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ORIGINAL ARTICLE

Antifungal activity of silver nanoparticles green biosynthesis from the extract of *Zygophyllum album* (L.f.) on Fusarium wilt

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Abstract

This study illustrates the antifungal activity of green biosynthesis of a silver nanoparticle solution using one of Sinai's natural plant extracts, namely *Zygophyllum album* which was used as a stabilizer and reducing agent to reduce Ag+ to metallic silver. In this study the plant extract was prepared by boiling in water for 10 min., 70% ethanol and wet autoclaving for 5 min. AgNPs were prepared using these three different extract methods. Transmission electron microscope (TEM) and zeta potential techniques were employed to characterize the synthesis of nanoparticles. The size of particles ranged from 6.28 nm to 28.89 nm at x100 and the zeta potential had one peak at –16.6 mean (mV) at area 100% for green synthesized AgNPs from *Z. album* prepared from boiling in water for 10 min. The size of particles ranged from 6.64 nm to 54.82nm at 100x and the zeta potential had one peak at – 12.9 mean (mV) at 100% area for green synthesized AgNPs from the plant ethanol extract. The size of particles ranged from 9.39 nm to 31.93 nm at 100x and the zeta potential had one peak – 19.8 mean (mV) at 100% area for green synthesized AgNPs from the wet autoclaved plant extract of *Z. album* for 5 min. All treatments of plant extract and AgNPs solutions, prepared from these plant extracts of *Zygophyllum album,* were compared with the positive control and Tachigaren – 30% W/P was conducted on the radial growth of *F. oxysporium* and caused antifungal activity with a high inhibition percent. There was a highly significant difference between the various extraction techniques. Increasing the concentration of treatments was accompanied with a significant effect on *Fusarium* wilt. Thus, this study may provide a good alternative approach to control Fusarium wilt disease in the field and under storage conditions of vegetables. Our study suggests that silver nanoparticles of plant extracts can be used for controlling *Fusarium* wilt.

Keywords: antifungal activity, *Fusarium oxysporum,* green synthesis, TEM, *Zygophyllum album*, zeta potential

Introduction

Fusarium wilt is one of the most serious diseases threatening crop production (Attia *et al.* 2022). *Fusarium oxysporum* is a widespread soil-borne fungal pathogen that invades roots and causes vascular wilt disease. The pathogen colonizes the vascular tissue and results in significantly important economic losses by causing extensive necrosis, typical wilting symptoms, rapid plant decline, accelerated fruit ripening, and plant death (Gomaa *et al.* 2021). The use of chemical compounds has resulted in side effects that may include environmental damage and pollution, in addition to pathogen resistance selection. The development of new disease control mechanisms is desperately needed, and nanotechnology advancements can benefit in the control of different pathogens, which the current study focused on. Natural product research is a great way to seek

bioactive metabolite molecules with potentially distinct chemical structures and mechanisms of action (Cragg and Pezzuto 2016). Egyptian flora offers numerous advantages including stabilizing compounds for preparing AgNPs. Polyphenolic compounds act as reducing agents while amino acids act as stabilizing agents. The plant family Zygophyllaceae is quite large. It consists of around 27 genera and 285 species, with 80 species belonging to the genus Zygophyllum (Hal *et al.* 2022). To maximize the benefits of natural products, particularly those derived from *Z. album*, nanotechnology is an excellent tool for improving therapeutic efficacy while minimizing side effects, as well as modifying the therapeutic response of herbal extracts (Anjum *et al.* 2017).

Biological research on *Zygophyllum sp* has revealed significant antioxidant, antitumor and anti-inflammatory properties which influenced their phytochemical constituents. Various compound classes such as oil, sterols, phenolic, saponins, triterpenes, essential flavonoids, and esters were isolated from various Zygophyllum species (Anjum *et al.* 2017; Shawky *et al.* 2019).

Nanoparticles are known for their antimicrobial properties. They are considered to be fundamental building blocks for nanotechnology. Green biosynthesis of nanoparticles is safe and eco-friendly. Green biosynthesis of silver nanoparticles by bacteria, fungi and plants was carried out (Yu *et al.* 2019; ElSharawy *et al.* 2023). There are several methods of preparing silver nanoparticles. Biosynthesis is thought to be the best way to combine the benefits of nanoparticles with natural products. Green synthesis and assembly of nanoparticles to develop clean, nontoxic, and environmentally friendly nanoparticle preparation procedures is known as biosynthesis (Mohanpuria *et al.* 2008; Mokhtari-Hosseini *et al.* 2022).

Recently, green synthesis of AgNPs solutions from plant extracts has attracted interest for a variety of applications in different fields and gained much attention from chemists and researchers (Alharbi *et al.* 2022). In the green production of AgNPs, the plant extracts act as reducing as well as stabilizing agents. UV-visible spectroscopy, FT-IR, XRD, TEM, and SEM confirmed the generation of AgNPs (Hembram *et al.* 2021). With biochemistry scientists have developed green synthesis in nanotechnology to reduce the production of hazardous harmful compounds (Ahmed *et al.* 2016).

Natural plant products have a tremendous influence on current agrochemical studies. They are becoming some of the most effective ways to protect crops and the environment from fungicides (Fortunati *et al.* 2019).

Silver nanoparticles were evaluated for their antimicrobial activity against *F. oxysporum* f. sp. *lycopersici* (Gomaa *et al.* 2021), *F. oxysporium* f.sp., betae, *Sclerotium rolfsii* and *Rhizoctonia solani* (El-Argawy *et al.* 2017), *F. oxysporium* f.sp., *ciceris* (ElSharawy *et al.* 2023), and *Escherichia coli, Bacillus mycoides* and *Candida albicans* (Othman *et al.* 2019). They have strong antifungal activity against *F. graminearum* (Gomaa *et al.* 2021; Gezaf *et al.* 2022).

Disease-causing microbes that have developed resistance to drug therapy are becoming a growing public health concern. As a result, new pesticides are desperately needed. Silver nanoparticles take advantage of silver's oligodynamic effect on microbes. In our work, we compared three methods of green synthesis using *Z. album* L plant extract which grows in North Sinai deserts, Egypt. The effectiveness of the prepared nanoparticles on the growth of *F. oxysporum* was determined. In addition, the biological activity of the *Z. album* was also measured.

Materials and Methods

Fungal isolation and identification

Fusarium oxysporum was isolated from naturally diseased tomato plants with typical symptoms of wilt disease from North Sinai Governorate. The causal organism was isolated from discolored vascular stems of infected plant tissues. Small pieces of infected plant tissue were surface sterilized for 2-3 minutes with sodium hypochlorite (0.5%), washed several times with sterilized distilled water, dried between sterilized Whatman No.1 filter paper, placed on the surface of sterilized potato dextrose agar media (PDA) and incubated for 7 days at 25°C ± 1. Isolated fungus was purified by hyphal tip technique (Gomaa *et al.* 2021). The pathogen was identified as *F. oxysporum* based on morphological and cultural characterization according to Wharton *et al.* (2006).

DNA extraction and PCR amplification

Fusarium oxysporum identity was confirmed by molecular analysis. The genomic DNA extracted from powdered mycelium using the CTAB (Cetyl Trimethyl Ammonium Bromide) protocol established by Aamir *et al.* (2015). PCR analysis was performed using the sequences of primer pair ITS 1 and ITS 2 according to White *et al.* (1990). PCR was conducted using a volume 25 µl Master Mix. The purified PCR products were sequenced at the DNA sequencing unit of the Assiut University Moubasher Mycological Centre (AUMMC). Nucleotide sequences were matched to suitable existing sequences found at the database of the National Center for Biotechnology Information (NCBl) (www.ncbi.nih.gov). The BLAST (Basic Local Alignment Search Tool) program

(BLASTN, http://www.ncbi.nlm.nih.gov) was used to find their closest relatives.

Collection and preparation of *Zygophyllum album*

Wilted *Zygophyllum album* (Zygophyllaceae) plants were collected from Al-Arish region, North Sinai, Egypt. The plant was verified at the Department of Botany College of Science, Arish University, and was identified as *Zygophyllum album (*L.f.), by Prof. Adel Ibrahim Hamed El-Gazzar (Fig. 1A).

Green synthesis of nanoparticles from *Zygophyllum album* extract

The plant extract of *Z. album* was obtained by applying the method of Mousavi *et al*. (2018). Plant parts (stems, leaves and flowers), with the exception of roots, were washed several times with tap water to remove all dirt and debris from the plants before rinsing with deionized water. After that, plant tissues were cut into fine pieces and then oven dried separately in shade at 40°C for 48 h (Fig. 1A). The dried plants cut into small pieces (Fig. 1B). Three indicative assays (A, B and C) were used in this study to assess and compare the extraction methods. Plant parts were ground to fine powder and three extraction methods were used. A – 10 g were transferred to a 250 ml flask containing 100 ml 70% ethanol and then boiled in water at 60°C for 10 min. The suspension was then filtered through Whatman No.1 filter paper. B – 10 g were transferred to a 250 ml flask containing 100 ml distilled boiling water for 10 min. and then filtered through Whatman No.1 filter paper. C – 10 g were transferred to a 250 ml flask containing 100 ml distilled water and autoclaved at 121°C at 1.5 pa for 5 min. and then filtered through Whatman No.1 filter paper (Kalantari *et al.* 2019).

Preparation of biosynthesis of silver nitrate solution (AgNPs)

AgNPs were created using the green biosynthesis method, as described by Elsharawy *et al.* (2023). As a reagent solution, an aqueous solution of $AgNO_3$ was used. As a stabilizing and reducing agent, a 20% plant extract solution was slowly mixed. We optimized the $AgNO_3$. The mixture was stirred for 72 h, and incubated at room temperature. The control groups were also kept under the same conditions. Briefly, 1 mm of solution was prepared and utilized for the green synthesis of AgNPs. Five ml of prepared plant extract was added and mixed slowly on a magnetic stirrer with 25 ml of prepared 1 mm $AgNO_3$ in an Erlenmeyer flask at room temperature under dark conditions for 48 h. The first sign of nanoparticle formation was a change in color. AgNPs were produced by centrifugation at 10,000 rpm for 10 min. (Fig. 1C), followed by carefully washing with distilled water and then storing in a dark controlled room at 4°C. The composite mixture was then placed in a microwave oven (1200W, 50Hz) for complete bio reduction at 300 W for 4 min. at a four time to prevent pressure buildup (Ali *et al.* 2015; Nguyen *et al.* 2020).

Characterization of green synthesized silver nanoparticles from *Zygophyllum album*

Characterization of AgNPs with zeta potential distribution were examined at the Nanotechnology and Advanced Materials Central Lab. (NAMCL), Agricultural Research Center (ARC), Egypt. Because of surface plasmon resonance, metal nanoparticles absorb a lot of electromagnetic waves in the visible range. Zeta potential distribution was used to assess the stability of biologically synthesized AgNPs that had been stored and to characterize the silver nanoparticles. The zeta

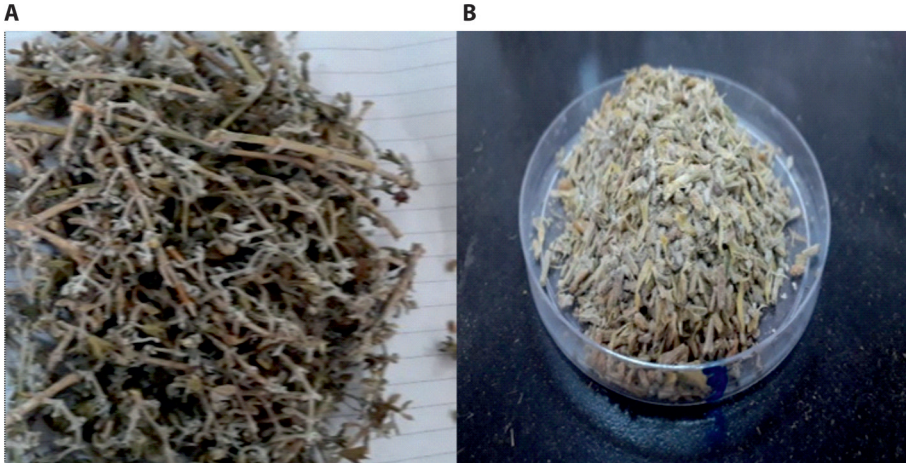

Fig. 1. A – dry branches of *Zygophyllum album* plant; B – small pices of *Zygophyllum album*

potential of AgNPs was measured with a zetasizer using the same procedures as the hydrodynamic mean diameter (Lakshmanan *et al.* 2018).

Transmission electron microscope (TEM) examination (JEM_2100 HR ELECTRON MICRESCOPE made in Japan) was performed in the Electron Microscope Unit, National Research Center (NRC), Egypt. Methods according to Yu *et al.* (2019) were used to image the AgNPs to study the shape and size of green synthesized AgNPs. In brief, sample solutions in distilled water were prepared on carbon-coated copper grids. The water was allowed to evaporate at room temperature for a photo with TEM. Furthermore, the presence of metals in the sample was determined using energy-dispersive spectroscopy (EDS) on an INCA Energy TEM 200 with analysis software (JEOL), at an accelerating voltage of 80 Kv (Elbahnasawy *et al.* 2021).

Effect of plant extract and AgNPs solution of *Zygophyllum album* on *Fusarium oxysporum in vitro*

Antifungal activity of different concentrations of plant extract and AgNPs preparations of *Z. album* in vitro against *F. oxysporum* were examined by using a standard protocol agar well diffusion method (Ali *et al.* 2015; Hashem *et al.* 2022). Each Petri dish had different concentrations (10, 20, 40, 80, 150, 200, and 250 µl · plate^{-1} (10 ml of media) and were supplemented to PDA media prior to pouring. Wells were also made for the positive controls (Tachigaren –30% W/P), and negative control (Sterile DIW). Disks (0.8 cm) were obtained from the edge of 7-day old *F. oxysporum* mycelial growth and were transferred to the middle of a Petri dish of PDA media. The plates were incubated at 28 ± 2°C. The diameters (cm) of fungus colonies on PDA plates for treatments and controls were measured.

The plates were incubated at 25 ± 2°C. The antifungal activity of the test agent was then determined by measuring the mean diameter of three repetitions in a completely randomized design. The percentages of inhibition rates of mycelium of fungus were calculated according to the following equation:

$$\text{Inhibition rate (\%)} = (R - r)/R \times 100,$$

where: R – the radial growth of mycelia hyphae for control plates and r – the radial growth of mycelia hyphae for treatment plates.

Statistical analysis

All data were analyzed using SPSS software (SPSS version 16.0, SPSS Inc., Chicago, IL, USA). Two-way analysis of variance (ANOVA) for Mean ± SD (n = 3) and significant differences between the means of the treatments were determined using Duncan's Multiple Range test ($p \leq 0.05$).

Results and Discussion

Molecular identification

Identifications were confirmed by sequence analysis at the Assiut University Moubasher Mycology Center (AUMMC). BLAST results of this study's ITS region and sequences in NCBI revealed relationships and similarities with reference sequences in GenBank. The amplified sequences were submitted to GenBank and given the accession number OP177954.1 with 98% similarity to *F. oxysporum*.

Specifications of the biosynthesis of green AgNPs from plant extract of *Zygophyllum album* at obtained optimum conditions

Silver nanoparticles (AgNPs) appeared yellowish brown in an aqueous medium after incubation with moderate stirring at room temperature. The color of the solution changed from faint light to yellowish brown to red dish brown to colloidal brown, indicating AgNP formation The color change of plant extract to brown after adding 1 mm $AgNO_3$ was a result of the reduction process of silver atoms to silver nanoparticles as an indicator of Ag-nanoparticle formation. The change of color of silver nanoparticle solution (AgNPs) to yellowish brown in aqueous medium after incubation with moderate stirring at room temperature indicated AgNPs formation. Similar color changes were observed in previous studies (Gezaf *et al.* 2022; Khane *et al.* 2022). The color change of the plant extract was caused by the reduction of silver ions to silver nanoparticles. The extract solution containing reducing compounds play the main role in the conversion of silver ions into AgO nanoparticles This observation has been recorded by many researchers (Othman *et al.* 2019; Yu *et al.* 2019).

Characterization of silver nanoparticles

TEM for AgNPs obtained extract methods

According to the TEM images, all of the prepared extracts contained very small nanoparticles less than 100 nm in size. TEM of green synthesized AgNPs from *Z. album* prepared by boiling in water for 10 min., gave nanoparticle sizes ranging from 6.28 nm to 28.89 nm at x100 and 200x (Fig. 2A, B). Also, TEM found almost spherical silver NP shapes around the metrics, indicating that the nanoparticles were not contact in their aggregation. It illustrates capping of nanoparticles which may be some secondary metabolites from the

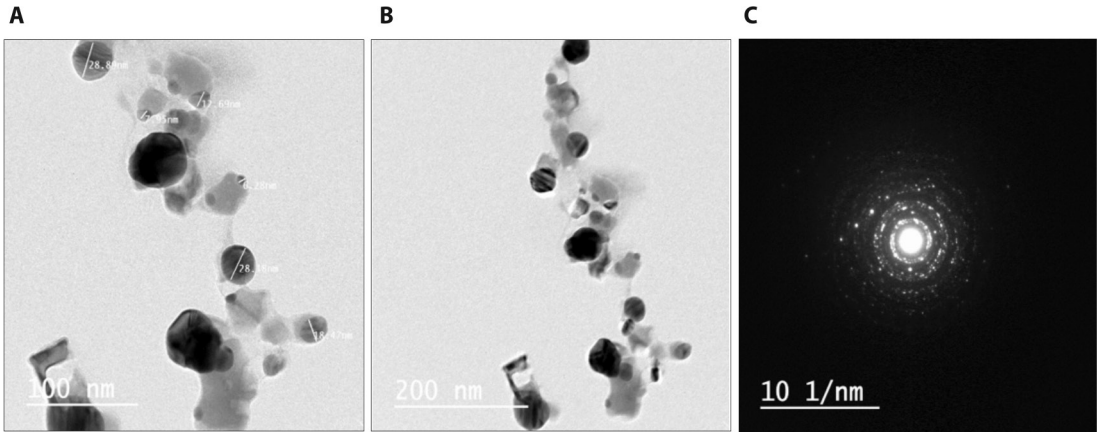

Fig. 2. TEM of green synthesized AgNPs from *Zygophyllum album*, prepared boiling in water for 10 min.; A–B – the size of particles is ranged from 6.28 nm to 28.89nm at x100 and 200x; C – images illustrate the nanoparticles are crystalline

plant extract (Fig. 2A, B). In TEM image (Fig. 2C), the selected area of electron diffraction (SAED) pattern of AgNPs obtained from boiling in water for 10 min. illustrates that the nanoparticles were arranged in distinct diffraction rings which illustrated they are crystalline. TEM images of green synthesized AgNPs from *Z. album* extract was obtained by 70% ethanol (Fig. 3A, B). The particles of nanoparticles ranged in size from 6.64 nm to 54.82 nm at x100 and 200x (Fig. 3A, B). TEM estimated the particle size and particle distribution. Furthermore, the TEM images revealed almost spherical silver NP shapes around the metrics. Typically, the nanoparticles are not contact in their aggregation, illustrating capping of nanoparticles which may be some secondary metabolites from the plant extract (Fig. 3A, B). In TEM image of SAED pattern of AgNPs obtained by 70% ethanol illustrates that the nanoparticles arranged in distinct diffraction rings which illustrates they are crystalline (Fig. 3C). According to TEM image, TEM of green synthesized AgNPs from wet extract of Z. album autoclaved for 5 min., the diameter of nanoparticles ranged from 9.38 nm to 31.93 nm at 100x and 200x (Fig. 4A, B). Using TEM we found almost spherical silver NP shapes around the metrics, indicating that the nanoparticles were not contact in their aggregation, which illustrated capping of nanoparticles that may be some secondary metabolites from the plant extract (Fig. 4A, B). In a selected area of diffraction pattern recorded from green synthesized AgNPs prepared from wet autoclaving for 5 min, the nanoparticles arranged in distinct diffraction rings which illustrates that they are were crystalline (Fig. 4C). The results of TEM images revealed that all of the prepared extracts contained very small nanoparticles less than 100 nm in size. A closer look revealed that the particles were surrounded by a thin layer of plant extract. This suggested that the particles were stabilized by the presence of a non-metallic organic capping agent.

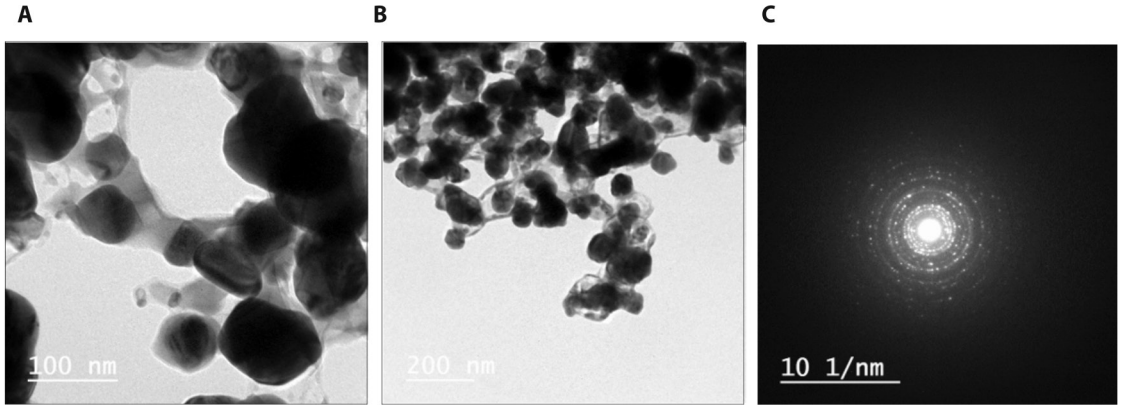

Fig. 3. TEM of green synthesized AgNPs from *Zygophyllum album*, prepared by 70% ethanol; A–B – the size of particles is ranged from 6.64 nm to 54.82 nm at x100 and 200x; C – images illustrate the nanoparticles are crystalline

Zeta potential distribution for AgNPs prepared from *Z. album* by boiling in water for 10 min. confirmed that the charge had a significant impact on particle distribution. The photo illustrates diffraction between nanoparticles and the only peak appeared at 100% area at a width of 3.53 mV (Zeta deviation), the stability between nanoparticles was – 16.6mV, the negative values were good results (Fig. 5A). Zeta potential distribution for green synthesized AgNPs obtained by 70% ethanol of *Z. album*, clarified the diffraction between nanoparticles. The only peak which appeared was – 12.9 mean (mV) at 100% area at width 3.22 mV (Fig. 5B). Zeta potential distribution for green synthesized AgNPs from the wet autoclaved extract of *Z. album* for 5 min., illustrated the diffraction between nanoparticles. The only peak which appeared was – 19.8 mean (mV) at 100% area at width 3.98 mV (Fig. 5C). The morphology of synthesized AgNPs was illustrated

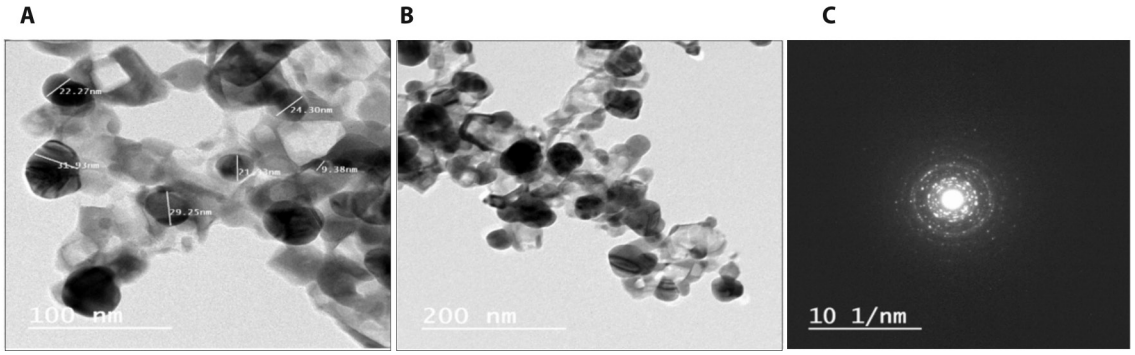

Fig. 4. TEM of green synthesized AgNPs from *Zygophyllum album*, prepared by wet autoclaved; A–B – the size of particles is ranged from 9.39 nm to 31.93 nm at x100 and 200x; C – images illustrate the nanoparticles are crystalline

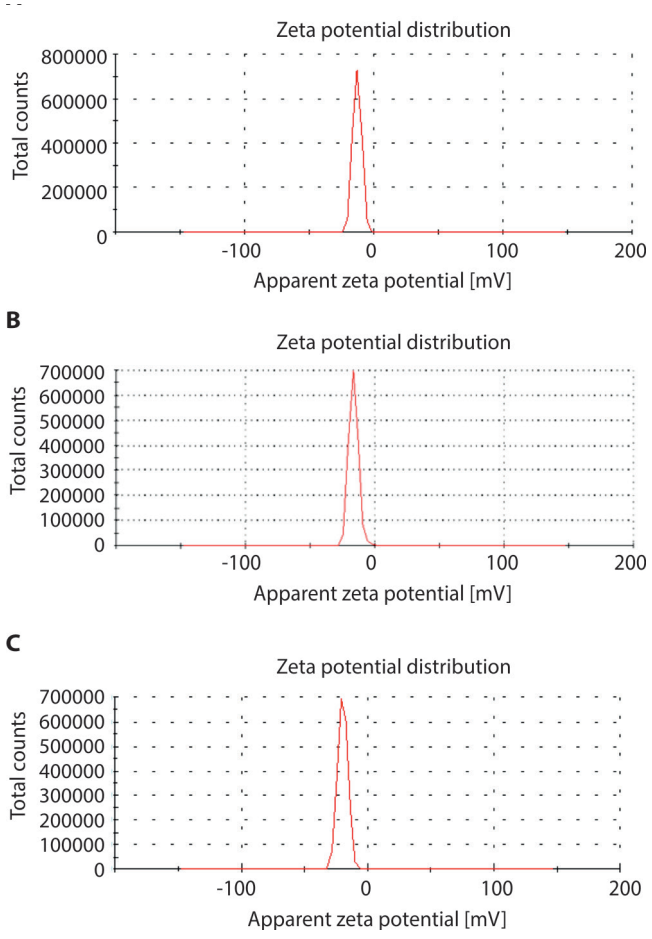

Fig. 5. Zeta potential of green synthesized AgNPs from *Zygophyllum album*: A – prepared by 70% ethanol; B – obtained by boiling in water for 10 min; C – from wet autoclaved

with TEM which illustrated that the size of nanoparticles ranged from 2 to 100 nm.

The synthesized AgNPs were clearly dispersed with spherical structures. In fact, spherical NPs outnumbered other shapes. The spherical shape indicated that the NPs were synthesized with low surface energy and high thermodynamic stability, confirming the high potential of the synthesized AgNPs. This suggested that the particles were stabilized by the presence of a non-metallic organic capping agent.

Zeta potential distribution for three AgNPs obtained from different types of plant extract: A – AgNPs obtained from boiling in water of plant extract for 10 min., B – AgNPs obtained from plant ethanol extract of *Z. album* and C – prepared from wet plant extract of *Z. album* autoclaved for 5 min. have one peak at area 100% the stability between nanoparticles at width (Zeta deviation) with negative charge for AgNPs. Since this peak appeared for metal nanoparticles these results can be explained by the fact that the surface of nanoparticles has free electrons which stimulate the surface of the plasmon resonance absorption (SPR) band. According to Thirumagal and Jeyakumari (2020), the adsorption of OH- ions from leaf phytochemicals causes nanoparticles to have a negative charge. The colloidal system is highly stable for surface charged they can cause either repulsion or attraction between particles, indicating colloidal system stability. Zeta potential analysis was used to collect data on the surface properties of the NPs. This equipment can examine the long-term stability of specific systems. For a physical suspension stabilized by electrostatic repulsion, a zeta value of around 30 mV is required (Izadiyan *et al.* 2018). The surface of NPs has a high electric charge, as recorded by an increase in absolute zeta potential, indicating strong repulsive forces between the NPs and thus stable particles. This explains their stability as a result of electrostatic repulsion, which prevents aggregation and agglomeration (Urnukhsaikhan *et al.* 2021).

Antifungal activity of biosynthesized green silver nanoparticles and plant extract of *Zygophyllum album* on *Fusarium oxysporum* mycelial growth *in vitro*

Zygophyllum album plant extract, prepared by boiling in water for 10 min. (B), had the antifungal ability of inhibition of *F. oxysporum* mycelial growth at different concentrations when compared with control plates. All treatments illustrated different degrees of inhibition of mycelial growth. The percentage of inhibition was increased by increasing the concentration of treatments. The percentages of inhibition were 9.05% ± 0.7, 17.28% ± 0.7, 21.39% ± 0.7, 27.98% ± 0.7, 31.27%± 0 .7 and 55.55% ± 1.2 for 10, 20, 40, 80, 150, 200 µl ·10 ml^{-1} of PDA media, respectively. In addition, it appeared that the fungicide Tachigaren-30% W/P significantly reduced mycelial growth by 66.76% ± 1.9. Furthermore, in the untreated control there was no inhibition (Table 1). The percentages of inhibition of 70% ethanol (A) of *Z. album* were 8.67% ± 1.1, 17.35% ± 0.12, 21.48% ± 1.2, 36.36% ± 0.6, 40.91% ± 0.2 and 62.39 ± 1.5 for 10, 20, 40, 80, 150, 200 µl ·10 ml^{-1} of PDA media, respectively. As compared to the antifungal activity, fungicide Tachigaren-30% reduced the mycelial growth to 76.92% ± 2.9 (Table 1). The extract of *Z. album* obtained by wet autoclaving for 5 min. (C) also had the ability of inhibiting *F. oxysporum* mycelial growth at different concentrations compared to the control plates. All treatments illustrated different degrees of inhibition for mycelial growth.

Table 1. Antifungal activity of bio synthesized green silver nanoparticles and plant extracts of *Zygophyllum album* on *Fusarium oxysporum* mycelial growth using three extraction methods *in vitro*

Plant extract conc.	Extraction method					
	70% ethanol [A]		boiling in water [B]		wet autoclaving [C]	
	plant extract	AgNPs	pla extract	AgNPs	Plant extract	AgNPs
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	8.6 ± 1.1	6.58 ± 1.8	9.05 ± 0.7	15.57 ± 1.1	2.05 ± 0.5	6.93 ± 0.5
20	17.35 ± 0.12	18.93 ± 0.7	17.28 ± 0.7	19.66 ± 0.5	7.81 ± 0.5	16.32 ± 0.8
40	21.48 ± 1.2	22.22 ± 1.2	21.39 ± 0.7	34.83 ± 1.3	13.16 ± 0.01	26.92 ± 0.1
80	36.36 ± 0.6	29.62 ± 2.4	27.98 ± 0.7	47.54 ± 0.6	20.16 ± 0.1	35.50 ± 0.0
150	40.9 ± 0.2	46.09 ± 0.7	31.27 ± 0.7	61.87 ± 1.4	23.86 ± 0.05	44.89 ± 0.1
200	62.39 ± 1.5	71.19 ± 2.8	55.55 ± 1.2	71.30 ± 0.6	29.21 ± 0.05	53.47 ± 0.1
Fungicide*	76.92 ± 2.9	82.52 ± 3.01	66.76 ± 1.9	79.65 ± 1.99	59.24 ± 1.11	68.38 ± 0.9

The values in the table above were the average of three replicates for % of inhibition of mycelial growth followed by "±" was standard error. Values were significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test of two-way ANOVA, *fungicide used was Tachigaren -30% W/P

The percentages of inhibition were increased by increasing the concentration of treatments. The percentages of inhibition were 2.05% ± 0.5, 7.81% ± 0.5, 13.16% ± 0.01, 20.16% ± 0.1, 23.86% ± 0.05 and 29.21% ± 0.05 for 10, 20, 40, 80, 150, 200 µl ·10 ml^{-1} of PDA media, respectively. At 200 µl ·10 ml^{-1} of PDA media, the percent of inhibition was only about 30%, followed by 150 µl ·10 ml^{-1} of PDA media of about 24% when compared to Tachigaren-30% plates 59% (Table 1).

In the present study there were highly significant differences between extraction methods for mycelium growth inhibition (%) using green AgNPs solutions of *Zygophyllum album*. All treatments illustrated different degrees of inhibition. The percentage of inhibition increased by increasing treatment concentration. The percentages of inhibition of green AgNPs solution of *Z. album* prepared by boiling in water for 10 min. (B) were 15.57% ± 1.1, 19.66% ± 0.5, 34.83% ± 1.3, 47.54% ± 0.6, 61.87% ± 1.4 and 71.30% ± 0.6 for 10, 20, 40, 80, 150, 200 µl ·10 ml^{-1} of PDA media, respectively. The results indicated that the optimal concentration for achieving the start transient inhibition was 80 µl ·10 ml^{-1} of PDA media with 47.54 ± 0.6 (Table 1). Also, we found that the mycelial growth inhibition increased with greater concentrations of the solution, which had obvious effects on inhibition efficiency by the 70% ethanol method (A). The percentages of inhibition were 6.58% ± 1.8, 18.93% ± 0.7, 22.22% ± 1.2, 29.62% ± 2.4, 46.09% ± 0.7 and 71.19 ± 2.8 for 10, 20, 40, 80, 150, 200 µl ·10 ml^{-1} of PDA media, respectively, with decreased growth of mycelial compared to control plates. The highest percent of inhibition appeared at 200 µl ·10 ml^{-1} of PDA media (more than 70%), followed by 150 µl ·10 ml^{-1} of PDA media (Table 1). The green AgNPs solution of *Z. album* prepared by wet autoclaving for 5 min. (C) clearly showed that the percentages of inhibition were 6.93% ± 0.5, 16.32% ± 0.8, 26.92% ± 0.1, 35.50% ± 0.0, 44.89% ± 0.1 and 53.47% ± 0.1 for 10, 20, 40, 80, 150, 200 µl ·10 ml^{-1} of PDA media, respectively (Table 1). Our work illustrated that the antifungal activity of *Z. album* plant extract prepared by 70% ethanol (A) was more effective against *F. oxysporum* than boiling in water for 10 min. (B) and wet autoclaving for 5 min. (C). The green AgNPs revealed that there were highly significant differences between boiling in water for 10 min. (B) and AgNPs prepared by 70% ethanol (A) and wet autoclaving for 5 min. (C), respectively (Table 1 and Table 2). This can be explained by stating that the nano-size of AgNPs play an important role in the effect against *F. oxysporum*. Prepared solutions of AgNPs from plant extracts obtained by various extraction methods were effective in preparing a fungicidal solution against *F. oxysporium*. It caused more than 50% inhibition of *F. oxysporum* mycelial growth in the case of AgNPs from plant extract by wet autoclaving for 5 min. and reached up to 70% in the case of AgNPs plant extract obtained by boiling in water for 5 min. and more than 70% in the case of AgNPs from ethanol plant extract. Our results showed that the extraction methods led to significant differences in the extract efficacy against *F. oxysporum* mycelial growth. There were significant differences in the impacts of various nanoparticles depending on the extraction methods. An evaluation of the fungal diameter, showed that there was a highly significant difference between the various extraction techniques. Increasing plant content was more efficient for fungal suppression (Table 1 and Table 2). Plant pests can cause significant annual reduction that may reach up to 40% of crop production worldwide. Thirteen percent of these losses may be attributed to plant pathogens (Flood 2010). Pest management still relies mainly on the use of different forms of pesticides. Various harmful effects have been uncovered for these

Table 2. Effect of green silver nanoparticles and plant extract and extraction method on *Fusarium oxysporum* mycelial growth diameter (mm)

Plant extract conc.	Extraction method					
	70% ethanol [A]		boiling in water [B]		wet autoclaving [C]	
	plant extract	AgNPs	plant extract	AgNPs	plant extract	AgNPs
0	8.07 ± 0.03 p	8.10 ± 0.0 p	8.10 ± 0.0 p	8.13 ± 0.03 p	8.10 ± 0.0 p	8.17 ± 0.03 p
10	7.37 ± 0.03 n	7.57 ± 0.09 o	7.37 ± 0.03 n	6.87 ± 0.03 mn	7.93 ± 0.03 p	7.60 ± 0.00 o
20	6.67 ± 0.04 m	6.57 ± 0.03 l	6.70 ± 0.06 m	6.53 ± 0.03 l	7.47 ± 0.03 n	6.83 ± 0.03 m
40	6.33 ± 0.03 k	6.30 ± 0.06 k	6.37 ± 0.03 k	5.30 ± 0.06 h	7.03 ± 0.03 mn	5.97 ± 0.09 j
80	5.13 ± 0.03 g	5.70 ± 0.12 ij	5.83 ± 0.04 ij	4.27 ± 0.03 d	6.47 ± 0.07 kl	5.27 ± 0.03 h
150	4.77 ± 0.02 f	4.37 ± 0.03 d	5.57 ± 0.03 i	3.10 ± 0.06 b	6.17 ± 0.03 j	4.50 ± 0.06 e
200	3.03 ± 0.07 b	2.33 ± 0.13 a**	3.60 ± 0.06 c	2.33 ± 0.07 a**	5.73 ± 0.03 i	3.80 ± 0.06 c
Fungicide*	2.45 ± 0.02	1.95 ± 0.0	2.99 ± 0.06	1.96 ± 0.10	3.87 ± 0.04	2.79 ± 0.03

Mean values with the same letter are not significant at $p \leq 0.05$. Mean values with (**) represent highly efficient concentration and method of extraction for fungal inhibition at $p \leq 0.001$; *fungicide used was Tachigaren -30% W/P

chemical pesticides such as effects against non-target organisms, the development of resistance to this disease, and the resurgence of disease populations. In addition, some researchers recorded that 90% of applied pesticides may be lost and never contact the target organisms (Soylu *et al.* 2010). Therefore, nanotechnology, provides new insights into the development of high performing fungicides that are more cost-efficient and less harmful to the environment.

In this study, three different methods were described for biosynthesis of AgNPs prepared from *Z. album* plant extract. Each method led to the production of a different size and formation of the nanoparticles. This variation led to a significant difference in the antifungal efficacy of each nanoparticle product. The observed increase in efficacy of nanopesticides could be due to the fact that the larger the surface area, the smaller the particle size in contact with the microorganism.

The highest percent of inhibition appeared at 200 µl ·10 ml^{-1} of PDA media where it was more than 70%, and it was more than 70% for two AgNPs prepared from A and B, respectively, followed by 150 µl ·10 ml^{-1} of PDA media. Green AgNPs solution of *Z. album* prepared by wet autoclaving plant extract for 5 min. illustrated antifungal activity against *F. oxysporum*. The percentage of inhibition increased by increasing the concentration of AgNPs solution in media. It was 50% and followed by 150 µl ·10 ml^{-1} of PDA media. This result agreed with El-Argawy *et al.* (2017). Antifungal activity of green AgNPs was greater than that of plant extract. This result is in agreement with Ali *et al.* (2015).

Finally, this study illustrated that green synthesis of AgNPs is rapid, dependable, non-toxic and hence, very promising. Our findings revealed that *Z. album* L. extract had higher antioxidant activity. These findings could be attributed to the higher levels of flavonoids and phenolic compounds found in plant extract because flavonoids and phenolic compounds in general have high antioxidant activity.

Conclusions

Green AgNPs synthesis methods based on microorganisms are inexpensive, simple, completely safe, and environmentally friendly. In fact, the synthesis of NPs from microbes has aided in the advancement of nanotechnology research.

In this study, a crude extract of *Z. album* was prepared. The antifungal activity of *Z. album* plant was determined in vitro using 70% ethanol, boiling in water, and wet autoclaving. Our results showed that *Z. album* possessed significant antifungal activity, which may be due to its high phenolic and flavonoid content. Furthermore, biosynthesized AgNPs were successfully prepared using a crude extract of *Z. album*. All of the extracts yielded AgNPs with small particle sizes, and the colloidal dispersions were stable with a high surface charge. It may be an alternative to conventional fungicides in controlling *F. oxysporum* wilt infection of plants. The AgNPs synthesis method developed in this study can be widely used in the synthesis of other noble metal NPs. Furthermore, plant extracts and AgNPs have great antifungal potential, making them an excellent source for the development of new herbal medicine compounds that can help humanity. It is hoped that the use of plant extract for the synthesis of nanoparticles will have a significant impact in the coming decades. The conjugation of silver nanoparticles with other control practices may enable us to construct an effective integrated management program for the control of some hard-to-eradicate diseases such as *Fusarium* wilt.

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