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Original article

# Polyphenolic extracts from pomegranate and watermelon wastes as substrate to fabricate sustainable silver nanoparticles with larvicidal effect against *Spodoptera littoralis*

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## ABSTRACT

The agricultural wastes adversely affect the environment; however, they are rich in polyphenols; therefore, this study aimed to employ polyphenol-enriched waste extracts for silver nanoparticles synthesis, and study the larvicidal activity of silver nanoparticles fabricated by pomegranate and watermelon peels extracts (PPAgNPs and WPAgNPs) against all larval instars of *Spodoptera littoralis*. The polyphenol profile of pomegranate and watermelon peel extracts (PP and WP) and silver nanoparticles was detected by HPLC. The antioxidant activity was estimated by DPPH, and FARP assays and the antimicrobial activity was evaluated by disc assay. The Larvicidal activity of AgNPs against Egyptian leaf worm was performed by dipping technique. The obtained AgNPs were spherical with size ranged 15–85 nm and capped with proteins and polyphenols. The phenolic compounds in silver nanoparticles increased about extracts; therefore, they have the best performance in antioxidant/reducing activity, and inhibit the growth of tested bacteria and yeast. The PPAgNPs were the most effective against the first instar larvae instar (LC<sub>50</sub> = 68.32 µg/ml), followed by pomegranate extract with (LC<sub>50</sub> = 2852 µg/ml). The results indicated that obvious increase in polyphenols content in silver nanoparticles enhance their larvicidal effect and increasing mortality of 1<sup>st</sup> larval of *S. littoralis* Egyptian leafworms causing additive effect and synergism. We recommend recycling phenolic enriched agricultural wastes in producing green silver nanoparticles to control cotton leafworm that causes economic losses to crops.

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## 1. Introduction

African cotton leafworm (*Spodoptera littoralis*) is a lepidopteran insect. It has economic importance because the wide range of host plants of different families besides feeding on the plant with a high

population can cause leaves skeletonization, losing crops, consumed shoot system, and causing economic losses. Chemical controlled caused development resistance to several groups of insecticides and adverse effects on the beneficial animals and the environment (Bakr and Al Yousef, 2015). Therefore, the researchers try to find new promising approach to suppress the population of *S. littoralis* by using agricultural wastes in fabricating nanoparticles. The peels of fruit and vegetables have considerable polyphenols content (Saad et al., 2021). Pomegranate and watermelon peels contain extensive amounts of phenolic compounds (Jung, 2015; Rolim et al., 2018; Yuan and Fang, 2018). The polyphenols are secondary metabolites that act as first defense line to protect plants from various stress conditions and bacterial and fungal infections (Dahham et al., 2010; Devi et al., 2011). Besides, they may act as

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potential insecticides (Ateyyat et al., 2012; Ghaly et al., 2014). Flavonoids are known to modulate the feeding and egg laying behavior of insects (Goławska and Łukasik, 2012). Therefore, the extracts of these peels contain significant content of phenolic compounds, which can act as reducing and protective agents for nanoparticles preparation. Synthesis of nanoparticles using physical and chemical methods is costly, has adverse impacts on the environment and needs high energy (El-Saadony et al., 2018, El-Saadony et al., 2019; El-Saadony et al., 2020). On the other hand, biosynthesis of nanoparticles is considered a clean and an economic alternative route when fabricated using bacteria (Reda et al., 2020; Sheiha et al., 2020; El-Saadony et al., 2021a; Reda et al., 2021), fungi (El-Saadony et al., 2021b) and plants is cheaper and favors large-scale production, as it requires simpler downstream processing. The application of botanicals for the synthesis of nanostructured materials offers numerous benefits than the use of safe chemicals in the synthesis (Borase et al., 2014). Moreover, these green synthesized nanoparticles are reported to be more effective insecticides (El-Saadony et al., 2020), less expensive, biodegradable, and safe for humankind and the environment than synthetic counterparts (Sabbour et al., 2015; Murugan et al., 2016). In search of environmentally friendly and effective pesticides, nanoparticles could be a new and good strategy. Nanoparticles are a particle with a size range between 1 and 100 nm, exhibit completely new or improved properties as compared to the original materials that differ in specific characteristics such as size, distribution, and morphology (El-Saadony et al., 2021a; El-Saadony et al., 2021c). Saranya et al. (2020) stated that nanosized particles with a wide range of use in insecticides with different mode of actions (Benelli, 2018). Newly transformed nanoparticles acquired a new property e.g. large specific surface area, thermal stability, biodegradability, and high affinity with the target site, hence; penetrate rapidly enhancing the biocidal activities (Benelli, 2018). Silver nanoparticles (AgNPs) have been used in many medical, pharmaceutical and agricultural applications as a pure free metal or a compound forms because it possesses broad-spectrum antioxidant and antimicrobial activities, and they are non-toxic to humans (Tian et al., 2018; Jacob et al., 2019). Recently, nanomaterials received great attention showed antimicrobial and biocidal activities (Bhattacharyya et al., 2010; Rai and Ingle, 2012; Bodaiah et al., 2016). The literature survey confirms scarce studies focusing watermelon and pomegranate peels phenolic compounds to use in fabrication of silver nanoparticles and the usage of these nanoparticles as insecticidal. Due to the vast potentiality of plants, the study aimed to employ phenolic compounds isolated from watermelon and pomegranate peels aquas extracts for silver nanoparticles synthesis. Estimate the antioxidant, antimicrobial of polyphenol extracts and silver nanoparticles. Besides the larvicidal activity of watermelon and pomegranate extracts, and silver nanoparticles were tested against all larval instars of *S. littoralis*. In addition, the study assessed the effects of these materials on the antifeedant of the 6<sup>th</sup> larval instar.

## 2. Materials and methods

The peels of pomegranate (*Punica granatum* L.) and watermelon (*Citrullus lanatus*) were obtained from a juice factory in 10<sup>th</sup> Ramadan City, Sharkia, Egypt. All chemicals and cultures used in this study were of analytical grade. Egyptian cotton leafworm rearing: A laboratory colony of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), (Lepidoptera: Noctuidae) was obtained from Plant Protection Institute, Agriculture Research Center, Zagazig, Sharkia Government, Egypt. The colony was reared according to Eldefrawi et al. (1964). The resulting larvae were fed on castor leaves.

### 2.1. Extraction of phenolic compounds from pomegranate and watermelon aquas extract

The extraction method were followed Saad et al., 2020. The pomegranate and watermelon peels were washed, dried in an oven at 105 °C for 3 h, and ground in a mixer, then sieved through 20-mesh screen. Ten grams of powdered peels were homogenized in 150 ml of distilled water and stirred for 4 h at 40 °C in a conical flask. The liquid extract was vacuum filtrated through Whatman No. 1 filter paper. The filtrates evaporated by a rotary evaporator then lyophilized and weighted to analyze the total extract yield (%) as following equation (1)

$$\text{Total extract yield (\%)} = \frac{\text{weight of dried extract}}{100\text{g of pp or wp}} \times 100 \quad (1)$$

where (pp) is pomegranate peel powder, and (wp) is watermelon peel powder

### 2.2. Green synthesis of silver nanoparticles

100 mg of phenolic extracts of pomegranate and watermelon peels was homogenized in 100 ml of silver nitrate solution (1 mM), and stirred overnight at room temperature. Then, silver nanoparticle solutions were centrifuged at 13,000 rpm for 15 min (Sigma 3–30 k, Germany). The supernatant was discarded and the silver nanoparticles pellets were rinsed several times with deionized distilled water, and then centrifuged again and the best samples were selected based on their appearance properties, such as intensity of darkness and applied for further experiments.

### 2.3. Characterization of green silver nanoparticles

Five advanced instruments were characterized the pomegranate silver nanoparticles (PPAgNPs) and watermelon silver nanoparticles (WPAgNPs). UV–VIS spectroscopy analysis to estimate the optical property of PPAgNPs and WPAgNPs mixture by Laxco™ dual-beam spectrophotometer in the range of 200–700 nm. The size and shape of silver nanoparticles were estimated using JEOL 1010 TEM (Japan). FTIR analysis was used to recognize the various groups of active compounds in pomegranate and watermelon peels extracts involved in the biotransformation and stabilization of AgNPs. The obtained PPAgNPs and WPAgNPs were analyzed by FT-IR spectroscopy (“Bruker Tensor 37, Kaller”, Germany) in the range of 4000 cm<sup>-1</sup> to 400 cm<sup>-1</sup> using KBr pellets. DLS analysis were carried out by using Nano “Z2 Malven, Malvern Hills, UK. The size was estimated based on the Brownian motion of the PPAgNPs and WPAgNPs in suspension. The zeta potential analysis was carried out to determine the surface charge of PPAgNPs and WPAgNPs, that ensure the stability of synthesized nanoparticles.

### 2.4. Total phenolic compounds in PPAgNPs, WPAgNPs and their substrate

The total polyphenols (TP) was evaluated according to Chen et al. (2015) with some modifications. In brief, 50 µL of each solution at (500 µg/mL) was mixed with 50 µL of diluted Folin-Ciocalteu reagent and 50 µL Na<sub>2</sub>CO<sub>3</sub> (7.5%) in microtiter plate wells, and incubated at room temperature for 60 min, then the absorbance was measured at 760 nm using microtiter plate reader (Bio-Tek Elx808, USA). The total polyphenols content was presented as mg gallic acid equivalent/mL of solutions.

Total flavonoids content (TF) was estimated as per Chen et al. (2017) with some modifications. 20 µL of each solution at (500 µg/mL) was mixed with 20 µL of sodium nitrite (5%) in micro-

titer plate wells and 20  $\mu\text{L}$  of ethanolic  $\text{AlCl}_3$  (10%) was added. The plate was incubated at room temperature for 10 min. 50  $\mu\text{L}$  of NaOH was added to stop the reaction. the absorbance was measured at 450 nm. The TF was expressed as mg quercetin equivalent per mL of each solution.

## 2.5. Phenolic compounds profile in PP, PW, PPAgNPs, and WPAgNPs

Phenolic profile was assessed by HPLC Shimadzu series (Shimadzu-prominence-20A, Japan) with separation column (Gemini, C18,  $4.6 \times 150 \text{ mm} \times 5 \mu\text{m}$ ) with 2 ml/min flow rate, and the mobile phase was 0.01% acetic acid in water (A) acetonitrile (B). The HPLC pumps, autosampler, column, oven, and diode array system were monitored and controlled, and the chromatographic data were collected using Class VP software (Shimadzu 5.0). The phenolics of PP, PW, PPAgNPs, and WPAgNPs were performed by HPLC at 280 nm (Goupy et al., 1999; Hassanin et al., 2020). Flavonoid compounds in samples were estimated at 370 nm (Mattila et al., 2000).

## 2.6. Biological activity of PPAgNPs, WPAgNPs and their substrate

### 2.6.1. Antioxidant activity

**2.6.1.1. DPPH radical scavenging assay.** The radical scavenging activity of PP, WP, PPAgNPs and WPAgNPs at concentration of (500  $\mu\text{g}/\text{mL}$ ) was estimated according to Bhakya et al. (2016) with some modifications. 100  $\mu\text{L}$  of each solution was mixed with 1  $\mu\text{L}$  of ethanolic DPPH was placed in the wells of microtiter plate and incubated at the room temperature in the dark for 30 min. the absorbance was measured at 517 nm using microtiter plate reader (Bio-Tek Elx808, USA) then applied in the equation (2)

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Abs.control} - \text{Abs.sample})}{(\text{Abs.control})} \times 100 \quad (2)$$

**2.6.1.2. Ferric reducing/antioxidant power assay.** The antioxidant activity of PP, WP, PPAgNPs, and WPAgNPs was measured by  $\text{Fe}^{+3}$  reduction ability to  $\text{Fe}^{+2}$  according to Benzie and Strain (1996) with some modifications. 100  $\mu\text{L}$  of PP, WP, PPAgNPs and WPAgNPs (500  $\mu\text{g}/\text{mL}$ ) was mixed with 100  $\mu\text{L}$  of the fresh prepared FRAP reagent (25 ml phosphate buffer, 2.5 ml PFC solution, and 2.5 ml  $\text{FeCl}_3$  solution) in microtiter plate. The plate was incubated in the dark at 37 °C for 30 min. Then absorbance of blue colored complex was determined at 595 nm against distilled water blank using microtiter plate reader (BioTek Elx808, USA).  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was used for calibration. Ascorbic acid was used as positive control. Results were expressed as mM  $\text{Fe}^{2+}$ / mg sample.

### 2.6.2. Antimicrobial activity

Antimicrobial activity of PP, WP, PPAgNPs and WPAgNPs (500  $\mu\text{g}/\text{mL}$ ) were carried out against different pathogenic bacteria; [Gram-positive bacteria (G+) (*Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*), Gram-negative bacteria (G-) (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), and yeasts (*Candida gelberta*, *Candida albicans*, and *Candida apis*). The bacterial cultures were grown over night in Muller Hinton broth (MHB) under shaking incubator at 37°C until ( $1 \times 10^8$  CFU/mL) concentration. The antimicrobial activity of PP, WP, PPAgNPs and WPAgNPs was performed by disc diffusion method (Akl et al., 2020; Ashour et al., 2020). The Muller Hinton plates were inoculated with 100  $\mu\text{L}$  of activated bacterial and *Candida* isolates by the spread plate method. Previously saturated paper discs (5 mm) with PP, WP, PPAgNPs and WPAgNPs (500  $\mu\text{g}/\text{mL}$ ) were placed on the surface of MHA plates. The plates were incubated

at 37 °C for 24 h. The inhibition zones around the discs were recorded by ruler (mm). The positive control was Levofloxacin antibiotic.

## 2.7. Larvicidal activity of plant extracts, silver nitrate and their combinations.

The 1<sup>st</sup> larval instar of Egyptian leaf worm was fed for 24 h on castor bean leaves treated with leaf dipping technique in solutions of PP and WP (1000, 2000 and 4000  $\mu\text{g}/\text{mL}$ ), PPAgNPs and WPAgNPs (8.5, 17 and 34  $\mu\text{g}/\text{mL}$ ) after air drying, whereas control treatments fed on castor bean leaves immersed in water. Each treatment was replicated three times (20 larvae/replicate). After 24 h exposure, the survival larvae were transferred to clean containers, fed on untreated leaves, and kept under observation till the end of each larval stage under constant conditions. The larval mortality was recorded and corrected according to Abbott (1925). The second experimental phase included the treatment of 1<sup>st</sup> larval instars with combinations of silver nitrate and plant extracts. The PPAgNPs or WPAgNPs (8.5  $\mu\text{g}/\text{mL}$ ) + 1000  $\mu\text{g}/\text{mL}$  (pomegranate and watermelon extract), PPAgNPs or WPAgNPs (17  $\mu\text{g}/\text{mL}$ ) + 2000  $\mu\text{g}/\text{mL}$  (pomegranate and watermelon extract) and PPAgNPs and WPAgNPs (34  $\mu\text{g}/\text{mL}$ ) + 4000  $\mu\text{g}/\text{mL}$  (pomegranate and Watermelon extract) at 1:1 mixing ratio were freshly prepared. The larval mortality values were determined as mentioned before. The joint action was determined according to the equation of the co-toxicity factor given by Mansour et al. (1966) as follows in equation (3):

Co – toxicity factor

$$= \frac{\text{Observed effect (\%)} - \text{Expected effect (\%)}}{\text{Expected effect (\%)}} \times 100 \quad (3)$$

A positive factor of 20 or more is considered synergism, a factor of –20 or less means antagonism, and a value between –20 and + 20 is an additive effect expected.

Benz equation (Benz, 1971) was used to calculate the expected mortality in the combined treatment using the observed mortality in the separate treatments and then compared to the observed percentage mortality in the combined treatment.

$$\text{Expected mortality} = \text{WGA} + \text{WGB} - \text{WGA}/10 \times \text{WGB}/10 \quad (4)$$

WG = Corrected mortality and A&B = Treatments.

## 2.8. Statistical analysis

Regression toxicity lines were established for the pesticides and the slope,  $\text{LC}_{25}$ , and  $\text{LC}_{50}$  values were determined through probit analysis (Finney, 1952). Data statistical analysis was performed using the Biostat 2009 software [version 5.8.4.3, 2010]. Data of the biological studies were analyzed using (ANOVA) test and the means were analyzed using LSD test at ( $p \leq 0.05$ ) (Steel and Torrie, 1960).

## 3. Results

### 3.1. Green synthesis of PPAgNPs and WPAgNPs from phenolic extracts

Pomegranate and watermelon peels extracts were pale yellow or green but with the addition of silver nitrate solution, their color converted to dark brown within 24 h. The establishment of dark brown color after mixing the extracts with silver ions was a clear indicator of the metal ions reduction and the formation of silver nanoparticles in the mixture. The addition of phenolic extracts to silver nitrate solution at a ratio of (1:100 v,v) achieve the best yield of nanoparticles. The transformation of silver nanoparticles

without any precipitations indicate that synthesized nanoparticles have a very small particle size. During our experiments no precipitations was observed in nanoparticle solutions of pomegranate and watermelon peels extracts.

### 3.2. Characterization of PPAgNPs and WPAgNPs

To confirm the formation and proper stability of silver nanoparticles, their absorption spectrum was read after 24 h in the range of 200–700 nm using a Laxco™ dual-beam spectrophotometer. Results of UV–Vis and TEM analyses presented in (Fig. 1) showed that the aqueous pomegranate and watermelon peels were produced different concentrations of silver nanoparticles. The highest absorbance at 420 nm was 1.5 a.u (absorbance unit) and recorded for PPAgNPs (Fig. 1A), which were synthesized using pomegranate peels extract, absorptions of 1.2 a.u observed at 430 nm for WPAgNPs synthesized by watermelon peels extract (Fig. 1C).

Fig. 1 (B, D) showed the morphological features of PPAgNPs and WPAgNPs, they were investigated via transmission electron microscope. The size of PPAgNPs was ranged from 15 to 70 nm; however, WPAgNPs size was in the range of 20–85 nm. The both silver nanoparticles were spherical. The difference in size mainly depended on the phenolic compounds content in extracts that employed to reduce silver ions, silver nitrate concentration, and temperature conditions.

FTIR spectrum of PPAgNPs and WPAgNPs was shown in Fig. 2 (A,B). The spectrum revealed that, the tested AgNPs were capped with reducing polyphenols in pomegranate and watermelon peels extracts. Eight bands (3436, 2918, 1646, 1542, 1179, 933, 744, and

419  $\text{cm}^{-1}$ ) were assigned to active groups in PPAgNPs suspension, however five bands (3436, 2918, 1646, 603, and 460  $\text{cm}^{-1}$ ) found in WPAgNPs FTIR spectrum. The active groups were NH of amide A overlapped with OH and CO of amide I at 3436, 1646  $\text{cm}^{-1}$ , respectively. In addition, aliphatic CH appeared at about 2918- $\text{cm}^{-1}$  Furthermore, the CCO appeared at 1179  $\text{cm}^{-1}$ . These data strongly revealed the presence of phenolic compounds and protein covering AgNPs which may play a role in the reduction and stabilization of biosynthesized AgNPs.

The DLS results of PPAgNPs were showed single peak in Fig. 3 (A, B). The average size was 61 nm with net negative charge of  $-25.46$  mV; however, Fig. 3 (C, D) showed the average size of WPAgNPs i.e., 75 and net negative charge  $-21.33$  Results indicated that PPAgNPs, which were synthesized using pomegranate peels extract, had smaller size than WPAgNPs.

### 3.3. Phenolic compounds content in PP, WP, PPAgNPs, and WPAgNPs

Table 1 showed the total phenolic and flavonoids content in pomegranate and watermelon peels extracts and silver nanoparticles. FTIR profile indicate the presense of phenolic compounds in nanoparticles suspension. Generally, the phenolic compounds significantly increased in silver nanoparticles about extracts where the total phenolic content in WPAgNPs and PPAgNPs increased with about 20% about PP and WP extract, and 15% increase in flavonoids content. The increase in polyphenols in nanoparticles because the quinoid compound produced due to the oxidation of the phenol group in phenolics can be adsorbed on the surface of nanoparticles increasing the phenolic content and accounting for

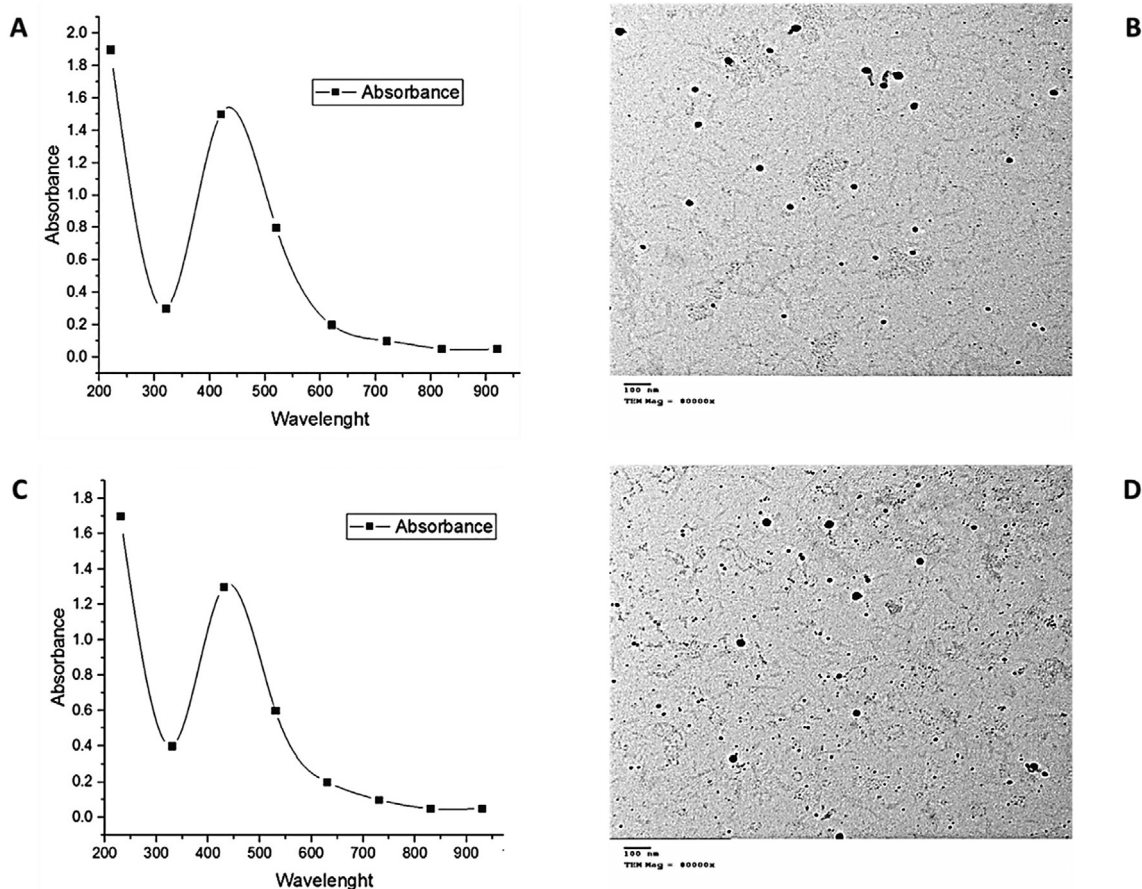
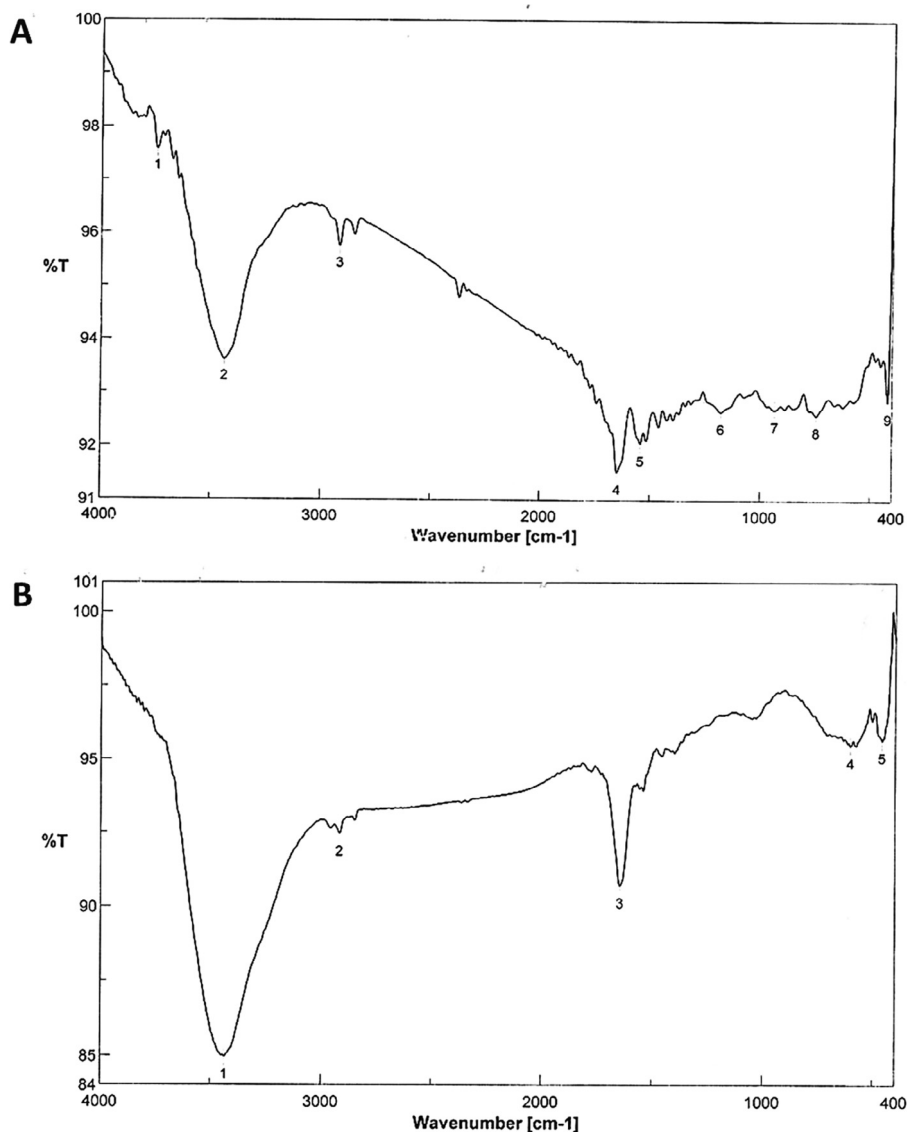


Fig. 1. (A, B) UV spectrum and TEM of silver nanoparticles synthesized by pomegranate peels extract; (C, D) UV spectrum and TEM of silver nanoparticles synthesized by watermelon peels extract.



**Fig. 2.** (A) FTIR spectrum of silver nanoparticles synthesized by pomegranate peels extract; (B) FTIR spectrum of silver nanoparticles synthesized by watermelon peels extract. Fourier Transform Infrared Spectroscopy (FTIR).

their suspension stabilization. On the other hand, Table 2 showed the phenolic profile in PP, WP extracts, PPAgNPs, and WPAgNPs. The results in Table 2 were correlated with results in Table 1 where the phenolic content in silver nanoparticles significantly increased with about 10–75% in PPAgNPs, and WPAgNPs as compared to PP and WP extract. Quercetin, Gallic acid, Catechin, Cyanidin-3-*o*-glu, Punicalagin, Ellagic acid were the highest phenolic compounds content in PP extract i.e. (1.10, 5.70, 3.60, 1.50, 2.40, and 0.90 mg/g), and i.e (1.45, 6.22, 4.10, 2.15, 2.98, and 1.74 mg/g) in PPAgNPs. However, Genistein, Ferulic acid, and Kampeferol in medium content and other phenolic compounds in lower content.

#### 3.4. Antioxidant activity of PP, WP, PPAgNPs, and WPAgNPs

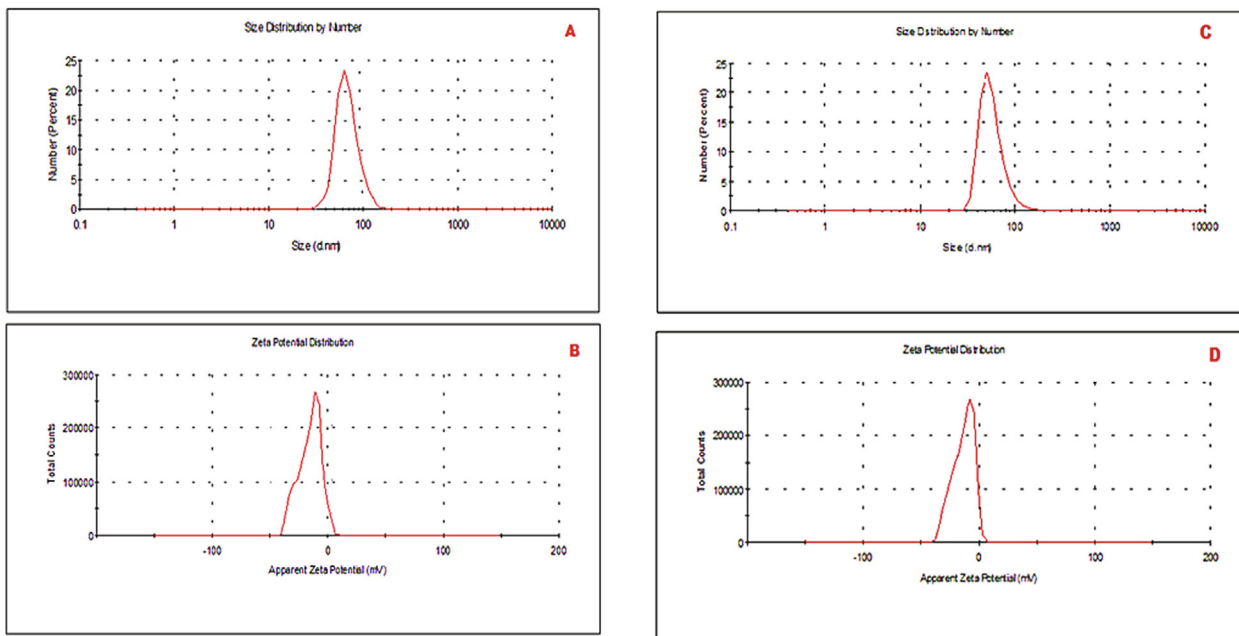
Fig. 4A showed that PP extract scavenged 65% of DPPH; however, WP extract scavenged 57% of DPPH radical. The scavenging activity of DPPH was significantly increased with 20% in PPAgNPs and WPAgNPs as compared to TBHQ. The same route in Fig. 4B where ferric reducing power increased in silver nanoparticles than extracts with about 25%.

#### 3.5. Antimicrobial activity of PP, WP, PPAgNPs, and WPAgNPs

Table 3 showed the antimicrobial activity of the polyphenolic extracts (PP and WP) and green silver nanoparticles (PPAgNPs and WPAgNPs). The polyphenolic extracts and silver nanoparticles at 500  $\mu$ g/mL significantly inhibited the bacterial and *Candida* growth with inhibition zones diameters in the range of (8–21 mm) as compared to levofloxacin 500  $\mu$ g/mL with a range of 18–28 mm. The most sensitive  $G^+$  and  $G^-$  bacteria were *S. aureus*, and *K. pneumoniae*; however, *L. monocytogenes*, and *P. aeruginosa* were the resistant ones to pomegranate and watermelon peels extracts and silver nanoparticles concentration. On the other hand, *C. gelbrta* was the most resistant yeast to polyphenolic extracts and silver nanoparticles. The PP extract has the best antimicrobial activity, consequently PPAgNPs because the highest phenolic content.

#### 3.6. Insecticidal application of polyphenolic extracts and green silver nanoparticles

Exposure of the 1<sup>st</sup> larval instar of *Spodoptera littoralis* to PP, WP, PPAgNPs and WPAgNPs showed in Table 4. The larvicidal activities



**Fig. 3.** DLS analysis of green AgNPs fabricated by PPWE, and WPWE; A: WPAgNPs size, C: PP AgNPs size; B: PPAgNPs zeta potential, D: WPAgNPs zeta potential; dynamic light scattering (DLS). Pomegranate peels water extract (PPWE); watermelon peels water extract (WPWE); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs).

**Table 1**  
Total phenolic and flavonoids in extracts and AgNPs (mean ± SD).

Sample	Total flavonoids mg/g	Total phenolic mg/g
PPWE	360 ± 0.9b	580 ± 1.2b
WPWE	286 ± 0.8d	350 ± 1.1d
PPAgNPs	420 ± 0.5a	701 ± 0.9a
WPAgNPs	325 ± 0.9c	415 ± 0.5c

Pomegranate peels water extract (PPWE); watermelon peels water extract (WPWE); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs). Data are means ± SD; Means with different lowercase letters in the same column are significantly different ( $p \leq 0.05$ ).

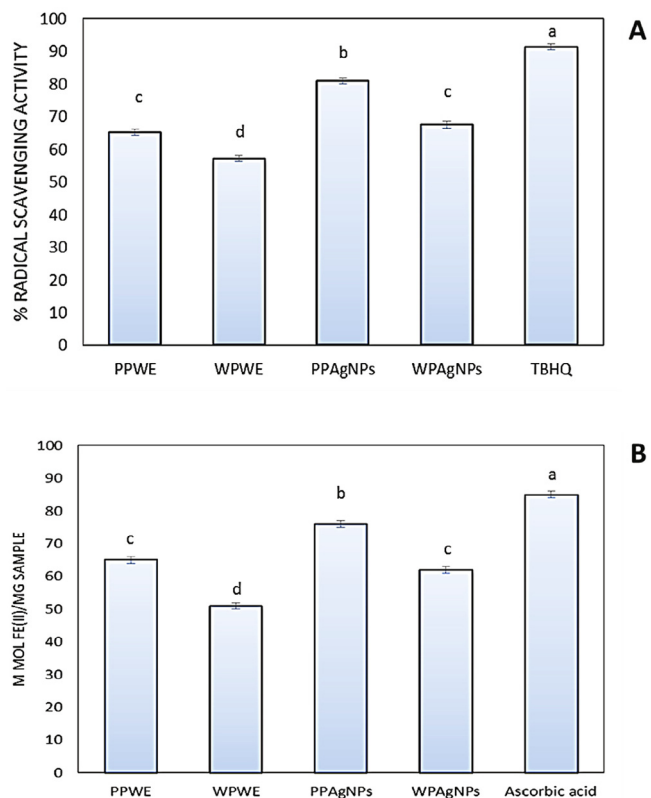
**Table 2**  
Phenolic compounds content in extracts and silver nanoparticles (mg/g).

Phenolic compound	PPWE	WPWE	PPAgNPs	WPAgNPs
Quercetin	1.10b	0.05d	1.45a	0.14c
Gallic acid	5.70b	2.60c	6.22a	2.88c
Benzoic acid	0.09b	0.03c	0.34a	0.12b
Genistein	0.47b	0.02d	0.72a	0.11c
Daidezein	0.31b	0.05d	0.56a	0.14c
Glycitein	0.20b	0.04c	0.45a	0.13b
Ferulic acid	0.52b	0.15d	0.77a	0.24c
Catechin	3.60b	1.50c	4.10a	1.77c
Vanillic	0.04c	0.03c	0.29a	0.12b
3,4,5-methoxy-cinnamic	0.45b	0.17d	0.70a	0.26c
Rutin	0.30b	0.08d	0.55a	0.17c
Cyanidin-3-o-glu	1.50b	nd	2.15a	0.01c
Punicalagin	2.40	nd	2.98a	0.03
Luteolin-7-glucose	0.13b	0.08c	0.38a	0.17b
Apiginin-7-glucose	0.16b	0.05c	0.41a	0.14b
P-oh- benzoic	0.09b	0.06c	0.34a	0.15b
Chlorogenic	0.15b	0.09c	0.40a	0.18b
Syringic	0.09b	nd	0.34a	0.01c
Ellagic acid	0.90b	0.75c	1.74a	0.94b
Kampeferol	0.60b	0.56b	0.85a	0.75a

Pomegranate peels water extract (PPWE); watermelon peels water extract (WPWE); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs). Means with different lowercase letters in the same row are significantly different ( $p \leq 0.05$ ).

of tested substances against 1<sup>st</sup> instar larvae are presented in terms of mortality, LC<sub>25</sub>, LC<sub>50</sub>, LC<sub>90</sub>, and slope values in Fig. 5. In all treatments, the mortality rates significantly increased with increasing concentrations. The obtained result revealed that PPAgNPs was the most effective substance against the 1<sup>st</sup> instar larvae (LC<sub>50</sub> = 68.32 µg/ml), followed by WPAgNPs with LC<sub>50</sub> = 130.45 µg/ml whereas watermelon peel extract was the least effective substance (LC<sub>50</sub> = 73681 µg/ml) however, pomegranate peel extract occupied the middle situation among the compounds (LC<sub>50</sub> = 285.2 µg/ml).

The combined effect of PPAgNPs + PP and WPAgNPs + WP enhance their larvicidal activity and increasing mortality causing additive effect and synergism. The synergistic effect showed clearly with the lower combined concentration of PPAgNPs + PP and



**Fig. 4.** (A) DPPH scavenging activity of extracts and AgNPs after 30 min, (B) reducing power of extracts and AgNPs; 2,2-diphenyl-1-picrylhydrazyl (DPPH). Pomegranate peels water extract (PPWE); watermelon peels water extract (WPWE); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs). Data are means  $\pm$  SE means with different letters indicate significant differences ( $p < 0.05$ ).

WPAgNPs + WP while increasing the combined concentration reduced their larvicidal effect and reduced mortality against 1<sup>st</sup> larval of *S. littoralis* Egyptian leafworms (Table 5).

#### 4. Discussion

Fruit and vegetables peels and wastes are a valuable source of phytochemicals, and their extracts exhibit considerable antioxidant and antimicrobial activity. Al-Sayed and Ahmed (2013) identified four phenolic compounds in sharlyn melon peels extract namely, 4-hydroxybenzoic acid, vanillin, chlorogenic acid, and coumaric acid. Also, the phenolic compounds, 3 hydroxybenzoic, chlorogenic, neochlorogenic, and isovanillic acids, also, apigenin7 $\alpha$ glu-

coside, luteolin-7-o-glucoside, and quercetin-3-galactoside in three melon flesh samples (Ganji et al., 2019), but, Major compounds in pomegranate peels extract were tannins and flavonoids such as; illogic acid, gallic acids, punicalin, and punicalagin (Al-Rawahi et al., 2014). Therefore, these phenolic extracts were employed to synthesize various nanoparticles. Marchiol et al. (2014) used extracts of different organs (leaves, stems and roots) of various plants including *Brassica juncea*, *Festuca rubra* and *Medicago sativa* for synthesis of silver nanoparticles. Extracts of different plant organs indicated different performances during synthesis of nanoparticles. In *Brassica juncea* and *Festuca rubra*, root extracts showed a better performance, while, in *Medicago sativa*, leaf extracts were the best. In this study, the fabrication mechanism of AgNPs by polyphenols in pomegranate and watermelon peels extracts briefed by the higher total phenolics content in pomegranate and watermelon peels extracts that facilitate the reduction of silver ions to nanoscale-sized silver particles. The electron donating ability of the phenolic compounds as they are strong antioxidants with high reducing capacity (Martínez-Castañón et al., 2008). The physical and chemical properties of obtained AgNPs in this study are in agreement with Pirtarighat et al. (2019) who found that silver nanoparticles synthesized by *Salvia spinosa* extract exhibited surface plasmon resonance found at 450 nm confirmed the formation of AgNPs. Also, Padalia et al. (2015) produced silver nanoparticles by *Calendula officinalis* extract with size between 10 and 90 nm and they had spherical, hexagonal, and irregular shapes. In addition, Chidambaram et al. (2014) prepared silver nanoparticles from petal extracts of *Calendula officinalis* with 2–20 nm size ranges. Furthermore, Baghizadeh et al. (2015), reported the synthesis of silver nanoparticles from marigold seed extracts with spherical shape and diameter of 5 to 25 nm. The active groups in AgNPs in our study similar to the same conclusion reported by Balakumaran et al., (2016) and El-Bendary et al. (2020). The negative charge on the nanoparticles size ensure the stability of the colloidal solutions. The samples of highly positive or negative values (+30 mV or –30 mV) remain stable without aggregation tendency (El-Saadony et al. 2019; Kratošová et al. 2019).

Abdel-Aziz et al. (2014) stated that the total phenolic compounds and total flavonoides were higher in AgNPs-containing *Che-nopodium murale* leaf extract compared to the plant extract. The highest phenolic content in AgNPs and waste extracts is attributed to their antioxidant and reducing activity. Adoni et al. (2020) found that green AgNPs synthesized by *Artemisia annua* leaf extract scavenged 84.99% of DPPH however; the scavenging activity of *Artemisia annua* extract was detected to be 73.04% at 500  $\mu$ g/mL.

Similar results obtained by Adoni et al. (2020) who found that silver nanoparticles synthesized by *Artemisia annua* extract showed moderate inhibitory activity against *E. coli*, *B. subtilis*, *S. aureus*, and *K. pneumoniae* as compared to levofloxacin. Also,

**Table 3**  
Antimicrobial activity of PPWE, WPWE, and green AgNPs (mean  $\pm$  SD).

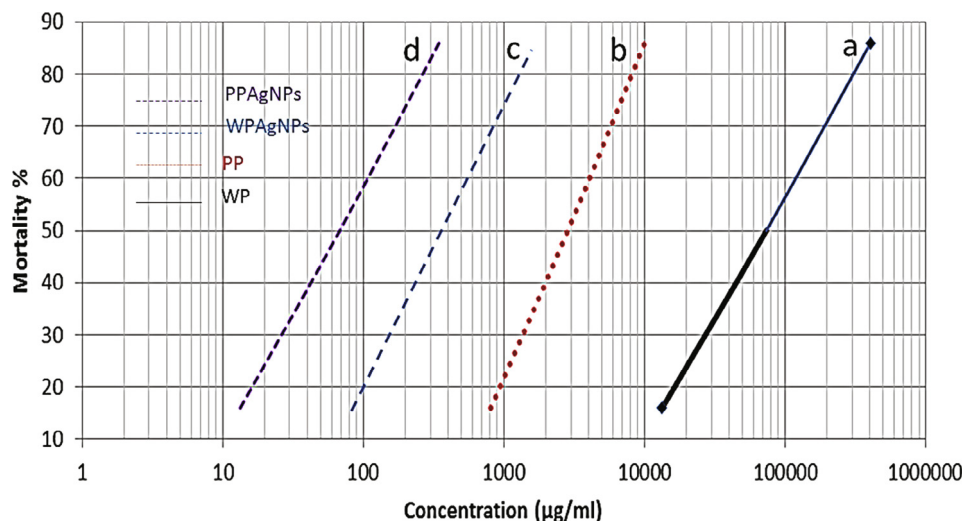
Sample	Microorganisms								
	Bacteria						Fungi		
	G+			G-			<i>C.g elbrta</i>	<i>C. apis</i>	<i>C. albicans</i>
<i>B. cereus</i>	<i>S. aureus</i>	<i>L. monocytogensis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>				
PPWE	15 $\pm$ 0.1d	18 $\pm$ 0.2d	14 $\pm$ 0.6d	9 $\pm$ 0.8d	11 $\pm$ 0.7d	8 $\pm$ 0.2d	8 $\pm$ 0.2d	10 $\pm$ 0.1d	12 $\pm$ 0.2d
WPWE	12 $\pm$ 0.2e	15 $\pm$ 0.3e	11 $\pm$ 0.1e	-	8 $\pm$ 0.1e	-	-	-	9 $\pm$ 0.0e
PPAgNPs	22 $\pm$ 0.5b	25 $\pm$ 0.7b	21 $\pm$ 0.2b	17 $\pm$ 0.3b	18 $\pm$ 0.2b	15 $\pm$ 0.2b	16 $\pm$ 0.5b	17 $\pm$ 0.5b	19 $\pm$ 0.1b
WPAgNPs	18 $\pm$ 0.6c	21 $\pm$ 0.1c	17 $\pm$ 0.9c	13 $\pm$ 0.5c	14 $\pm$ 0.3c	11 $\pm$ 0.3c	12 $\pm$ 0.2c	13 $\pm$ 0.4c	15 $\pm$ 0.9c
Levofloxacin	25 $\pm$ 0.1a	28 $\pm$ 0.5a	24 $\pm$ 0.1a	20 $\pm$ 0.7a	21 $\pm$ 0.4a	18 $\pm$ 0.5a	19 $\pm$ 0.5a	20 $\pm$ 0.2a	22 $\pm$ 0.3a

Pomegranate peels water extract (PPWE); watermelon peels water extract (WPWE); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs). Data are means  $\pm$  SD; Means with different small letters in the same column are significantly different ( $p < 0.05$ )

**Table 4**  
Larval toxicity of silver nitrate and plant extracts (Pomegranate and Watermelon) against Egyptian cotton leafworm.

Mortality rate	PP	WP	PPAgNPs	WPAgNPs
LC <sub>25</sub>	1211b	23156a	22.69d	60.51c
LC <sub>50</sub>	2852b	73681a	68.32d	130.45c
LC <sub>90</sub>	14524b	665319a	555.32d	1000c
Slope	1.813	1.3412	1.4085	1.3941

Lethal concentration killing 25% of insects (LC<sub>25</sub>); Lethal concentration killing 50% of insects (LC<sub>50</sub>); Lethal concentration killing 90% of insects (LC<sub>90</sub>). Means with different lowercases at the same raw indicate significant differences ( $p < 0.05$ )



**Fig. 5.** Toxicity lines of silver nitrate and plant extracts (Pomegranate and Watermelon) against Egyptian cotton leafworm, Pomegranate peels water extract (PP); watermelon peels water extract (WP); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs). Means with different lowercases indicate significant differences.

**Table 5**  
The joint action of silver nanoparticles with pomegranate and watermelon peels extracts. (mean ± SD).

Treatments	Combined concentration (µg/ml)	Observed mortality (%)	Expected mortality (%)	co-toxicity factor	Interaction
PPAgNPs + PPWE	8.5 + 1000	20.00e	10.00e	+ 100.00 ± 0.0a	synergism
	17 + 2000	56.67c	52.00b	+ 8.98 ± 0.1d	additive
	34 + 4000	90.00a	78.33a	+ 15.38 ± 0.3c	additive
WPAgNP + WPWE	8.5 + 1000	18.00e	12.00e	+ 50.86 ± 0.9b	synergism
	17 + 2000	33.33d	22.66d	+ 47.06 ± 0.8b	synergism
	34 + 4000	62.00b	45.55c	+ 12.52 ± 0.4c	additive

Pomegranate peels water extract (PPWE); watermelon peels water extract (WPWE); silver nanoparticles fabricated by pomegranate peels extract (PPAgNPs); silver nanoparticles fabricated by watermelon peels extract (WPAgNPs). Data are presented means ± SD. Means with different lowercases at the same column indicate significant differences

Phongtongpasuk et al. (2016) reported that eco-friendly AgNPs fabricated by dragon fruit peels extract have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. El-Bendary et al. (2020) found that silver nanoparticles have good performance against *Candida* species.

Watermelon rind extracts has mosquitocidal and antimicrobial properties. Watermelon extract showed potency against *Culex quinquefasciatus* (LC<sub>50</sub> = 51.31 µg/ml) and lower effective on larvae of *Aedes aegypti* (LC<sub>50</sub> = 73681 µg/ml) (Aruna et al., 2014). Watermelon outer peel consists of various bioactive components, including citrulline and carotenoids (Quek et al., 2007; Mort et al., 2008). Many parts of pomegranate e.g. bark, leaves, fruit, and fruit peel have antimicrobial and antifungal (Al-Zoreky, 2009), molluscicidal (Tripathi and Singh, 2000) and insecticidal effect (Gandhi and Pillai, 2011). The insecticidal effect against larvae of the red flour beetle, *Tribolium castaneum* treated with aqueous pomegranate fruit peel extracts using topical application and ingestion treatment (10 and 26% mortality) with anti-feeding effects (53.26%) (Hamouda

et al., 2014). The bioactivity of pomegranate peels extracts related to considerable levels of phenolic compounds (Jung, 2015) which are produced during cell metabolism as defense and signaling compounds as well as protecting the plant from various distress conditions besides their role relieve stresses against bacterial and fungal diseases (Devi et al., 2011). In addition, they may act as a potential insecticide (Ghaly et al., 2014). Flavonoids are also known to modulate the feeding and oviposition behavior of insects (Goławska and Łukasik, 2012). Flavanone naringine and the two flavanols, quercetin dehydrate and rutin hydrate prove aphicidal effect increase nymph's mortality with raising the concentrations (Ateyyat et al., 2012; Gupta et al., 2017). Also, peel extracts pomegranate disrupting development and causing deformities involved in vital activities like feeding, walking, or flying, making the insect vulnerable to several sorts of mortality agents and preventing them from causing damage to the crop (Hamouda et al., 2014). The phytochemical constituents present in petroleum ether extract of *Punica granatum* peel were phenols and saponins caused



mortality against third instar larvae of *Culex pipiens* ( $LC_{50} = 95.66$  32  $\mu\text{g/ml}$ ) (Farag et al., 2018) beside tannins (Koide et al., 1998).

The synergism mechanism may be due to plant aqueous extract synthesized AgNPs with insecticidal potentiation (Gul et al., 2016). AgNPs are more reactive because of their increased surface to volume ratio and excess of the phenolic compounds that attached to nanoparticles surface (Vani and Brindhaa, 2013; Di Ilio and Cristofaro, 2021). Besides they can absorbed easily into the cuticular lipids causing physical damage (Barik et al., 2008) and causing partial lysis of the midgut epithelial cells; vesicles and damaged membranes at the apical side of epithelial cells (Foldbjerg et al., 2015; Sultana et al., 2018). While the permeable free silver ions can induce the oxidative stress and detoxification genes (Nair et al., 2013). Another proposed mechanism for AgNPs could be due to the denaturation of proteins containing the sulfur or phosphorous compounds like DNA that drove to the denaturation of enzymes and organelles (Choi et al., 2008; Sondi and Salopek-Sondi, 2004) causing stop ATP synthesis.

## 5. Conclusions

Agricultural wastes are rich in polyphenols. In this study pomegranate and watermelon peels extracts were employed to synthesize green silver nanoparticles with considerable antioxidant and antimicrobial activity because of higher content of phenolic compounds. The obtained PPAgNPs proved the insecticidal effectiveness against the first instar of Egyptian cotton leafworm with synergistic effect when combined with the tested extracts (pomegranate and watermelon) especially at lower concentration. Increasing the concentrations of combination lower potency causing additive effect.

## Author contributions:

A.M.S.; M.T.E-S.; M. M.R.; A.M.E.T and T.F.T. designed the study plan. A.M.S.; M.T.E-S.; M. M.R.; and T.F.T. helped in conducting the experiment and collected literature. A.M.S.; M.T.E-S.; S.S.; A.E.T; and M.A.M.M. analyzed the data and drafted the manuscript. S.E. E.F., A.A.N, S.O.A, F.M.A., and M.E.S. provided technical help in writing the manuscript. All the authors read and approved the final version of the manuscript.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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