aid in reduction [16]. A number of plants are being currently investigated for their role in the use of nanoparticle. Gold nanoparticles with a size range of 2–20 nm have been synthesized using the live alfalfa plants [17]. Nanoparticles of silver, nickel, cobalt, zinc, and copper have also been synthesized inside the live plants of *Brassica juncea* L. (Indian mustard), *Medicago sativa* L. (Alfalfa), and

Helianthus annuus L. (Sunflower). Of the plants investigated, *Brassica juncea* had better metal accumulating ability compared to other plants [18]. Silver (Ag NPs) and sulfur (S NPs) nanoparticles have attracted researchers due to their potential properties and applications in the fields of biology, biotechnology, chemistry, agriculture, physics, and medicine [14]. Various approaches were developed for synthesis of these nanoparticles such as thermal decomposition, electrochemical, microwave assisted process, chemical reduction, sol-gel, hydrothermal synthesis, ultrasonic technique, microemulsion, and precipitation method [8, 18–24]. These methods have many disadvantages due to the difficulty of scaling up the process, hazardous chemicals, low material conversion, and high energy requirements. Therefore, there is a growing need to develop environmentally friendly methods for nanoparticles synthesis without using hazardous materials. Green synthesis of silver nanoparticles using plant extracts could be more advantageous than other methods. Recent researches reported using different plant leaf extracts for synthesizing silver nanoparticles such as Pine, Persimmon, Ginkgo, Magnolia and Platanus [25], *Coriandrum sativum* L. [26], *Nicotiana tobaccum* L. [27], *Ocimum sanctum* L. [24], *Stevia rebaudiana* Bert. [23], *Arbutus unedo* L. [28], *Ficus benghalensis* L. [29], *Ocimum tenuiflorum* L. [22], *Gloriosa superba* L. [30], *Syzygium aromaticum* L. [31], *Citrus limon* L. [21], *Annona squamosa* L. [32], *Ixora coccinea* L. [33], *Sesbania grandiflora* L. [34], Loquat [35], Mulberry [16], Carob [36], *Alternanthera dentata* Moench [19], and *Tephrosia purpurea* L. [20].

The fruit fly *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) is one of the most valuable organisms in biological research, particularly in genetics and developmental biology [37]. *D. melanogaster* has been used as a model organism for research for almost a century [38]; it is easy to handle, a small animal with a short life cycle, and cheap and easy to keep at large numbers [39].

The main objectives of this study were to design a simple, rapid, and environmentally synthesized methods of silver (Ag NPs) and sulfur (S NPs) nanoparticles at ambient temperature using reducing, stabilizing, and capping plant leaf extracts and to investigate the toxicity effect of those nanoparticles on different stages of *D. melanogaster*.

2. Materials and Methods

2.1. Preparation of the Silver and Sulfur Nanoparticles

2.1.1. Materials. The AR grade silver nitrate ($AgNO_3$), sodium thiosulfate ($Na_2S_2O_4{\cdot}5H_2O$), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich chemicals. Fresh olive (*Olea europaea*), fig (*Ficus carica*), loquat (*Eriobotrya japonica*), citrus (*Citrus limon*), pistachio (*Pistacia vera*), and mulberry (*Morus nigra*) plant leaves were collected locally from the campus of Royal Scientific Society, El Hassan Science City, Amman, Jordan. Deionized water was used for all solution preparations. Glassware was washed in dilute nitric acid (HNO_3), rinsed thoroughly with distilled water, and dried in hot air oven prior to use.

2.1.2. Preparation of Plant Leaves Extract. Fresh plant leaves of olive, fig, loquat, citrus, pistachio, and mulberry were washed several times with distilled water to remove the dust particles and then sun dried to remove the residual moisture. The leaf extracts used for synthesis of nanoparticles were prepared by placing 5 gram of the washed, dried, and fine cut leaves in 200 mL glass beaker along with 100 mL of sterile distilled water. The aqueous mixture was then boiled for 5–10 minutes until the color of aqueous solution changes to light yellow or brown. Then the aqueous extracts were cooled to room temperature and filtered with Whatman filter paper No. 1 filter paper before centrifugation at 1200 rpm for 5 minutes to remove the heavy biomaterials. The leaf extracts were stored at room temperature in order to be used for further experiments.

2.1.3. Synthesis of Silver Nanoparticles. In a typical reaction procedure, 5 mL of plant leaf extract was added to 100 mL of 1 mM of aqueous solution of silver nitrate ($AgNO_3$) with stirring magnetically at room temperature or heating at 20–80°C. The resulting solution became brown and then changed to grey-black in color, indicating the formation of silver nanoparticles (Ag NPs). The concentrations of silver nitrate solutions, the quantity of plant extract, and temperature were also varied at 1–4 mM, 5–10% by volume and at temperature 20–80°C. The UV-vis spectra showed strong surface plasmon resonance (SPR) band at 420–425 nm, thus indicating the formation of silver nanoparticles. The silver nanoparticles obtained by the plant leaf extracts were centrifuged at 15,000 rpm for 5 minutes and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

2.1.4. Synthesis of Sulfur Nanoparticles. In a typical reaction synthesis, sulfur nanoparticles (S NPs) synthesized as follows: an appropriate amount of sodium thiosulfate ($Na_2S_2O_3{\cdot}5H_2O$) was dissolved in 100 mL of sterile deionized water in a 250 mL beaker under mild stirring with magnetic stirrer at room temperature and atmospheric pressure. 10 mL of aqueous solution of citrus leaf extract acidified with dilute hydrochloric acid (HCl) was added to the aqueous solution of sodium thiosulfate with rate 1 mL/min with mild stirring for allowing the sulfur precipitations uniformly. The suspended sulfur particles obtained were separated by centrifugation at 1000 rpm/min for 5 minutes and then repeatedly washed with sterile distilled water to remove any biological materials. The sulfur nanoparticles are divided into two parts. In the first part, the sulfur nanoparticles remained in the sterile distilled water without any additives added. In the second part, the sulfur nanoparticles after purification were dried in a vacuum at 80°C for 2 h. The product was a light yellow powder used for SEM and FT-IR analysis.

2.1.5. Characterization of Synthesized Nanoparticles. UV-vis absorption spectra were measured using a Shimadzu UV-1601 spectrophotometer (Kyoto, Japan). Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Shimadzu, XRD-6000) equipped with Cu Kα radiation source using Ni as filter at a setting of 30 kV/30 mA. All

X-ray diffraction (XRD) data were collected under the experimental conditions in the angular range $3^\circ \leq 2\theta \leq 50^\circ$. Fourier transform infrared (FT-IR) spectra for leaves extracts powders and silver nanoparticles were obtained in the range from 4,000 to 400 cm^{-1} with an IR-Prestige-21Shimadzu FT-IR spectrophotometer by KBr pellet method. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a Hitachi S-4500 SEM machine (Chiyoda-ku, Japan). Concentration of silver ions was analyzed by Atomic Absorption Spectroscopy (AAS; AA-6300, Shimadzu).

2.2. Toxicity against Drosophila melanogaster

2.2.1. Rearing Drosophila melanogaster for Bioassay Tests. About 100 *D. melanogaster* adults were obtained from an available colony at the Biotechnology Laboratory at Al-Balqa' Applied University and were introduced into new culture bottles to breed. The contents of the *Drosophila* artificial diet used per one liter were 700 mL distilled water, 48 gm sucrose, 18 gm bacteriological agar, 18 gm yeast, 4 mL propionic acid, and 54 gm wheat cream.

2.2.2. Silver and Sulfur Nanoparticles Treatments for Insecticidal Activity. The silver nanoparticles of olive, fig, loquat, pistachio, and mulberry at concentrations of 10, 50, 100, and 200 ppm for each were bioassayed for toxicity against *Drosophila melanogaster*. The sulfur nanoparticle of citrus at the same concentrations of 10, 50, 100, and 200 ppm was also assayed.

(1) Silver and Sulfur NPs Toxicity against Larvae and Pupae. Volumes of 400 μL of each of the above mentioned nanoparticles were spread on the surface of the artificial diet in 3 cm in diameter Petri dishes. Each treatment was repeated three times. Also, diet was treated with distilled water in five repetitions for control treatment. Ten freshly hatched *Drosophila* larvae were transferred to each Petri dish containing the nanoparticle impregnated in the diet. The Petri dishes were incubated at 25°C, 60% relative humidity and 16: 8 lights to dark period. Larvae mortality was recorded after 72 hr. In addition, pupal mortality was recorded to those larvae that succeeded in pupation. Larval and pupal development periods were also checked every 6 hours and recorded. The larvae that succeeded in pupation were numbered on the outside surface of the Petri dish opposite to the pupation site with permanent marker in order to follow its development period. Moreover, abnormalities in morphology, color, and weight of the emerged flies were noticed, if present.

(2) Silver and Sulfur NPs Toxicity to Pupae. Groups of ten larvae prior to pupation were transferred to empty Petri dishes. Larvae were left to pupate inside the dishes. Volumes of 0.5 μL of each concentration of both silver and sulfur nanoparticles were applied to pupae using the droplet-imbibing assay. Each treatment was repeated 3 times and the control treatment was repeated 5 times. Pupal mortality was recorded ten days later in order to investigate the NPs toxicity during the early pupal stage without previous feeding of larvae on NPs.

(3) Silver and Sulfur NPs Toxicity against Adults and Number of Hatched Eggs. Groups of flies of five (3-days) old females were anesthetized using carbon dioxide and released inside Petri dishes containing media impregnated with the nanoparticles at the concentrations mentioned above. Each treatment was repeated three times and the water treatment was repeated five times as control. The flies were left without food for 12 hours before releasing them into the Petri dishes to enhance their feeding. Female mortality was recorded every day until the flies in the control treatment were dead. The number of hatched larvae from eggs laid by females in each treatment was counted to investigate the NPs egg-laying deterrence effect.

(4) Statistical Analysis. Hatched eggs, pupae, and adults' mortality were analyzed using GenStat 16 software followed by Repeated Measures Analysis of Variance and Least Significance Difference tests to compare effects among treatments. The results were expressed as means of untransformed data and considered significantly different at $P < 0.05$. Data from larvae and pupae development were analyzed using a one-way ANOVA and Least Significance Difference tests to compare effects among treatments.

3. Results and Discussion

3.1. UV-Vis Absorbance Studies. The addition of plant leaf extracts of olive, fig, loquat, pistachio, and mulberry to silver nitrate solution resulted in color change of the solution from transparent to dark yellow or dark brown due to the formation of silver nanoparticles. These color changes arise due to the excitation of surface plasmon vibrations of the silver nanoparticles. The Surface Plasmon Resonance (SPR) of silver nanoparticles produced peaks centered around 425–430 nm indicating the formation of silver nanoparticles.

3.2. XRD Analysis of Silver Nanoparticles (Ag NPs). The crystalline nature of Ag NPs synthesized by using olive, fig, loquat, pistachio, and mulberry leaf extracts was confirmed from the XRD patterns by the characterization peaks observed in the XRD image, Figure 1. XRD analysis showed four distinct diffraction peaks at θ values of 38.12°, 43.98°, 64.09°, and 77.58° which correspond to crystal facets of (111), (200), (220), and (311) of face-centered cubic silver. No extra diffraction peaks of other phases are detected indicating the phase purity of Ag NPs. The average crystallite size of the synthesized silver nanoparticles was calculated using Debye-Scherrer equation [35, 36]:

$$D = \frac{K\lambda}{\beta \cos\theta}, \quad (1)$$

where D is the crystallite size of silver nanoparticles, λ represents wavelength of X-ray source 0.1541 nm used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with value from 0.9 to 1, and θ is the Bragg angle. The average particles size of the synthesized silver nanoparticles by different plant leaf extracts and calculated by Scherrer equation are shown in

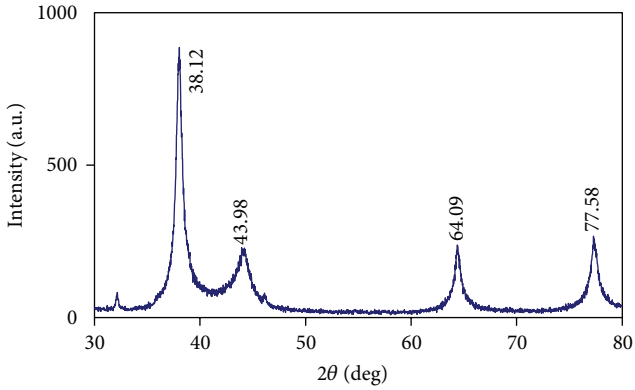

Figure 1: XRD of silver nanoparticles synthesized by loquat leaf extract.

Table 1. It was observed that loquat leaf extract gave the smallest nanoparticles.

3.3. XRD Analysis of Sulfur Nanoparticles (S NPs). The XRD analysis of sulfur nanoparticles synthesized is illustrated in Figure 2. The 2θ peaks at 15.4°, 23.08°, 25.8°, 27.76°, 31.45°, 37.02°, and 42.08°are attributed to the crystal planes of sulfur at 113, 222, 040, 313, 044, 422, and 319, respectively. The sulfur nanoparticles are well-crystalline and the position and the relative intensity of the diffraction peaks match well with the standard monoclinic phase sulfur diffraction pattern. The average particle sizes of the synthesized sulfur nanoparticles were calculated using Debye-Scherrer equation. It was found that the average size of sulfur nanoparticles calculated from XRD data is approximately 8 nm. The presence of structural peaks in XRD pattern illustrates that S NPs synthesized by this novel approach were nanocrystalline in nature. Scanning electron microscopy (SEM) analysis revealed that the sulfur nanoparticles were spherical in shape. The unassigned peaks in XRD pattern are thought to be related to crystalline and amorphous organic phases of citrus leaf extract.

3.4. Fourier Transform Infrared Spectroscopy Analysis (FT-IR)

3.4.1. FT-IR Analysis of Plants Leaves Extract. The FT-IR spectrum obtained for plant leaf extracts displays a number of absorption peaks reflecting its complex nature. The FT-IR data for olive, fig, loquat, pistachio, and mulberry leaf extract are shown in Figure 3. Strong absorption peaks at 3429 cm^{-1}–3336 cm^{-1} result from stretching of the –NH band of amino groups or are indicative of bonded –OH hydroxyl group. The absorption peaks at about 2920 cm^{-1} and 2850 cm^{-1} could be assigned to stretching vibrations of $-CH_2$ and CH_3 functional groups. The peaks at about 1647 cm^{-1}, 1755 cm^{-1}, and 1543 cm^{-1} indicate the fingerprint region of CO, C–O, and O–H groups. The intense band at about 1037 cm^{-1}–1022 cm^{-1} could be assigned to the C–N stretching vibrations of aliphatic amines. The FT-IR spectrum also shows bands at 1543 cm^{-1} and 1435 cm^{-1} identified as amide I and amide II which arise due to carbonyl (C=O) and amine (–NH) stretching vibrations in the amide linkages of the proteins, respectively. FT-IR indicates that

Table 1: Average particle size diameter of the synthesized silver nanoparticles using different plant leaf extracts.

Plant leaf extract	Average diameter size
Fig	8 nm
Mulberry	18 nm
Olive	10 nm
Pistachio	22 nm
Loquat	5 nm

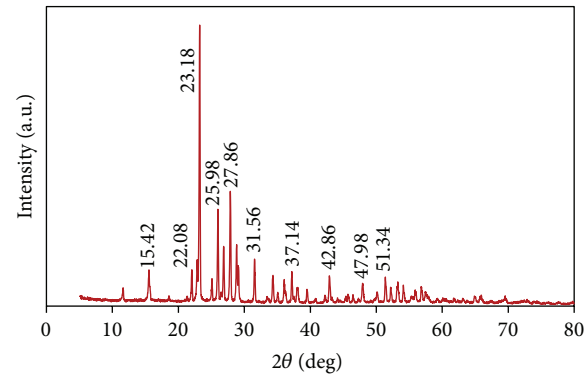

Figure 2: XRD pattern of synthesized sulfur nanoparticles using citrus leaf extract.

the carboxylic group (–C=O), hydroxyl (–OH), and amine (N–H) groups in olive, fig, loquat, pistachio, and mulberry leaf extract are mainly involved in reduction of Ag^+ ions to Ag^o nanoparticles. The FT-IR spectroscopic study also confirmed that the protein present in plant leaf extracts acts as a reducing agent and stabilizer for the silver nanoparticles and prevents agglomeration. The carbonyl group of amino acid residues has a strong binding ability with metal suggesting the formation of a layer covering silver nanoparticles and acting as a stabilizing agent to prevent agglomeration in the aqueous medium.

3.4.2. FT-IR Analysis of Synthesized Ag NPs. Figure 4 shows the FT-IR of silver nanoparticles synthesized using *E. japonica* leaf extract. The peaks at 3344 cm^{-1} and 1639 cm^{-1} in plant leaf extracts were shifted to 3417 cm^{-1} and 1616 cm^{-1} in the synthesized silver nanoparticles. The results indicate that the amine (–NH), hydroxyl (–OH), and carboxyl (–C=O) groups of plant leaf extracts are mainly involved in fabrication of silver nanoparticles.

3.4.3. FT-IR Spectrum Analysis of Citrus Leaf Extract. Citrus leaf extract was analyzed by FT-IR. The powder of citrus leaf extract displays a number of absorption peaks, reflecting its complex nature, Figure 3(f). The band at 3371 cm^{-1} could be ascribed to the stretching absorption band of amino (–NH) and hydroxyl (–OH) groups. The bands at 2920 cm^{-1} and 2850 cm^{-1} could be assigned to –CH stretching vibrations of $-CH_2$ and $-CH_3$ functional groups. The peak appears as a shoulder at 1701 cm^{-1} is due to carbonyl stretching vibration of the acid groups of fatty acids present in citrus leaf extract.

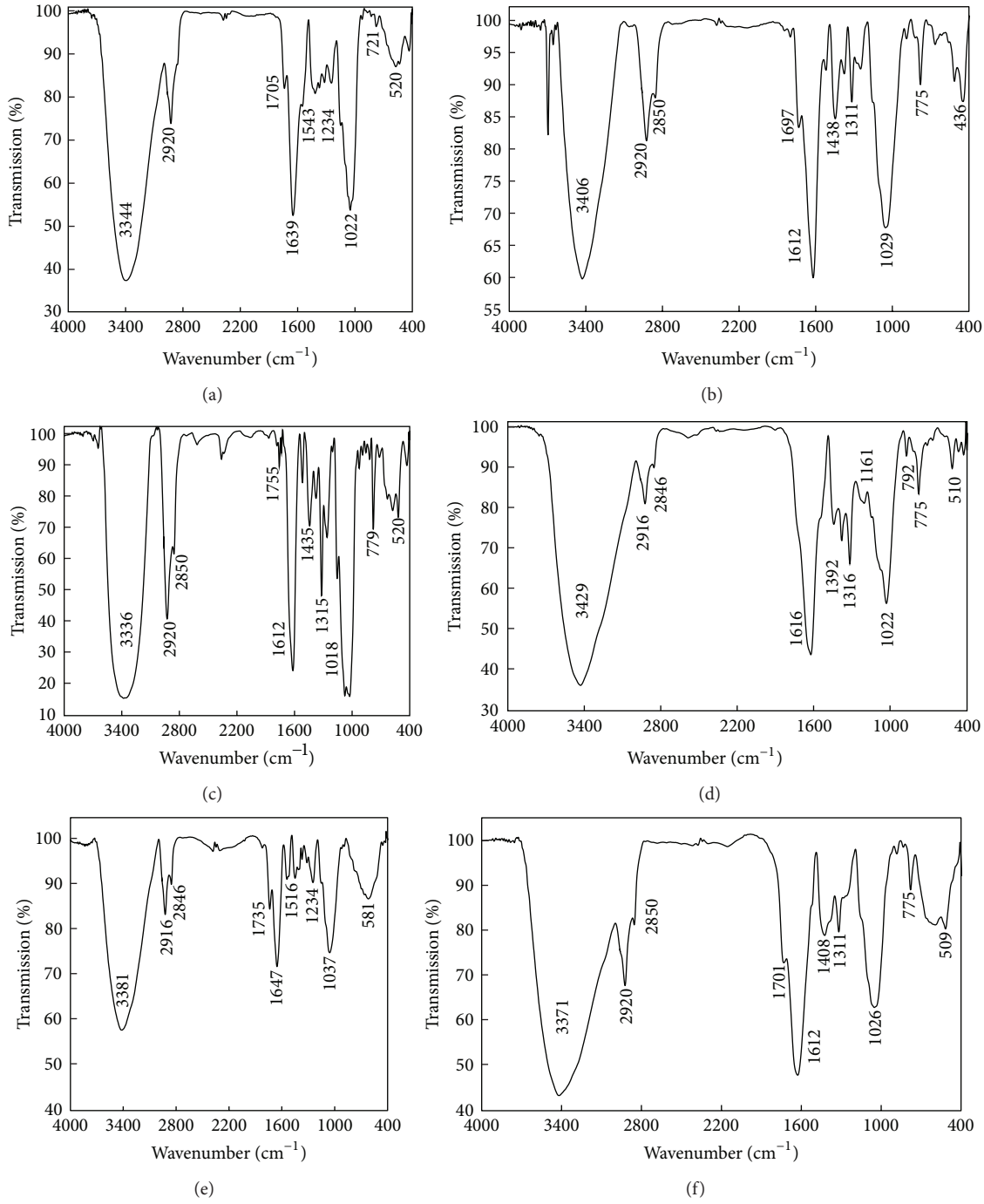

Figure 3: FT-IR spectra of plants leaves extract: (a) loquat, (b) fig, (c) olive, (d) pistachio, (e) mulberry, and (f) citrus.

The band at 1612 cm^{-1} is characteristic of amide I and amide II. The amide band I is assigned to the stretch mode of the carbonyl group coupled to the amide linkage while the amide II band arises as a result of the N–H stretching modes of vibration in the amide linkage. The band at 1408 cm^{-1} is assigned to the methylene scissoring vibrations from the proteins. The peak at 1311 cm^{-1} can be due to C–O vibrations of alcohols, phenols, and C–N vibrations of amides. The strong band at 1026 cm^{-1} assigned to the C–O stretching vibrations of alcohols. Additional peaks at 755 cm^{-1}, and 509 cm^{-1} can be assigned to bending modes of aromatic compounds.

3.4.4. FT-IR Spectrum Analysis of Sulfur Nanoparticles (S NPs). Figure 5 shows the FT-IR spectrum of the synthesized sulfur nanoparticles. It is observed that every sulfur character peak of S NPs is present in the FT-IR spectra of monoclinic sulfur and the shapes of the peaks are identical with those of

Table 2: Mortality of larvae of *Drosophila melanogaster* due to 72-hour feeding on different concentrations of the silver and sulfur nanoparticles of plant origin and subsequent mortality to pupae succeeded in development.

Nanoparticle	Plant	% Mean larval mortality Concentration (ppm)*				% Total mean larval mortality[1] per treatment	% Total mean pupal mortality[1] per treatment
		10	50	100	200		
Silver (Ag)	Fig	0.0a	13.3bc	13.3b	93.3b	30.0b	49.2d
	Loquat	0.0a	6.7ab	13.3b	20.0a	10.0a	46.7cd
	Mulberry	0.0a	20.0c	26.7c	100.0b	36.7b	40.8b
	Olive	43.3b	73.3d	93.3d	100.0b	77.5c	42.5b
	Pistachio	0.0a	0.0a	6.7ab	10.0a	4.2a	21.7abc
Sulfur (S)	Citrus	0.0a	0.0a	0.0a	0.0a	0.0a	19.2ab
Control (water)						1.7a	0.0a
LSD				11.77		20.0	26.55

*Values followed by the same letter within a row are not significantly different ($P < 0.05$).
[1]Values followed by the same letter within a column are not significantly different ($P < 0.05$).

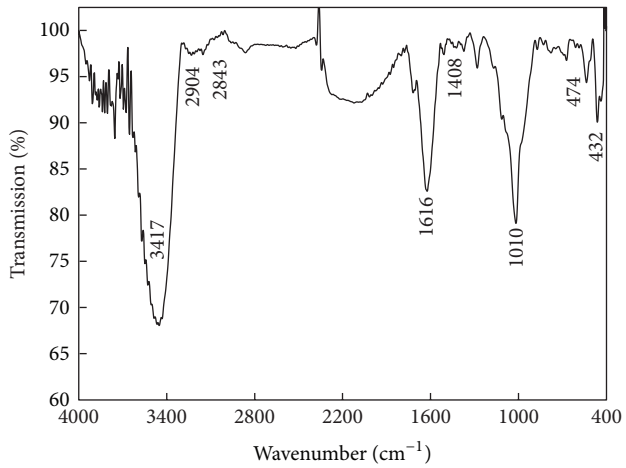

Figure 4: FT-IR of silver nanoparticles synthesized by loquat leaf extract.

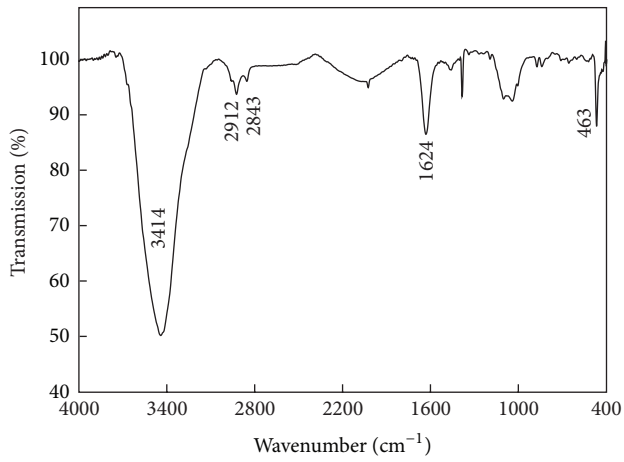

Figure 5: FT-IR of the synthesized sulfur nanoparticles using citrus leaf extract.

the monoclinic sulfur. The FT-IR spectra of S NPs indicate a new chemistry linkage on the surface of sulfur nanoparticles. This suggests that citrus leaf extract can bind to sulfur nanoparticles through free amine groups in the protein of citrus leaf extract, therefore acting as stabilizer and dispersing agent for synthesized sulfur nanoparticles.

3.5. Toxicity against D. melanogaster

3.5.1. Silver and Sulfur NPs Toxicity against Larvae and Pupae. Olive, fig, and mulberry Ag NPs have the strongest effect on larvae mortality ($F_{6,71} = 14.81$, $P < 0.001$), whereas loquat and pistachio Ag NPs and citrus S NPs have no significant effect (Table 2). As the concentration of the nanoparticles increased, the mortality of the larvae increased ($F_{3,48} = 137.84$, $P < 0.001$) (Table 5b); olive appeared to be the strongest mortality factor in all tested concentrations ($F_{15,48} = 24.74$, $P < 0.001$) (Table 2).

Olive, fig, and mulberry and loquat Ag NPs have a significant effect on pupae mortality($F_{6,71} = 2.74$, $P = 0.019$),

Table 3: Development period of larvae of *Drosophila melanogaster* at different concentrations of the silver and sulfur nanoparticles of plant origin and subsequent development of pupae succeeded in pupation.

Nanoparticle	Plant	Mean larval development period (days)	Mean pupal development period (days)
Silver (Ag)	Fig	4.7c*	5.3a*
	Loquat	4.4b	5.3a
	Mulberry	4.7c	5.2a
	Olive	4.9c	4.8a
	Pistachio	4.3b	5.2a
Sulfur (S)	Citrus	4.2a	5.2a
Control (water)		4.0a	5.0a
LSD		0.25	0.56

*Values followed by the same letter within a column are not significantly different ($P < 0.05$).

Table 4: Contact toxicity of the silver and sulfur nanoparticles of plant origin at different concentrations to *Drosophila melanogaster* pupae during its early pupation without previous experience with NPs.

Nanoparticle	Plant	% Mean pupal mortality Concentration (ppm)*				% Total mean pupal mortality[1] per treatment
		10	50	100	200	
Silver (Ag)	Fig	0.0a	46.7d	50.0c	53.3d	37.5c
	Loquat	3.3a	20.0b	30.0b	43.3bc	24.2b
	Mulberry	10.0b	33.3c	46.7c	46.7cd	34.2bc
	Olive	23.3c	40.0cd	53.3c	63.3e	45.0c
	Pistachio	0.0a	0.0a	3.3a	36.7b	10.0a
Sulfur (S)	Citrus	0.0a	0.0a	3.3a	10.0a	3.3a
Control (water)						0.0a
LSD				9.67		12.56

*Values followed by the same letter within a row are not significantly different ($P < 0.05$).
[1]Values followed by the same letter within a column are not significantly different ($P < 0.05$).

Table 5: *Drosophila melanogaster* flies oral mortality to silver and sulfur nanoparticles at different concentrations after 7 days of exposure.

Nanoparticle	Plant	% Mean adult mortality Concentration (ppm)*				% Total mean adult mortality[1] per treatment
		10	50	100	200	
Silver (Ag)	Fig	0.0a	0.0a	0.0a	3.3a	0.8a
	Loquat	0.0a	0.0a	26.7b	53.3c	20.0b
	Mulberry	0.0a	0.0a	0.0a	0.0a	0.0a
	Olive	0.0a	33.3b	60.0c	73.3d	41.7c
	Pistachio	0.0a	0.0a	0.0a	20.0b	5.0a
Sulfur (S)	Citrus	0.0a	0.0a	0.0a	0.0a	0.0a
Control (water)						0.0a
LSD				27.60		12.21

*Values followed by the same letter within a row are not significantly different ($P < 0.05$).
[1]Values followed by the same letter within a column are not significantly different ($P < 0.05$).

whereas pistachio silver and citrus S NPs have no significant effect (Table 2).

Olive, fig, and mulberry and loquat Ag NPs have decreased larval longevity significantly ($F_{6,61} = 7.82$, $P < 0.001$) except citrus S NPs and pistachio Ag NPs compared with control (Table 3). On the other hand, none of the tested nanoparticles have a significant effect on pupae longevity ($F_{6,61} = 0.50$, $P = 0.80$) (Table 3).

3.5.2. Silver and Sulfur NPs Toxicity to Pupae. All tested nanoparticles have increased pupae mortality significantly ($F_{6,77} = 15.91$, $P < 0.001$) except citrus S NPs and pistachio Ag NPs compared with control, olive, and fig Ag NPs which have the strongest effect followed by mulberry and loquat Ag NPs ($F_{15,48} = 8.18$, $P < 0.001$) (Table 4). As the concentration of the nanoparticles increased, the mortality increased linearly ($F_{3,48} = 119.51$, $P < 0.001$) (Table 4).

3.5.3. Silver and Sulfur NPs Toxicity against Adults and Number of Hatched Eggs. All tested nanoparticle have no significant effect on adults' mortality except olive and loquat Ag NPs compared with control ($F_{6,77} = 13.40$, $P < 0.001$) (Table 5). As the concentration of the nanoparticles increased, the mortality increased linearly ($F_{3,48} = 91.04$, $P < 0.001$) (Table 5). Olive Ag NPs have significant effect on adult mortality even with lower concentrations (50 ppm) while loquat Ag NPs began to be effective at (100 pm) and pistachio Ag NPs at 200 ppm ($F_{15,48} = 8.18$, $P < 0.001$) (Table 5).

All tested nanoparticles have reduced the hatched larvae number significantly ($F_{6,71} = 131.24$, $P < 0.001$) and fig Ag NPs has the strongest effect followed by olive, mulberry, and loquat Ag NPs and then citrus Ag NPs. The minimum effect was for pistachio Ag NPs (Table 6). As the concentration of the nanoparticles increased the number of hatched larvae decreased ($F_{3,48} = 8.49$, $P < 0.001$) (Table 6). Fig Ag NPs appeared to be the strongest inhibitor of hatching in all tested concentrations ($F_{15,48} = 1.47$, $P = 0.16$) (Table 6).

4. Conclusions

Silver nanoparticles (Ag NPs) were successfully obtained from bioreduction of silver nitrate solutions using fresh olive, fig, loquat, and mulberry leaf extracts, while sulfur

Table 6: Egg-laying deterrent of the silver and sulfur nanoparticles of plant origin at different concentrations to *Drosophila melanogaster*.

Nanoparticle	Plant	Mean number of larvae Concentration (ppm)*				Total mean number of larvae[1] per treatment
		10	50	100	200	
Silver (Ag)	Fig	3.7a	3.7a	2.3a	0.3a	2.5a
	Loquat	12.3c	12.7c	12.3c	10.3c	11.9d
	Mulberry	9.0b	9.0b	7.7b	10.0c	8.9c
	Olive	8.3b	8.0b	7.0b	4.7b	7.0b
	Pistachio	17.3d	16.7d	15.3d	16.0d	16.3e
Sulfur (S)	Citrus	13.7c	13.0c	12.0c	11.7c	12.6d
Control (water)						20.2f
LSD				2.13		1.30

*Values followed by the same letter within a row are not significantly different ($P < 0.05$).
[1]Values followed by the same letter within a column are not significantly different ($P < 0.05$).

nanoparticles (S NPs) were successfully obtained from bioreduction of sodium thiosulfate using citrus leaf extracts. Owing to varying properties of these six plant species, Ag NPs and S NPs obtained from them also varied in size, the smallest being yield using loquat leaf extracts. Ag NPs and S NPs have been appropriately characterized using UV-vis spectroscopy, XRD, FT-IR, and SEM analysis. Results indicated loquat leaf extract to be a better reducing agent in comparison to the rest of leaf extracts. FT-IR analysis revealed the efficient capping and stabilization properties of these Ag NPs and S NPs. All tested nanoparticles have reduced the *D. melanogaster* hatched larvae number significantly. Also, olive, mulberry, and fig Ag NPs have a significant effect on larvae, pupae, and adult mortality and they have decreased larval longevity significantly. Hence, due to their benign and stable nature and insecticidal property, these Ag NPs and S NPs may be well utilized in business and curative purposes. However, plant uptake and utilization of Ag NPs and S NPs require more detailed research on many issues like uptake potential of various species, translocation, and the activities of the Ag NPs and S NPs at the cellular and molecular levels.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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