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Facile Green Synthetic Route to the Zinc Oxide (ZnONPs) Nanoparticles: Effect on Green Peach Aphid and Antibacterial Activity

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Abstract

In this study, zinc oxide nanoparticles (ZnONPs) were synthesized using *Punica granatum* peel extract in one–step reaction at room temperature. Zinc oxide nanoparticles were characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), ultraviolet visible spectroscopy (UV-vis) and scanning electron microscopy (SEM). The UV-vis absorption spectrum shows an absorption band at 278 nm due to ZnO nanoparticles. XRD characterized the final product as highly crystalline ZnO with sizes in the range 10-40 nm. The SEM results reveal a presence of network of randomly oriented ZnO nanoplatelets with an average size of 40 nm and thicknesses of about 8 nm. This study determined the effect of zinc oxide nanoparticles on green peach aphid and antibacterial activity.

Keywords: green synthesis, zinc oxide nanoparticles, *Punica granatum* peel, antibacterial activity, green peach aphid

1. Introduction

Zinc oxide has a potential application in sensors, solar cells, UV lasers, semiconductors, hydrogen storage devices, and catalysts. These application led many researchers to develop different routes to synthesis zinc oxide nanoparticles such as chemical route (Singh & Gopal, 2008; Abbasi et al., 2017), hydrothermal route (Ipeksac et al., 2013; Peng et al., 2016), sol-gel template process (Kumari et al., 2010; El Ghoul et al., 2012), photoluminescence emission technique (Rocha et al., 2014), microwave-assisted hydrothermal and decomposition (Tseng et al., 2012; Mousa et al., 2013), aerosol process (Ozcelik & Ergun, 2014), sonochemical synthesis (Zak et al., 2013), laser ablation (Thareja & Shukla, 2007), microemulsion method (Yıldırım & Durucan, 2010), precipitation method (An et al., 2014), hydrolyzed in polar organic solvents (Ehlert et al., 2014), solid-state thermal decomposition (Soofivand et al., 2013), microwave synthesis (Sutradhar et al., 2016). These routes have many disadvantages due to difficulty of scale up the synthesis process, separation and purification of nanoparticles from surfactants, co-surfactants, organic solvents, high energy consumption, and toxic by-products. Developing green methods for synthesis ZnONPs are of importance and still a challenge for materials researchers. Recently, plant extracts have been suggested as possible eco-friendly, alternative to chemicals in synthesis of nanoparticles. Different plants extracts have been reported in the open literature for green synthesis of zinc oxide nanoparticles, such as *Olea europea* (Awwad et al., 2014), *Eichhornia crassipes* leaf (Vanathi et al., 2014), *Aloe barbadensis* Miller leaf (Sangeetha et al., 2011), *Hibiscus subdariffa* leaf (Bala et al., 2015), *Solanum nigrum* leaf (Ramesh et al., 2015), *Camelia sinesis* leaf (Shah et al., 2015), *Hibiscus rosa-sinensis* (Devi & Gayathri, 2014), *Cassia fistula* plant extract (Suresh et al., 2015), *Ocimum tenuiflorum* leaves (Raut et al., 2015), *Trifoliumpratense* flower extract (Dobrucka & Dugaszewska, 2016), *Mimosa Pudica* leaves and coffee powder (Fatimah et al., 2016), *Rambutan* peel extract (Karnan & Selvakumar, 2016) *Jacaranda mimosifolia* flowers (Sharma et al., 2016), *Terminalia chebula* fruits (Rana et al., 2016) and *Azadirachia indica* (Madan et al., 2016).

In this research work, we developed a green and fast route for synthesis zinc oxide nanoparticles using *Punica granatum* peel aqueous extract. Also this research work determined the effect of synthesized ZnONPs on green peach aphid (GPA) and antibacterial efficacy against standard strains of Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*.

2. Materials and Methods

2.1 Materials

Zinc acetate dihydrate ($ZnC_4H_6O_2{\cdot}2H_2O$) and potassium hydroxide (KOH) are pure grade purchased from SIGMA-Aldrich and used without further purification. Distilled water was used in all experimental work.

2.2 Preparation of Punica granatum Peel Extract

Fresh fruits of healthy *Punica granatum* were collected from local market, Amman, Jordan. Peels were washed with water to remove dust particles and then dried in shade for two weeks to remove the residual moisture. Peels aqueous extract was prepared by placing 20 g of dried fine powder in 500 ml glass beaker along with 400 ml of sterile distilled water. The mixture was boiled for 10 min until the color of aqueous solution changed from watery to brown. Then the mixture was cooled to room temperature and filtered with Whatman No. 1 filter paper before centrifuging at 1,200 rpm for 5 minutes to remove biomaterials. The aqueous extract was stored at room temperature in order to be used for further experiments.

2.3 Green Synthesis of Zinc Oxide Nanoparticles (ZnONPs)

In a typical reaction mixture, 1.2 g of zinc acetate dihydrate was dissolved in 100 ml of the distilled water in 250 mL conical flask and stirred magnetically at room temperature for 5 min. *Punica granatum* peel aqueous extract (pH 5) was adjusted by potassium hydroxide to pH > 12. Afterwards, the alkaline peels aqueous extract was added drop wise under stirring, as soon as, the peels extract comes in contact zinc ions spontaneous change the colorless of zinc ions to yellow color. The obtained yellow mixture was left under stirring at room temperature. After one min, the yellow mixture started changing to a yellow-white suspended mixture, indicating the formation of water soluble monodispersed zinc oxide nanoparticles.

2.4 Characterization Techniques

Scanning electron microscopy (SEM) analysis of synthesized zinc oxide nanoparticles was done using a Hitachi S-4500 SEM machine. Powder X-ray diffraction (XRD) was performed using a X-ray diffractometer, Shimadzu, XRD-6000 with CuKα radiation $\lambda = 1.5405$ Å over a wide range of Bragg angles ($3^o \leq 2\theta \leq 80^o$). Fourier transform infrared (FT-IR) spectroscopic measurements were done using Shimadzu, IR-Prestige-21 spectrophotometer. UV-vis spectrum of zinc oxide nanoparticles was recorded, by taking 0.1 ml of the sample and diluting it with 2 ml deionized water, as a function of time of reaction using a Schimadzu 1601 spectrophotometer in the wave length region 200 to 700 nm operated at a resolution of 1 nm.

2.5 Green Peach Aphid

Zinc oxide nanoparticles synthesized using aqueous *Punica granatum* peels was tested against the green peach aphid. Five concentrations of ZnONPs were used to find their effect on this global pest. The testing method is similar to that adopted by Ghidan et al. (2016). Two ways analysis of variance was used to compare between mortalities caused by the ZnONPs concentrations.

2.6 Antibacterial Activity

Zinc oxide nanoparticles synthesized using aqueous *Punica granatum* peel extract was tested for its potential antimicrobial activity against some selected microbes. To analyze the antimicrobial activity of the sample, the samples were subjected to Agar well Diffusion method. Diameter of the zone of inhibition was measured in mm and expressed as Mean ± Standard Deviation.

3. Results and Discussion

3.1 XRD Analysis

The X-ray diffraction (XRD) pattern of synthesized ZnO nanoparticles is illustrated in Figure 1. The XRD pattern revealed the orientation and crystalline nature of zinc oxide nanoparticles. The peak position with 2θ values of 31.76°, 34.47°, 36.25°, 47.54°, 56.58°, 62.82°, 65.55°, 67.94°, 68.88°, 72.33, and 76.67° are indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes, which are in good agreement with those of powder ZnO obtained from the International Center of Diffraction Data card (JCPDS-36-1451) confirming the formation of a crystalline monoclinic structure. No extra diffraction peaks of other phases are detected, indicating the phase purity of ZnO nanoparticles. The average crystallite size of the synthesized zinc oxide nanoparticles was calculated to be 20 nm using Debye-Scherrer equation (Awwad et al., 2014; Raut et al., 2015).

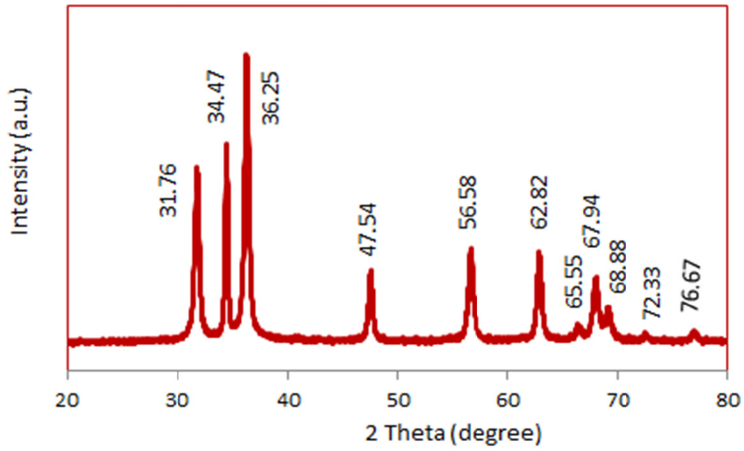

Figure 1. XRD pattern of synthesize zinc oxide nanoparticles

3.2 FT-IR Analysis

FT-IR Spectrum of *Punica granatum* peels, Figure 2 display strong and abroad absorption bands at 3518-3232 cm^{-1}, which could be ascribed to the stretching absorption bands of amino (-NH) and hydroxyl (-OH) stretching H bonded alcohols and phenols. The strong absorption peak at 2928 cm^{-1} could be assigned to the asymmetric and symmetric stretching of $-CH_2$ and $-CH_3$ functional groups of aliphatic. Peak at 1728 cm^{-1} corresponds to stretching carboxyl groups. The band at 1616 cm^{-1} is characteristic of amide carbonyl group in amide I and amide II. The bands at 1520 cm^{-1} and 1446 cm^{-1} is assigned to the methylene scissoring vibrations from the proteins. C-N stretch of aromatic amines and carboxylic acids gives rise to band at 1350 cm^{-1}. The band at 1234 $cm^{-1}$is due to CO vibrations of alcohols, phenols and C-N stretching vibrations of amine. The band at 1026 cm^{-1} assigned to the C-O stretching vibrations of alcohols. The peaks at 879 cm^{-1}, 768 cm^{-1}, and 509 cm^{-1} can be assigned to aromatic compounds. These functional groups act as dispersing, capping and stabilizing agents for ZnONPs during the process of synthesis. FT-IR spectrum of the synthesized ZnONPs, Figure 3 indicated a new chemistry linkage on the surface of ZnO nanoparticles. This suggests that *Punica granatum* peel extract can bind to zinc oxide nanoparticles through carbonyl of the amino acid residues in the protein of the extract, therefore acting as stabilizer and dispersing agent for synthesized zinc oxide nanoparticles and prevent agglomeration of nanoparticles. The main characteristic peaks of *Punica granatum* peel extract were observed in FT-IR spectrum of zinc oxide nanoparticles; the FT-IR spectrum of the ZnO nanoparticles show strong and sharp peaks at 567 cm^{-1} and 440 cm^{-1}.

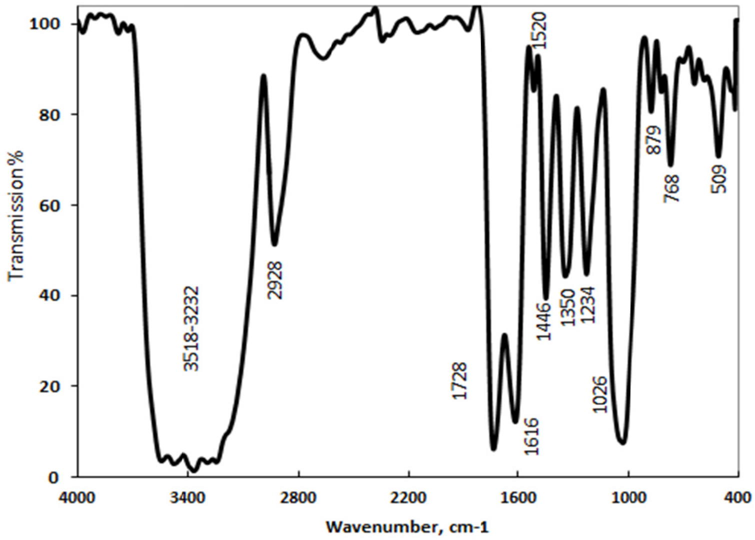

Figure 2. FT-IR spectrum of *Punica granatum* peel extract

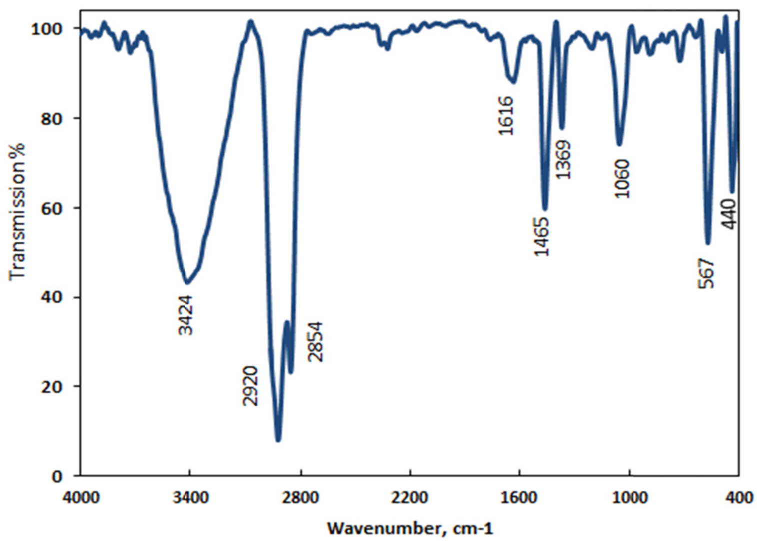

Figure 3. FT-IR spectrum of synthesized ZnONPS

3.3 UV-vis Spectroscopy

It is known that UV-vis spectroscopy is the most widely used technique for the structural characterization of nanoparticles. The absorbance of the ZnO reaction mixture was monitored in the range of 200-700 nm. Typical excitation absorption at 278 nm was observed at room temperature.

3.4 SEM Images Analysis

Scanning electron microscopy (SEM) images of synthesized zinc oxide nanoparticles by this green method are shown in Figure 4. It was found that the *Punica granatum* peel extract strongly influences the morphologies of the resultant zinc oxide nanoparticles. As-synthesized zinc oxide were mainly composed of Nano-flowers and Nano-platelets sizes ranges with the average size in the range of 40 nm and primary particles coalesced together to form larger-sized secondary particles,. While using larger quantities of *Punica granatum* peel extract in synthesis process, zinc oxide Nano-flowers were obtained (Figure 4A). Smaller quantities with quite surface were synthesized with an average size of ca. 40 nm and average thickness of 8 nm. Figure 4B showed a quite dense morphology compromised of randomly oriented overlapping, thin sheets of ZnONPs where the individual sheets appear to have a lateral dimension of less than 300 nm. A good estimate of the Nano-sheets thickness is in the range of 10 to 30 nm. However, it is possible that the thicker sheets may consist of several thinner sheets aggregated to form Nano-platelets networks. Such ZnONPs platelet format form a useful baseline in the sense of demonstrating that ZnO of very high crystallinity and near-perfect stoichiometry which agrees well with the XRD results.

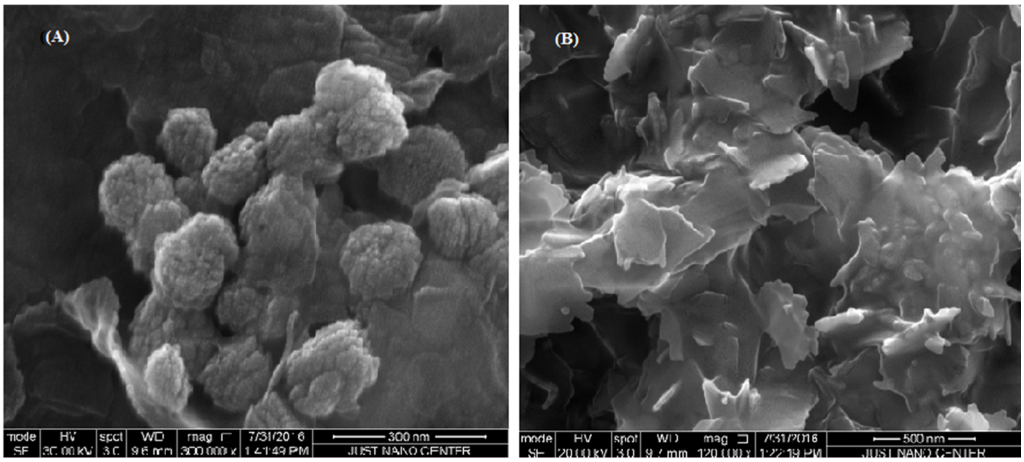

Figure 4. SEM images of synthesized ZnONPs: (A) flowers shape and (B) platelets shape

3.5 Antibacterial Activity

The green synthesized zinc oxide nanoparticles showed a significant antibacterial activity against Gram positive and Gram negative bacteria. The antibacterial activity results against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*. Chloramphenicol was used as a control antibacterial agent. Maximum zone of inhibition (MZI) are listed in Table 1. It was observed that an increase in ZnONPs concentration increases the MZI of *Staphylococcus aureus* and *Escherichia coli*. The results indicated that green synthesized zinc oxide nanoparticles showed effective antibacterial activity against *S. aureus* and *E. coli*. Also our results indicated that the inhibitory effect increase with an increase in the concentrations of ZnONPs.

Table 1. Antibacterial activity of ZnONPs against selected bacteria

ZnONPs (µg/L)	*S. aureus*	*E. coli*
200	16±0.21	11.8±0.05
100	14±0.06	10.7±0.06
50	12±0.07	9±0.07
Reference drug	20±2.44	20±2.28

3.6 Effect on Green Peach Aphid

The effect of different concentrations of ZnONPs on GPA is shown in Tables 2 and 3. Means of mortality % of the 1^{st} and 2^{nd} nymphal instars caused by the five concentrations were different significantly, Table 2. Means of mortality % of 3^{rd} and 4^{th} nymphal instars of the same aphid caused by the same concentrations were also different significantly. However, mortality % of both aphid categories in all concentrations was greater significantly than the control treatment.

The highest mortality % of 1^{st} and 2^{nd} nymphal instars was in 8000 µg/ml concentration. It was 75.5% after 24 h, then reached 100% after 48 h and 72 h, while mortality increased at 4000 µg/ml concentration reached 90 and 100 after 48 h and 72 h respectively. The lowest mortality was in case of using 250 µg/ml after 24 h then increased to 43 and 75% after 48 h and 72 h, respectively. The mortalities in case of control were significantly the least after 24 h, 48 h and 72 h compared with the other treatments.

The highest mortality % of 3^{rd} and 4^{th} nymphal instars was in 8000 µg/ml concentration, Table 3. It was 74.0% after 24 h, then reached 65%, 100% after 48 h and 72 h, respectively, while mortality increased at 4000 µg/ml concentration reached 55.0% after 48 h and 72 h respectively. The lowest mortality was in case of using 250 μg/ml after 24 h then increased to 35.0% and 58.5% after 48 h and 72 h, respectively. The mortalities in case of control were significantly the least after 24 h, 48 h and 72 h compared with the other treatments.

Table 2. Means of mortality percent (%) of 1st and 2nd nymphal instars of the green peach aphid by five different concentrations of ZnONPs

Concentration (μg/ml)	Mortality % of 1st and 2nd nymphal instars		
	After 24 h	After 48 h	After 72 h
Control	4.0d	5.0e	5.0d
250	29.5c	43.0d	75.0c
1000	34.5c	55.0c	83.0bc
2000	60.5b	92.0b	92.0ab
4000	66.0ab	90.0b	100a
8000	75.5a	100a	100a

Note. * Means within the same colum of the same period of time sharing the same letter do not differ significantly at 5% level using Frisher protected LSD test.

Table 3. Means of mortality percent (%) of 3rd and 4th nymphal instars of the green peach aphid by five different concentrations of ZnONPs

Concentration (μg/ml)	Mortality % of 3rd and 4th nymphal instars		
	After 24 h	After 48 h	After 72 h
Control	3.0e	4.0c	5.0c
250	17.5d	35.0ab	58.5b
1000	25.0d	31.0ab	61.0b
2000	38.0c	65.0a	55.0b
4000	58.0b	55.0a	55.0b
8000	74.0a	65.0a	100a

Note. * Means within the same colum of the same period of time sharing the same letter do not differ significantly at 5% level using Frisher protected LSD test.

4. Conclusion

In the present work, we first report an eco-friendly and simple method for the synthesis of zinc oxide nanoparticles using *Punica granatum* peel extract. FTIR analysis of aqueous *Punica granatum* peel extract indicated the presence of phyto-constituents such as amines, aldehydes, phenols, and alcohols which were the surface active molecules stabilized the zinc oxide nanoparticles. XRD analysis reveals that the average size of the nanoparticles was found to be 20 nm which was calculated by Debye-Scherrer equation. FT-IR and XRD results corroborated the purity of the synthesized ZnONPs. Green synthesized ZnONPs was evaluated against green peach aphid, the results showed significant effect on this aphid compared with the control treatment. Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* showing significant effective activity. The method of the present study offers several important advantageous features. First, the synthesis route is economical and environmentally friendly, because it involves inexpensive and non-toxic materials for second, large scale synthesis.

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