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SYNERGISTIC EFFECT OF ESSENTIAL OILS AND SILVER NANOPARTICLES SYNTHESIZED USING GELATIN FOR ANTIBACTERIAL, SOIL RESPIRATION AND SOIL ENZYME ACTIVITIES

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ABSTRACT: This study aims to investigate the synthesis of gelatin (Gel) and gelatin-glucose (Gel-Glu) mediated silver nanoparticles (AgNPs) and to investigate their synergies with different essential oils (EO) for antibacterial activity as well as their effects on soil respiration and soil enzyme activities. The antibacterial activities were evaluated using the agar diffusion test. The results of STEM analysis revealed that Gel-Glu-AgNPs in the range of 5–25 nm had a smaller size than Gel-AgNPs. Furthermore, we found that both AgNPs were positively charged by zeta analysis. In addition, at least one of the combinations of Gel-AgNPs and Gel-Glu-AgNPs with EO increased the antibacterial activity. The results also showed that AgNPs reduced soil respiration at the end of 120 h and that combinations of AgNPs and essential oils caused a significant reduction in alkaline phosphatase activities of soil samples compared to dehydrogenase activity, particularly at higher exposure times and concentrations. In conclusion, gelatin played an important role as a reducing and stabilizing agent in the synthesis of AgNPs. Finally, it was evaluated that combining nanoparticles and essential oil led to different results in the interaction of AgNPs with bacteria, which was additionally confirmed by soil respiration and enzyme analysis. The results justify further developing new strategies to uncover the effects of silver nanoparticles in different applications.

Keywords: *Silver nanoparticles, Gelatin, Essential oils, Antibacterial activity, Soil enzymes*

Jelatin Kullanılarak Sentezlenen Gümüş Nanopartiküller ile Esansiyel Yağların Antibakteriyel, Toprak Solunum Ve Toprak Enzim Aktivitelerinde Sinerjistik Etkisi

ÖZ: Bu çalışma, jelatin (Jel) ve jelatin-glukoz (Gel-Glu) aracılı gümüş nanopartiküllerin (AgNP'ler) sentezini araştırmayı ve bunların esansiyel yağlar (EO) ile sinerjilerinin toprak solunumu ve toprak enzim aktiviteleri üzerine etkilerini araştırmayı amaçlamaktadır. Antibakteriyel aktiviteler, agar difüzyon testi kullanılarak değerlendirildi. STEM analizinin sonuçları, 5-25 nm aralığındaki Gel-Glu-AgNP'lerin Gel-AgNP'lerden daha küçük bir boyuta sahip olduğunu ortaya koydu. Buna ilaveten, zeta analizi ile her iki AgNP'nin de pozitif yüke sahip olduğunu bulduk. Ayrıca, Gel-AgNP'ler ve Gel-Glu-AgNP'lerin EO ile kombinasyonlarından en az biri antibakteriyel aktiviteyi arttırdı. Sonuçlar, AgNP'lerin 120 saat sonunda toprak solunumunu azalttığını ve AgNP'ler ile uçucu yağların kombinasyonlarının, özellikle daha yüksek maruz kalma süreleri ve konsantrasyonlarında, dehidrogenaz aktivitesine kıyasla toprak numunelerinin alkalin fosfataz aktivitelerinde önemli bir azalmaya neden olduğunu gösterdi. Sonuç olarak jelatin, AgNP'lerin sentezinde indirgeyici ve stabilize edici bir ajan olarak önemli bir rol oynamıştır. Son olarak, nanopartiküller ve uçucu yağ kombinasyonunun, AgNP'lerin bakterilerle etkileşiminde farklı sonuçlara yol açtığı değerlendirilmiş, ayrıca bu toprak solunumu ve enzim analizi ile de doğrulanmıştır. Elde edilen sonuçlar, farklı uygulamalarda gümüş nanopartiküllerin etkilerini ortaya çıkarmak için yeni stratejilerin daha da geliştirilmesini doğrulamaktadır.

Anahtar Kelimler: *Gümüş nanopartiküller, Jelatin, Uçucu yağlar, Antibakteriyel aktivite, Toprak enzimleri*

1. INTRODUCTION

Nanomaterials show distinctive physicochemical properties such as ultrasmall dimensions, high surface area-to-mass ratio and high chemical reactivity, so their applications in many fields are attracting attention (Song and Ge, 2019). Nanoparticles can be broadly classified as organic, inorganic, and carbon-based. Nanoparticles synthesized from nanometer-sized metals by either destructive or constructive methods are metal-based nanoparticles characterized by their small size and high surface area. Almost all metals can be synthesized into nanoparticles (Ealia and Saravanakumar, 2017; Jeevanandam et al., 2018). Thanks to their broad bactericidal activity and physicochemical properties against gram-negative and gram-positive bacteria, silver nanoparticles (AgNPs) are one of the most widely applied metallic nanoparticles for modern antimicrobial purposes (Burdușel et al., 2018; Gurunathan et al., 2014; Morones-Ramirez et al., 2013). Cationic silver, Ag^+, is a popular bactericide and its utilization in various fields also generates increased negative effects on microscopic biota (Wijnhoven et al., 2009). Additionally, AgNPs can eliminate multiple drug-resistant strains and inhibit biofilm formation, showing significant potential for antibacterial applications (Yun'an Qing et al., 2018) On the other hand, studies on the toxicity of AgNPs in terms of antibacterial activity have yielded mixed results. While most studies have found that Ag^+ released from nanoparticles is the main chemical species causing toxicity, some studies have argued that nanoparticles exert a toxic effect by causing precise effects such as oxidative stress (Aruguete and Hochella, 2010; Fabrega et al., 2009).

Soil enzymes have biochemical and microbiological functions as soil health sensors (Caldwell, 2005). The biological effects of nanoparticles in the soil largely depend on the physicochemical properties of the soil(Cornelis et al., 2014). As a result of their existence and accumulation in soil, the effect of AgNPs on the soil ecosystem is becoming an increasingly popular research area. It is important to understand activity variation in soil enzymes, especially to unveil the effectiveness of nanoparticles on soil biology(Peyrot et al., 2014). Microbial diversity can be expressed by soil enzymes, which have functional importance in various biogeochemical processes and metabolic pathways(Nannipieri et al., 2002). Contamination soils with AgNPs can affect many microorganisms in the soil directly but also lead to indirect effects through their action on soil enzymes. The overall effects of AgNP contamination in soils may be seen in nutrient cycling and many microbially mediated biogeochemical processes (Peyrot et al., 2014).

Green synthesis has attracted considerable interest in materials science as a reliable, sustainable, and environmentally friendly protocol for the synthesis of a variety of nanomaterials, such as metal/metal oxide nanomaterials (Erci and Cakir-Koc, 2021; Erci et al., 2020, 2018; Erci and Torlak, 2019). Therefore, green synthesis is recognized as an important tool that is extensively used in laboratories and industry to lessen the harmful effects associated with traditional nanoparticle synthesis methods(Singh et al., 2018). It is important to synthesize the synthesized silver nanoparticles at cheaper rates for their effective use by humans. An environmentally and economically viable synthesis method is needed to obtain these nanoparticles (Prabhu and Poulose, 2012). Since the amino acids, proteins, or secondary metabolites present in the biological synthesis of nanoparticles play an active role in preventing particle aggregation, the additional steps required for this process might be eliminated (Zhang et al., 2016). Gelatin, a natural biopolymer, is a preferred material for polymer implants due to its non-immunogenicity, biodegradability, and biocompatibility (Rath et al., 2016). Gelatin is typically made up of non-polar aliphatic amino acids such as glycine, proline, alanine, and hydroxyproline. The hydroxyl group of hydroxyproline facilitates $AgNO_3$ reduction, while non-polar amino acids promote the stability of AgNPs (Nagarajan et al., 2012; Yiwei et al., 2007). Herein we aimed to develop a framework to study the production of silver nanoparticles (AgNP) synthesized using gelatin and the synergistic activities of different essential oils against some bacterial strains. At the same time, as a further goal, the investigation of the effect of synthesized AgNPs on soil respiration with soil enzymes was carried out.

2. MATERIALS AND METHODS

2.1. Gelatin-mediated synthesis of silver nanoparticles

Gelatin (bovine skin, type B powder, catalog no. G6650), glucose (catalog no. G7528), and silver nitrate (catalog no. 209139) used to synthesize AgNPs were obtained from from Sigma-Aldrich. Clove (*Eugenia caryophyllata*) essential oil (CEO) and peppermint (*Mentha piperita*) essential oil (PEO) were purchased as a commercial product in a volume of 10 mL (100%) (Mecitefendi, Turkey). All glass containers used in the experiment were washed with distilled water before use. To prepare Gel-AgNPs, gelatin solution was used to reduce $AgNO_3$ to AgNPs. Briefly, an aqueous gelatin solution (1 g, 100 mL) was prepared by stirring on a magnetic stirrer for 30 min. To prepare Gel-Glu-AgNPs, 60 mg of glucose was added to the gelatin solution. Then a solution of 27 mg $AgNO_3$ (in 1 mL of ddH_2O) was added to the gelatin-glucose solution. Synthesis of Gel-AgNPs was completed using a household microwave for 25 min (Arçelik, MD 574, Turkey), while the synthesis of Gel-Glu-AgNPs was achieved at 80 °C for 90 min.

2.2. Characterization of AgNPs

UV-Visible (UV-Vis) spectra of the synthesized Gel-AgNPs were recorded with a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) in the wavelength range of 200–800 nm. The Fourier transform infrared spectroscopy (FTIR) examination was performed using a Shimadzu IR Prestige-21 FTIR-ATR spectrometer in the 400–4000 cm^{-1}. An X-ray diffraction (XRD) system (PANalytical Empyrean XRD) was used to determine the crystal structure of Gel-AgNPs in the range of 20-80 degrees and a step size of 0.02. The chemical composition of the synthesized Gel-AgNPs was analyzed using an EDS analyzer in conjunction with scanning electron microscopy (SEM, SU 1510, Hitachi High-Technologies, Japan). Simultaneously, morphology and size analyses of AgNPs were investigated in Scanning Transmission Electron Microscopy (STEM) mode on the ZEISS Gemini SEM 500 instrument (Carl Zeiss Co., LTD, Shanghai, China). Furthermore, Gel-AgNPs were diluted 1:2 with ultrapure water to define the average surface charge of Gel-AgNPs using a Malvern ZEN 3600 Nano ZS Zetasizer (Malvern Instruments, UK) and the measurement was performed at 633 nm wavelength and 173º retroreflection angle. The size distribution of the AgNPs synthesized on the same instrument was determined using dynamic light scattering (DLS).

2.3. Antibacterial activity of AgNPs

The microorganisms used in the study (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 25922) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Antibacterial activity was evaluated using the agar diffusion test. Bacterial cultures were adjusted to 0.5 McFarland standard, corresponding to a bacterial suspension of approximately 1×10^8 and 2×10^8 colony forming units (CFU/mL). 50 µL of the nanoparticle samples (400 µg/mL) and essential oils were used in each of the 7 mm diameter wells made on Mueller Hinton agar (MHA, Lab M, UK) plates. The essential oils provided were taken as 62.5 µL and completed with 1 mL of deionized water to obtain the working concentration. For synergistic activity, an equal volume of the mixture of nanoparticles and essential oils was mixed in a magnetic stirrer for one hour, and 50 µL of this mixture was added to each well. The diameter of the zones of inhibition (mm) was then measured using calipers after incubating the plates at 37° C. for 24 h. The experiments were carried out in triplicate.

2.4. Preparation of test soil and determination of soil properties

The soil samples used in the study were taken from the surface (0-30 cm depth) from 20 different locations on the campus area of Selçuk University in Konya Province, Turkey. After the soils were

thoroughly mixed, the residues were removed. After sampling, the soil samples were kept at 4 °C for biological analysis. Air-dried soil samples were passed across a 2 mm diameter sieve so that they were used for some physical and chemical analyses. 1 kg of fresh soil samples were placed in pots.

Soil properties were analyzed according to established standards. The Bouyoucos hydrometer method was used to determine the soil fractions as sand (50−2000 μm), silt (20−50 μm), and clay (<2 μm) (Gee and Bauder, 1986). The amount of organic matter was measured by applying Dumas dry combustion method via a LECO CN-2000 instrument (Wright and Bailey, 2001). Scheibler Calcimeter was used to measure calcium carbonate ($CaCO_3$) in soil (Nelson and Sommers, 1982). The soil aggregate stability was determined with artificial rainfall-simulation equipment (Gugino et al., 2009). Field capacity (FC) was determined as the percentage of moisture retained in soil under 33 kPa pressure. In comparison, the permanent wilting point (PWP) was calculated as a percentage of moisture retained in the soil at 1500 kPa pressure using a pressure plate apparatus (Klute, 1986). Then the available water capacity (AWC) was obtained by subtracting the PWP from the FC. Finally, pH and EC of the soil sample were measured in a 1:2.5 mixture (w/v) soil/pure water using a glass electrode on a digital pH meter and a conductivity meter, respectively (McLean, 1983; Rhoades, 1982). Soil properties are summarized in Table 1.

Table 1. Physiochemical properties of the soil samples

Physiochemical Properties	**Soil Mixture**
pH (1:2,5 soil: Water)	7.65
OM (%)	0.72
$CaCO_3$ (%)	16.20
EC (1:2,5 soil: Water) μ mhos cm^{-1}	1756
AS (%)	17.70
FC (%)	24.60
PWP (%)	12.80
AWC (%)	11.80

Abbreviations: OM, organic matter; EC, electrical conductivity; AS, aggregate stability; FC, field capacity; PWP, permanent wilting point; AWC, available water capacity

2.5. Measurement of soil respiration

Gel-AgNPs, Gel-Glu-AgNPs, clove essential oil, and peppermint essential oils and their combinations at a concentration of 0.2, 0.4, and 0.6 mL 250 g^{-1} dry soil were applied to soil samples in pots. The nanoparticle concentrations for volumes of 0.2, 0.4 and 0.6 mL were 200, 400 and 600 μg, respectively. An equal volume of the mixture of nanoparticles and essential oils was mixed in a magnetic stirrer for one hour and applied to soil samples as the same concentration to study the synergistic effect. Later, the water content of the soil was brought up to the determined field capacity and then mixed and incubated for the indicated periods. Soil samples in pots were taken for enzyme analysis after 24, 120 and 240 h. Only samples at the end of 120 h were evaluated for soil respiration. "The Cornell Soil Health Assessment Training Manual" was followed to assess the effect of the applications on soil respiration (Moebius-Clune et al., 2016). Briefly, the soil samples (20 g), air dried and sieved through a 2 mm sieve, were placed in a perforated metal container with filter paper at the bottom of a jar. The soil was incubated at 28°C for 96 h with 9 mL of 0.5 M potassium hydroxide (KOH) and 7.5 mL of deionized water. Only deionized water

was added to the control samples. Electrical conductivity (EC) measurements were then taken using an EC probe to determine the EC of the KOH trap for each sample, followed by a calculation in mg CO_2-C g^{-1} soil.

2.6. Effect of nanoparticles on the activities of soil enzymes

To evaluate the effect of applications on soil dehydrogenase and alkaline phosphatase activities, soil samples were first weighed and treated with samples in different doses of Gel-AgNPs, Gel-Glu-AgNPs, clove essential oil, and peppermint essential oil and their combinations. At the same time, the control group without samples was also incubated.

Enzyme activities in soil samples were assessed using colorimetric methods at the end of the 24, 120, and 240 h. Dehydrogenase and alkaline phosphatase activities were analyzed based on studies in the literature (Tabatabai and Bremner, 1969; Thalmann, 1968). Briefly, 1.0 g and 6.0 g of soil were transferred to a glass tube for alkaline phosphatase and dehydrogenase activity, respectively, and then suspended in buffered substrate solutions. To determine the alkaline phosphatase activity of the soils, 4 mL of MUB buffer (pH 11) and 1 mL of substrate solution (0.025 M p-nitrophenyl phosphate) were added to a 1 g soil sample and incubated at 37 °C for 1 h. After 15 min of centrifugation at 3500 rpm, 1 mL of 0.5 M $CaCl_2$ and 4 mL of THAMNaOH solution were added. Then the alkaline phosphatase activity was determined as an optical density in a spectrophotometer at 410 nm according to p-nitrophenol standard solutions. The results obtained were expressed as μg p-nitrophenol g^{-1} dry soil. For dehydrogenase activity, 6 g fresh soil samples were mixed with 1 mL of substrate solution (3% TTC (2,3,5-triphenyl tetrazolium chloride) and 2.5 mL of glucose solution (1.2%) and then incubated at 25 °C for 24 h. At the end of the incubation, the samples were completed with 10 mL of methanol, followed by filtration through Whatman 42 filter paper. After washing 3-4 times, the tubes were filled with methanol to a final volume of 50 mL. The optical densities of the samples were measured in a microplate reader using a spectrophotometer at a wavelength of 485 nm corresponding to standard TPF (triphenylformazine solution). The data obtained were expressed as μgTPF g^{-1} dry soil 24 h^{-1}. All these applications were performed in 3 repetitions.

3. RESULTS and DISCUSSIONS

3.1. Synthesis and characterization of AgNPs

In the current study, the synthesis of silver nanoparticles (AgNP) was successfully achieved by using gelatin as both a reducing and stabilizing agent. The reduction of $AgNO_3$ to AgNPs was confirmed by forming a reddish-brown color, a unique optical property for AgNPs. To monitor the synthesis of gelatin AgNPs, absorbance measurements were performed by UV-Vis spectroscopy at wavelengths between 200 and 800 nm. The synthesis of Gel-AgNPs carried out in the microwave was completed after 25 min, while the synthesis of Gel-Glu-AgNPs was completed at 80 °C for 90 min. As seen in Figure 1, the obtained characteristic peaks of Gel-AgNPs and Gel-Glu-AgNPs recorded at 446 and 435 nm, respectively, confirmed the presence of Gel-AgNPs and Gel-Glu-AgNPs. Furthermore, when comparing the results of this work with published data, good agreement was found (Behravan et al., 2019).

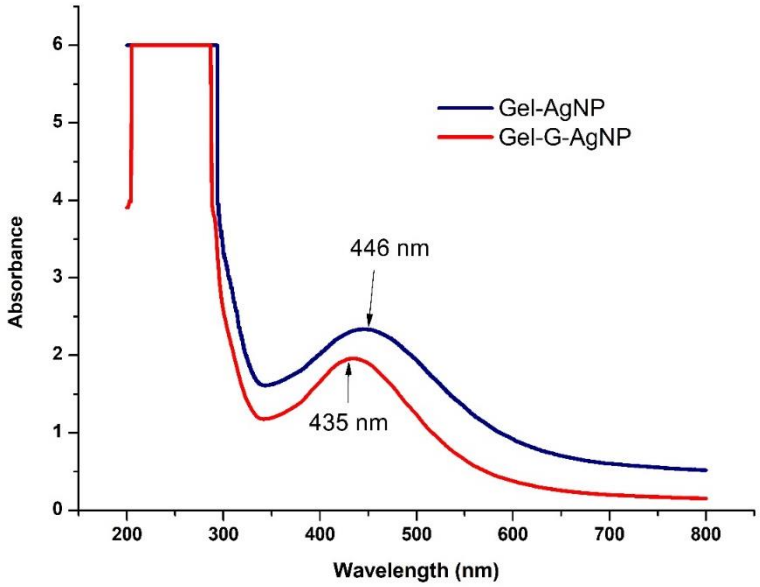

Figure 1. UV−Vis spectra of Gel-AgNPs and Gel-Glu-AgNPs.

Characterization by FTIR was performed to detect functional groups present in Gel-AgNPs and Gel-Glu-AgNPs (Figure 2). The bands of the FTIR spectra for Gel-AgNPs were recorded at 3338.78, 2987.74, 1643.35, 1504.48, 1226.73 and 1064.71 cm^{-1}. The FTIR analysis of Gel-Glu-AgNPs revealed the spectrum at 3358.07, 1643.35, 1516.05 1192.04, and 1066.64 cm^{-1}. In the spectrum of Gel-AgNPs, broadband at 3338 cm^{-1} corresponding to hydrogen bonding and NH stretching was observed (Aewsiri et al., 2009). The strong absorption peak at 1643 cm^{-1} indicated the C=O stretching vibration (Mahmoud and Abbo, 2013). Since gelatin is a polyamide protein, the peaks at 1643 and 1504 cm^{-1} indicated the existence of amide I and II groups of the proteins. These arise owing to the electrostatic interaction between AgNP and gelatin. The related peaks confirmed that the silver nanoparticles were coated with gelatin (Burt et al., 2004).

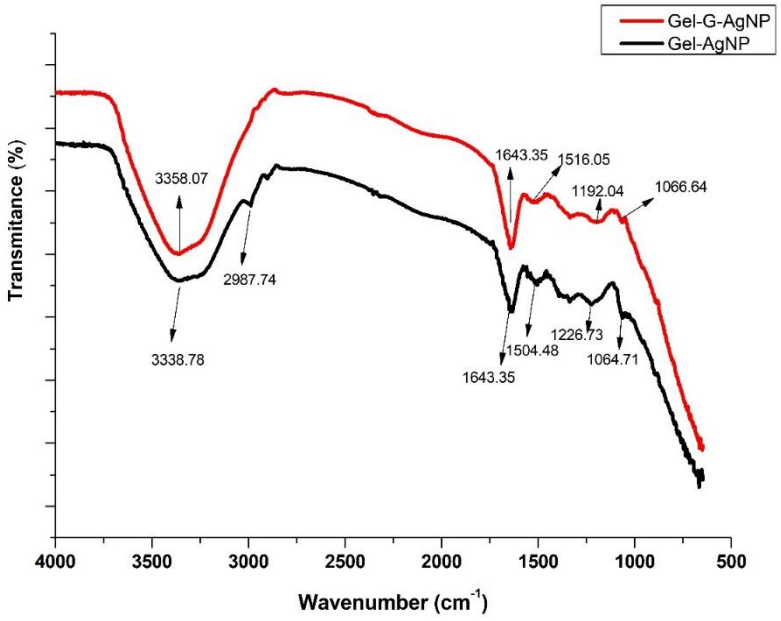

Figure 2. FTIR spectra of Gel-AgNPs and Gel-Glu-AgNPs.

In addition, the nanoparticles were characterized by XRD to reveal the crystal and nanostructure of AgNPs. The characteristic peaks observed in the XRD analysis further confirmed the formation of Gel-

AgNPs (Figure 3). Four different diffraction peaks of 38.51, 46.60, 64.78, and 77.72 corresponding to the crystallographic planes (111), (200), (220), and (311) indicated that silver nanoparticles were crystalline (Su et al., 2017). In addition, RD analysis showed a peak at 38.51 (2θ), indicating that the nanoparticles consisted of pure silver (Yılmaz Öztürk et al., 2020). Some unassigned peaks were also detected, indicating that the crystallization of the organic phase occurred on the surface of the silver nanoparticles.

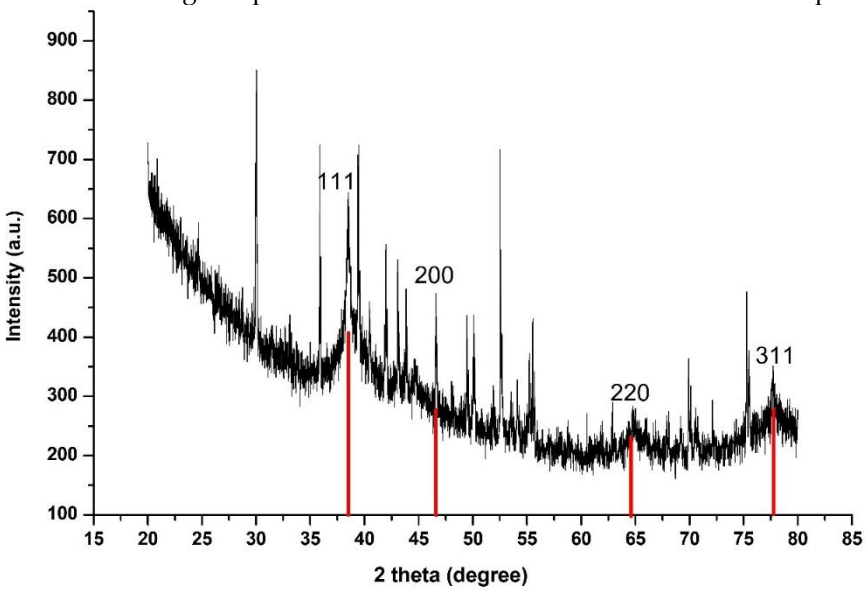

Figure 3. X-ray diffraction patterns of Gel-AgNPs.

As seen in Figure 4, EDS analysis of Gel-AgNPs revealed that the existence of high peaks indicates elemental silver with traces of other elements. The typical optical absorption peak at 3 keV is characteristic of metallic silver nanoparticles because of surface plasmon resonances (Gomaa, 2017). The appearance of C, N, and O-specific peaks is due to different components playing a role as stabilizing or capping agents in forming AgNPs (Galvez et al., 2021).

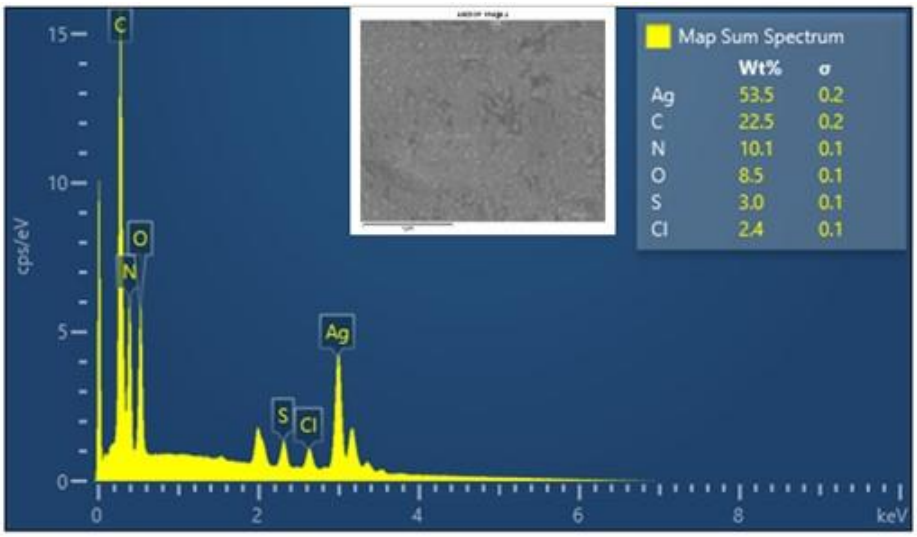

Figure 4. Energy dispersive spectroscopy (EDS) characterization of silver nanoparticles.

The STEM analyzes allowed us to obtain information about the shape and size structure of AgNPs (Figure 5). The size measurements determined that the sizes of Gel-AgNPs varied between 20 and 110 nm, while the sizes of Gel-Glu-AgNPs varied between 5-25 nm. The addition of glucose into the gelatin solution caused smaller silver nanoparticles. The results showed that the synthesized silver nanoparticles

had a spherical shape with a homogeneous distribution. The results of the zeta potential measurement showed that the synthesized Gel-AgNPs had a zeta potential of 5.21 (mV) (Figure 6a).

On the other hand, as shown in Figure 6c, Gel-Glu-AgNPs were found to have a zeta potential of 6.35 (mV). The zeta potential value of gel AgNPs could be caused by positively charged fragments on the gelatin surface. Also, the smaller zeta potential value of AgNPs showed chelating agents from cationic groups and non-ionic chelating gelatin groups (Pourjavadi and Soleyman, 2011).

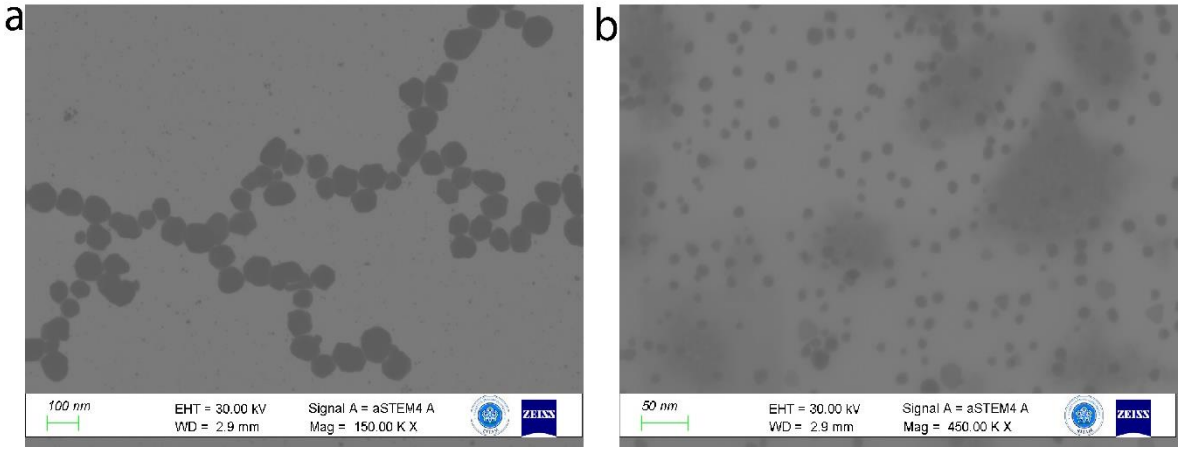

Figure 5. STEM micrographs of silver nanoparticles (**a**) Gel-AgNPs; (**b**) Gel-Glu-AgNPs.

The size and distribution of the Gel-AgNP and Gel-Glu-AgNPs were measured by dynamic light scattering (DLS), as shown in Figures 6b and 6d. DLS analysis revealed that the Gel-AgNPs were 158.4 nm in size and their polydispersity index (PDI) was 0.221. The polydispersity index is dimensionless and values above 0.7 indicate that the sample may have abroad size distribution (Nasiriboroumand et al., 2018). Gel-Glu-AgNPs were found to be 151.4 nm in size, and the polydispersity index (PDI) value was 0.224. The results of the DLS analyzes showed that Gel-AgNPs had a narrow size distribution compared to Gel-Glu-AgNPs.

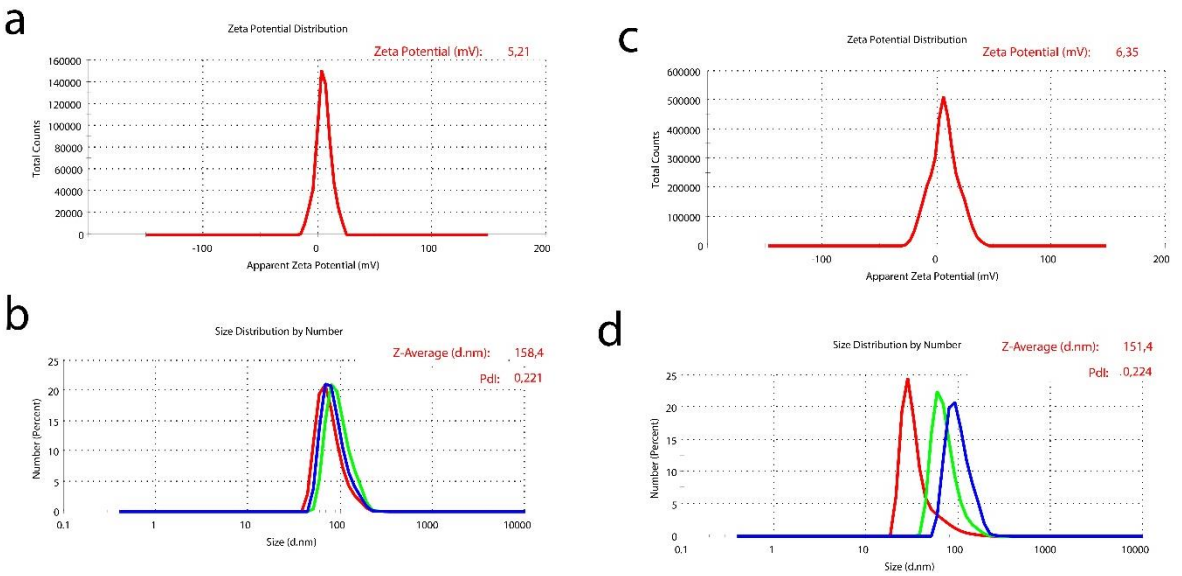

Figure 6. Zeta potential and DLS analysis of AgNPS (**a**) Zeta potential of Gel-AgNPs (**b**) Size distribution and PDI of Gel-AgNPs (**c**) Zeta potential of Gel-Glu-AgNPs (**d**) Size distribution and PDI of Gel-Glu-AgNPs.

3.2. Antibacterial activities of synthesized silver nanoparticles and synergistic effect with essential oils

The antibacterial activity of AgNPs against Gram-positive and Gram-negative bacteria was investigated using the agar diffusion method. A total of 50 μL of the samples was added to the 7 mm diameter well created on the agar plates. The diameter (mm) of the zone of inhibition formed around the wells was measured.

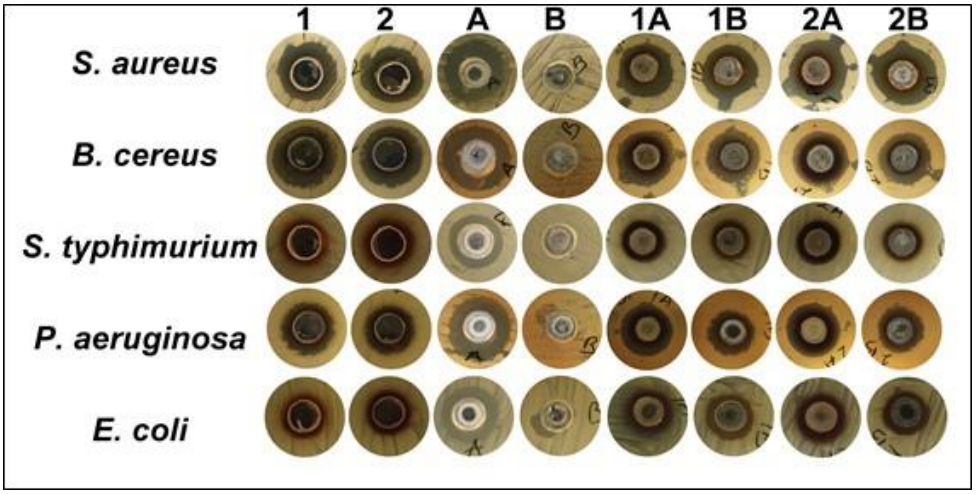

Figure 7. Inhibition zones of the silver nanoparticles. (**1**) Gel-AgNPs (**2**) Gel-Glu-AgNPs (**A**) CEO (**B**) PEO.

As seen from Figure 7 and Table 2, Gel-AgNPs showed a zone of inhibition of 17.88 ± 0.60 against *S. aureus*, while CEO showed a zone of inhibition of 18.49 ± 0.53. No inhibition was observed in PEO at the relevant concentration. The gel AgNP and CEO combination was found to have a zone of inhibition of 18.18 ± 0.33. Gel-Glu-AgNPs were found to show a synergistic effect with both clove and PEO with zones of inhibition of 19.37 ± 0.16 and 19.60 ± 0.53, respectively. Gel AgNPs against *B. cereus* were found to have a zone of inhibition of 16.79 ± 0.27 at the indicated concentration, while CEO had a zone of inhibition of 18.67 ± 0.28. It was shown that gel-AgNP-CEO and gel-AgNP-PEO formed a zone of inhibition of 20.04 ± 1.72 and 18.81 ± 0.46 and showed a synergistic effect against *B. cereus*. No inhibition against *B. cereus* was observed at the appropriate concentration of PEO.

On the other hand, Gel-Glu-AgNPs have been found to have a synergistic effect on both CEO and PEO. Gel-AgNPs were observed to show synergistic activity with CEO and PEO by showing 14.65 ± 0.17 and 12.87 ± 0.51 zones of inhibition against *S. typhimurium*, respectively. Furthermore, Gel-Glu-AgNPs have been shown to have synergistic activity with both essential oils. On the other hand, PEO showed no inhibition against *S. typhimurium* at the relevant concentration.

Table 2. Inhibition zones of the silver nanoparticles against tested microorganisms by agar well diffusion method. Each data point (diameter) in mm was expressed in terms of mean ± standard deviation (mean ± SD). (**1**) Gel-AgNPs (**2**) Gel-Glu-AgNPs (**A**) CEO (**B**) PEO

Samples	*S. aureus*	*B. cereus*	*S. typhimurium*	*P. aeruginosa*	*E. coli*
1	17.86 ± 0.61	16.80 ± 0.28	12.39 ± 0.17	15.80 ± 0.19	12.09 ± 0.29
2	18.02 ± 1.33	16.90 ± 0.36	12.29 ± 0.15	14.78 ± 0.13	12.59 ± 0.40
A	18.49 ± 0.54	18.68 ± 0.29	15.20 ± 0.40	20.02 ± 0.34	16.64 ± 0.23
B	ND	ND	ND	ND	ND
1A	18.19 ± 0.33	20.05 ± 1.73	14.65 ± 0.18	18.70 ± 0.49	13.72 ± 0.38
1B	17.94 ± 0.15	18.81 ± 0.46	12.87 ± 0.52	16.11 ± 0.75	14.28 ± 0.21
2A	19.38 ± 0.16	19.28 ± 0.35	15.15 ± 0.16	19.46 ± 0.25	15.63 ± 0.38
2B	19.60 ± 0.54	18.10 ± 0.38	13.02 ± 0.16	16.36 ± 1.11	13.28 ± 0.20

ND denotes no antibacterial activity

According to the obtained results, it was found that Gel-AgNPs performed better inhibition than Gel-Glu-AgNPs with an inhibition zone of 15.80 ± 0.19. It was found that CEO formed a zone of inhibition of 20.02 ± 0.49, while PEO did not show any activity. While nanoparticles did not show a synergistic effect with CEO against *P. aeruginosa*, they demonstrated a synergistic effect with PEO. Also, it was found that Gel-Glu-AgNPs showed better inhibition than Gel-AgNPs with an inhibition zone of 12.59 ± 0.39 against *E. coli*. While both nanoparticles were observed to exhibit a synergistic effect with PEO, it was found that the 16.64 ± 0.23 zone of inhibition revealed by CEO alone was reduced with nanoparticles.

In the course of this work, we discovered that Gel-AgNPs and Gel-Glu-AgNPs showed the highest antibacterial activity against *S. aureus*. In addition, it was found that Gel-AgNP and Gel-Glu-AgNPs showed the lowest activity against *E. coli* and *S. typhimurium*, respectively. While CEO was observed to show high antibacterial activity at the indicated concentrations, PEO was found to show no activity at the same concentration. The synergistic antibacterial activity of Gel-AgNP and CEO was only observed against *B. cereus*. Additionally, Gel-Glu-AgNPs were found to have a synergistic effect with CEO against *S. aureus* and *B. cereus*. On the other hand, it was found that all nanoparticles showed synergistic activity with PEO in antimicrobial activity against all microorganisms.

There have been several previous attempts to synthesize silver nanoparticles using gelatin. In a study, facile synthesis of AgNPs using gelatin was performed. It was observed that the obtained Gel-AgNPs showed high stability(Lee and Zhang*, 2013). A report published by Lavanya et al., 2020, offered a comparative analysis of chemically synthesized AgNPs and gelatin synthesized AgNPs. Their study showed that gelatin AgNPs at a concentration of 80 μg/mL inhibited the growth of *S. aureus*. It has been suggested that gelatin AgNPs lead to good bacterial inhibition even in low concentrations (Lavanya et al., 2020). Our findings suggest that Gel-AgNPs can serve as a potential antibacterial agent against Gram-positive and Gram-negative bacteria. Silver nanoparticles cause structural and morphological variations in bacterial cells, leading to cell death (Erci and Torlak, 2019). Recent research has shown that using essential oils and silver nanoparticles together produces a synergistic effect in antimicrobial activity. The antibacterial activity of a combination of biosynthesized silver nanoparticles produced by *Fusarium oxysporum* and essential oil of *Oreganum vulgare* against gram-positive and gram-negative bacteria, including multidrug-resistant strains, has been demonstrated experimentally.

Their results showed that the combination of the two compounds produced a synergistic effect that reduced MIC values and duration of action compared to AgNP used alone(Scandorieiro et al., 2016). Another study examined the effect of using eugenol alone or in combination with silver nanoparticles produced by *Fusarium oxysporum* on *Streptococcus agalactiae*. The overwhelming evidence showed that eugenol has bactericidal activity against planktonic cells of all strains. When this activity was combined with AgNPs, it resulted in a potent synergistic activity that significantly reduced the MIC values of both compounds(Perugini Biasi-Garbin et al., 2015).

3.3. Effect of AgNPs on soil respiration

Gel-AgNPs, Gel-Glu-AgNPs, CEO, PEO, and their combinations were applied to soil samples at 3 different volumes (0.2, 0.4 and 0.6 mL 250 g^{-1} soil). On days 1, 5, and 10 (24, 120, and 240 h), the activity of soil dehydrogenase and alkaline phosphatase and at the end of day 10, soil respiration was analyzed compared to the untreated control group. The results of the treated samples measuring soil respiration are shown in CO_2 (mg g^{-1}) in Figure 8.

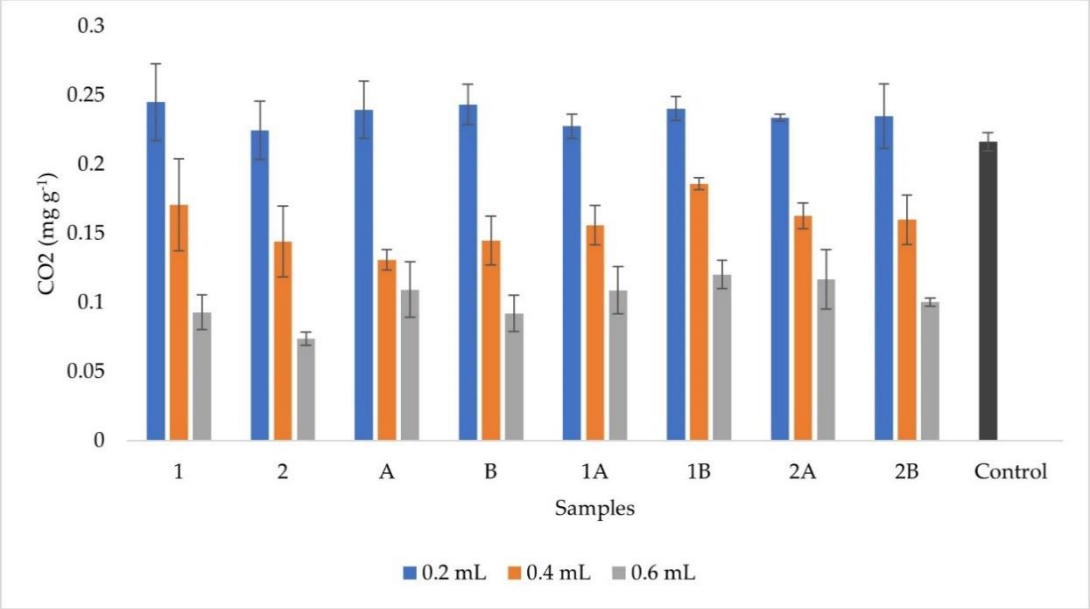

Figure 8. Respiration in soil affected by increasing sample doses. Values represent means standard deviations from three independent replicates. **1**) Gel-AgNPs (**2**) Gel-Glu-AgNPs (**A**) CEO (**B**) PEO.

The soil respiration value in the untreated soil sample was 0.22 ± 0.01. It was found that soil respiration decreased most when applying the highest dose of Gel-Glu-AgNP (0.6 mL). An increase in soil respiration was observed at the lowest dose (0.2 mL) of all samples compared to the control. The initially increased effect of treatments on soil respiration is believed to be due to the death of hypersensitive species (Rahmatpour et al., 2017). While the third dose of the Gel-AgNP-PEO combination caused an increase in soil respiration compared to both AgNPs and PEO, there was no synergistic effect with CEO. A synergistic effect was observed at the third dose of the combination of Gel-Glu-AgNP with PEO and CEO. Results showed that the respiratory reduction was dose-dependent in all samples.

3.4. Effect of AgNPs and essential oils on enzymatic activities in soils

In this study, concentration and time-dependent dehydrogenase and alkaline phosphatase activities were examined to assess the effect of Gel-AgNPs, essential oils, and their combined effect on soil. By applying the samples as 0.2, 0.4, and 0.6 mL 250 g^{-1} soil, the enzyme activities were measured during the exposure time of 24, 120, and 240 h and compared with the control group. Figure 9 shows the time-dependent variation of dehydrogenase activity with increasing application doses of AgNPs and essential oils (0.2, 0.4, and 0.6 mL 250 g^{-1} dry soil). The values were given in μg p-nitrophenol g^{-1} oven-dried soil $^{-1}$.

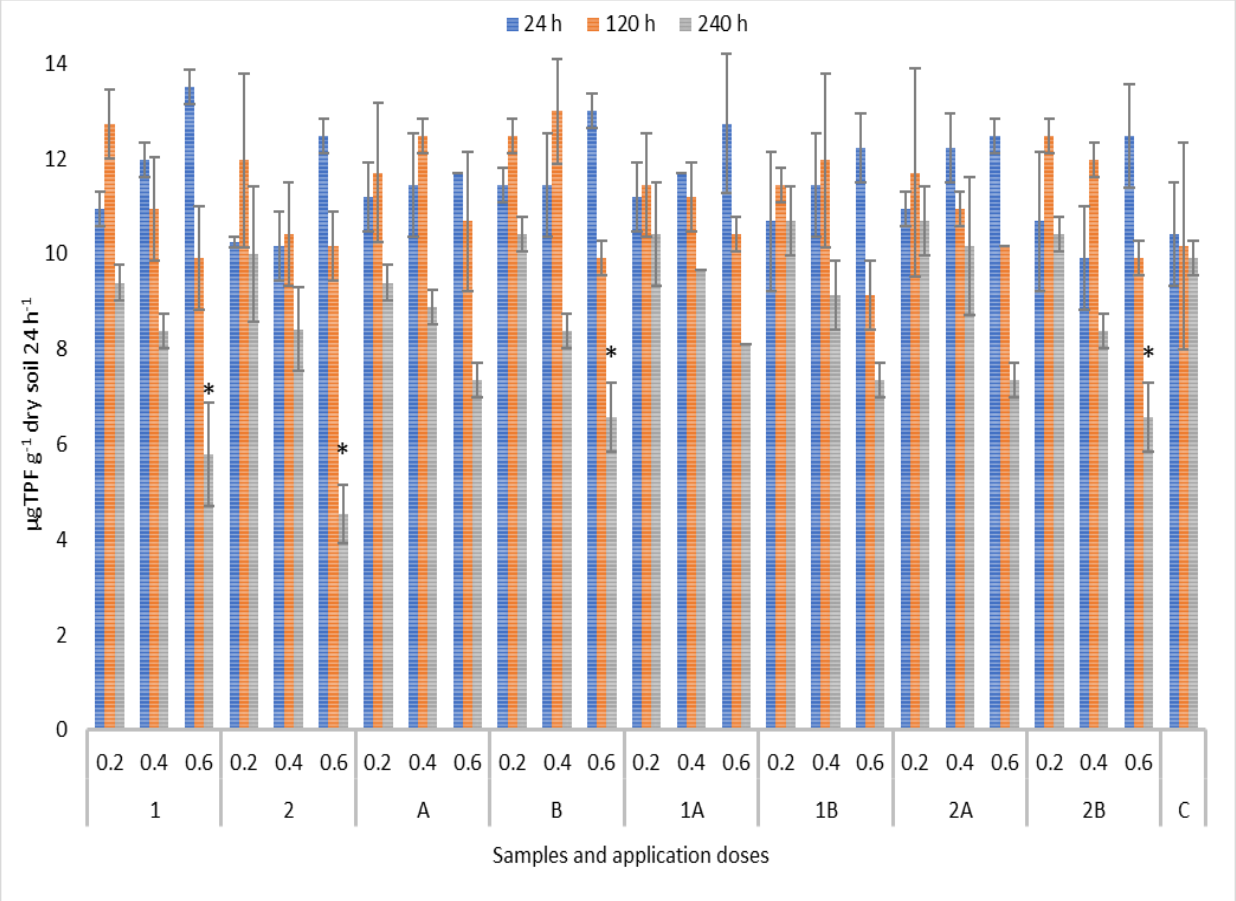

Figure 9. Dehydrogenase activity of soils treated by increasing the dose of the samples after incubation at different times. Values represent the means of three independent replicates ± standard deviations. The asterisk (*) indicates a significant difference between the treatments and control (Tukey's test; P< 0.05). **1**) Gel-AgNPs (**2**) Gel-Glu-AgNPs (**A**) CEO (**B**) PEO.

At the end of 24 h, it was observed that the Gel-Glu-AgNP-PEO combination reduced the dehydrogenase activity the most. AgNPs and essential oils also increased dehydrogenase activity at high doses compared to the control. Gel-AgNPs increased dehydrogenase activity with a dose increase at the end of 24 h. For Gel-Glu-AgNPs, the enzyme activity was found to be close at the end of the 24 h in the first two doses, while there was an increase at the highest application dose. For the first dose of Gel-AgNPs (200 µg), there was an increase by the end of 120 h and a decrease in dehydrogenase activity by the end of 240 h. In addition, these nanoparticles have been shown to reduce dehydrogenase activity when the application doses were increased at the end of 120 and 240 h. It was observed that this situation remained the same in Gel-Glu-AgNPs. In particular, it was found that the dehydrogenase activity of nanoparticles at the end of the 240 h was significantly lower than that of the control when high doses were applied. Overall, the results show that high concentrations of AgNPs were more toxic to microorganisms in the rhizosphere (Das et al., 2012). The effects of the samples on the dose- and time-dependent soil alkaline phosphatase activity were shown in Figure 10.

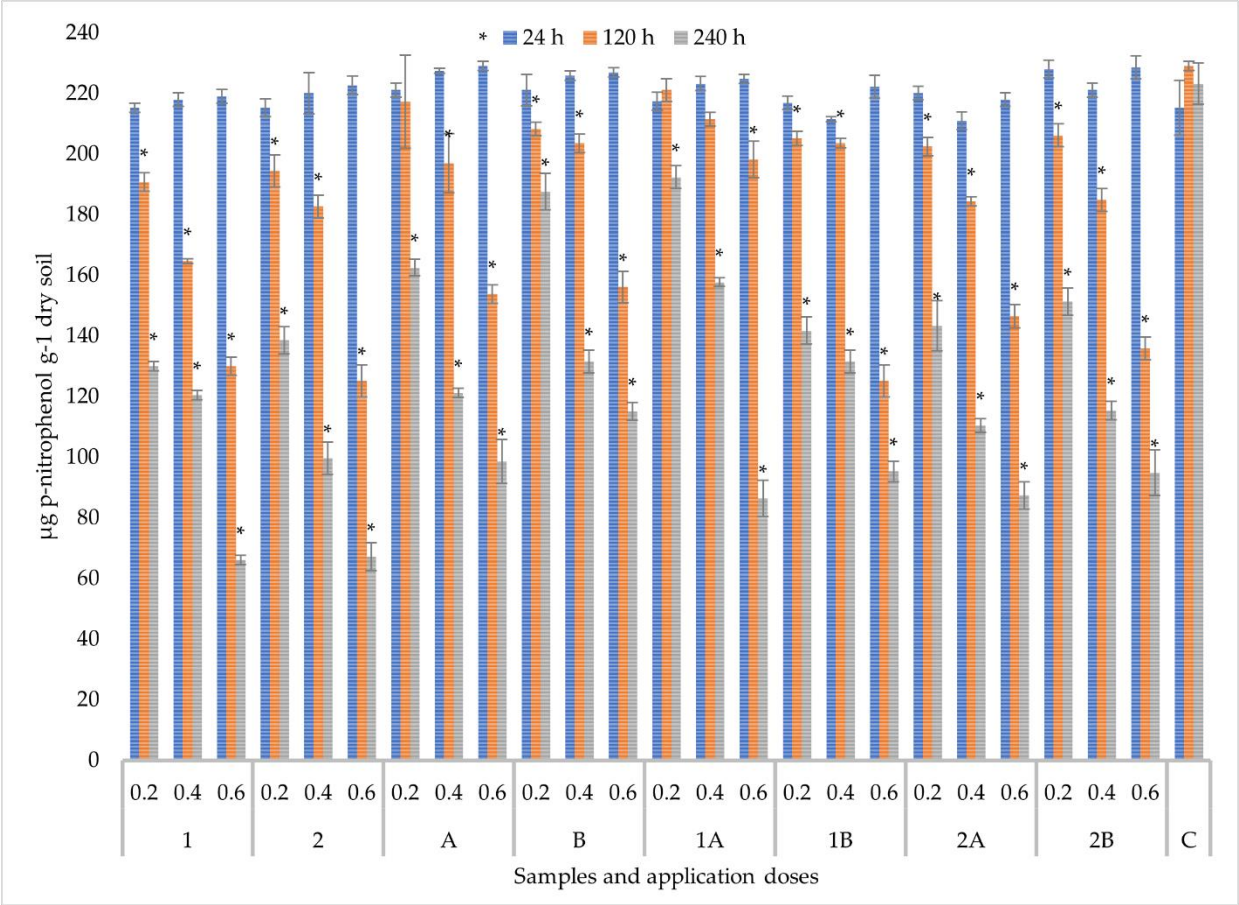

Figure 10. Alkaline phosphatase activity of soils treated by increasing the dose of the samples after incubation at different times. Values represent the means of three independent replicates ± standard deviations. The asterisk (*) indicates a significant difference between the treatments and control (Tukey's test; P< 0.05). **1**) Gel-AgNPs (**2**) Gel-Glu-AgNPs (**A**) CEO (**B**) PEO.

According to the results obtained, it was observed that the alkaline phosphatase activity increased at higher doses at the end of 24 h. On the other hand, it was observed that enzyme activity was inhibited in a time-dependent manner at all doses of nanoparticles and essential oils. In addition, it was found that the activity decreased as a function of the increase in dose due to the increase in time in all samples except 24 h. Analysis using one-way ANOVA and Tukey's test showed statistical significance ($p < 0.05$) between Gel-AgNPs (600 µg, 0.6 mL), Gel-Glu-AgNPs (600 µg, 0.6 mL), PEO (0.6 mL) and control for dehydrogenase activity at the end of 240 h incubation. On the other hand, the dose of 0.6 mL at the end of 240 h incubation was statistically different from the control in all samples ($p < 0.05$). Especially, it has been observed that alkaline phosphatase was most sensitive to AgNPs and essential oils. Exposure to high concentrations of AgNPs and a long period showed an inhibitory effect on the activities of both enzymes tested. This association has also been explored in prior studies (Mishra et al., 2021).

Several studies have explored the effects of nanoparticles on microorganisms and enzymes in pure soil. Shin et al. (2012) found that AgNPs showed obvious inhibition of dehydrogenase and urease activities at 100-1000 µg g^{-1} dry soil concentrations. In another study, five soil exoenzymes (dehydrogenase, urease, acid phosphatase, neutral phosphatase, and alkaline phosphatase) were examined in the rhizosphere of wetland plants treated with silver nanoparticles (0, 0.024, 0.24, 4.80 and 9.60 µg g^{-1} dry soil). AgNPs were found to inhibit all exoenzyme activities tested in their study, especially at high concentrations of AgNPs (4.80 and 9.60 µg g^{-1} dry soil); significant inhibitory activity was observed (Cao et al., 2017).

4. CONCLUSIONS

In this study, silver nanoparticles were synthesized in an aqueous solution of gelatin, which acts as a reducing and stabilizing agent. In addition, a second nanoparticle synthesis was performed by adding glucose to the gelatin solution to reveal the role of gelatin. The nanoparticles obtained were characterized by different analytical techniques. The study found that the nanoparticles were positively charged through zeta potential analysis. In addition, the DLS analysis of nanoparticles allowed us to conclude that the PDI values were below 0.3 and the AgNPs synthesized therein were quite stable; hence this helps to obtain better results, especially regarding the stability of nanoparticles. In the study, the antibacterial activity of the synthesized AgNPs and their synergistic effect with essential oils against both gram-positive and gram-negative were carried out. The results showed that Gel-AgNPs and Gel-Glu-AgNPs exhibited antibacterial activity against both gram-positive and gram-negative bacteria. In contrast, both AgNPs showed the most potent antibacterial activity against *S. aureus*. Also, high antibacterial activity was observed for CEO, while no antibacterial activity existed for PEO. The results also showed that the synergistic activity in antibacterial activity depended on the type of essential oils, AgNPs, and bacterial strain, and this should be considered in future experiments. To expand the scope of the study, soil respiration and enzymes were also examined to understand the effectiveness of silver nanoparticles on soil microorganisms. In the course of this work, we found that the lowest application dose (0.2 mL) of the samples caused an increase in soil respiration compared to the control and that soil respiration decreased in all samples with increasing application dose.

The enzymes represent one of the most active organic compounds in the soil. Therefore, the dehydrogenase and alkaline phosphatase activities of the samples treated with AgNPs and essential oils and their combinations were examined at different times and application doses. In the enzyme analysis, an increase in enzyme activities was observed at the end of 24 h, while time-dependent enzyme inhibition was observed at other incubation times. In addition, it was observed that the enzyme activity decreased proportionally as the dose of the samples increased. It was also found that combinations of nanoparticles and essential oils could synergistically affect enzyme activities.

Since there are no regulations and standards for using AgNPs in agriculture, especially for soil treatment, the study can be particularly useful to evaluate such nanomaterials obtained and stabilized with gelatin in different applications. As a result, the obtained nanoparticles are believed to be free from toxic substances derived from other chemical or physical synthesis methods of nanomaterials. This could create a new perspective on the use and evaluation of nanoparticles in environmental applications.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

Aewsiri, T., Benjakul, S., Visessanguan, W. 2009, "Functional Properties of Gelatin from Cuttlefish (*Sepia pharaonis*) Skin as Affected by Bleaching Using Hydrogen Peroxide", *Food Chemistry*, Vol. 115, No. 1, pp. 243–249.

Aruguete, D. M., Hochella, M. F. 2010, "Bacteria–Nanoparticle Interactions and Their Environmental Implications", *Environmental Chemistry*, Vol. 7, No. 1, pp. 3–9.

Behravan, M., Hossein Panahi, A., Naghizadeh, A., Ziaee, M., Mahdavi, R., Mirzapour, A. 2019, "Facile Green Synthesis of Silver Nanoparticles Using *Berberis vulgaris* Leaf and Root Aqueous Extract and Its Antibacterial Activity", *International Journal of Biological Macromolecules*, Vol. 124, pp. 148–154

Burduşel, A.-C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., Andronescu, E. 2018, "Biomedical Applications of Silver Nanoparticles: An up-to-Date Overview", *Nanomaterials*, Vol. 8, No. 9, p. 681.

Burt, J. L., Gutiérrez-Wing, C., Miki-Yoshida, M., José-Yacamán, M. 2004, "Noble-Metal Nanoparticles Directly Conjugated to Globular Proteins", *Langmuir*, Vol. 20, No. 26, pp. 11778–11783.

Caldwell, B. A. 2005, "Enzyme Activities as a Component of Soil Biodiversity: A Review", *Pedobiologia*, Vol. 49, No. 6, pp. 637–644.

Cao, C., Huang, J., Cai, W.-S., Yan, C.-N., Liu, J.-L., Jiang, Y.-D. 2017, "Effects of Silver Nanoparticles on Soil Enzyme Activity of Different Wetland Plant Soil Systems", *Soil and Sediment Contamination: An International Journal*, Vol. 26, No. 5, pp. 558–567.

Cornelis, G., Hund-Rinke, K., Kuhlbusch, T., van den Brink, N., Nickel, C. 2014, "Fate and Bioavailability of Engineered Nanoparticles in Soils: A Review", *Critical Reviews in Environmental Science and Technology*, Vol. 44, No. 24, pp. 2720–2764.

Das, P., Williams, C. J., Fulthorpe, R. R., Hoque, M. E., Metcalfe, C. D., Xenopoulos, M. A. 2012, "Changes in Bacterial Community Structure after Exposure to Silver Nanoparticles in Natural Waters", *Environmental Science & Technology*, Vol. 46, No. 16, 2012, pp. 9120–9128.

Ealia, S. A. M., Saravanakumar, M. P. 2017. "A review on the classification, characterization, synthesis of nanoparticles and their application". In *IOP Conference Series: Materials Science and Engineering* (Vol. 263, p. 032019). IOP Publishing.

Erci, F., Cakir-Koc, R. 2021, "Rapid Green Synthesis of Noncytotoxic Iron Oxide Nanoparticles Using Aqueous Leaf Extract of *Thymbra spicata* and Evaluation of Their Antibacterial, Antibiofilm, And Antioxidant Activity", *Inorganic and Nano-Metal Chemistry*, Vol. 51, No. 5, pp. 683-692.

Erci, F., Cakir-Koc, R., Yontem, M., Torlak, E. 2020, "Synthesis of Biologically Active Copper Oxide Nanoparticles as Promising Novel Antibacterial-Antibiofilm Agents", *Preparative Biochemistry & Biotechnology*, Vol. 50, No. 6, pp. 538–548.

Erci, F., Koc, R., Isıldak, I., 2018, "Green Synthesis of Silver Nanoparticles Using *Thymbra spicata* L. Var. *spicata* (Zahter) Aqueous Leaf Extract and Evaluation of Their Morphology-Dependent Antibacterial and Cytotoxic Activity", *Artificial Cells, Nanomedicine, and Biotechnology*, Vol. 46, No. sup1, 2018, pp. 150–158.

Erci, F., Torlak, E., 2019, "Antimicrobial and Antibiofilm Activity of Green Synthesized Silver Nanoparticles by Using Aqueous Leaf Extract of *Thymus serpyllum*", *Sakarya University Journal of Science*, Vol. 23, No. 3, pp. 333-339.

Fabrega, J., Fawcett, S. R., Renshaw, J. C., Lead, J. R., 2009, "Silver Nanoparticle Impact on Bacterial Growth: Effect of PH, Concentration, and Organic Matter", *Environmental Science & Technology*, Vol. 43, No. 19, pp. 7285–7290.

Galvez, A. M., Ramos, K. M., Teja, A. J., Baculi, R., 2021, "Bacterial Exopolysaccharide-Mediated Synthesis of Silver Nanoparticles and Their Application on Bacterial Biofilms", *Journal of Microbiology, Biotechnology and Food Sciences*, Vol. 2021, pp. 970–978.

Gee, G. W., Bauder, J. W., 1986, "Particle-Size Analysis", Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods, Vol. 5, Editor: Arnold, K., the American Society of Agronomy, Inc. Soil Science Society of America, Inc., 383–411.

Gomaa, E. Z., 2017, "Antimicrobial, Antioxidant and Antitumor Activities of Silver Nanoparticles Synthesized by *Allium cepa* Extract: A Green Approach", *Journal of Genetic Engineering and Biotechnology,* Vol. 15, No. 1, pp. 49–57.

Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., van Es, H.M., Wolfe, D.W., Moebius-Clune, B.N., Thies, J.E., Abawi, G.S., 2009, Cornell Soil Health Assessment Training Manual, Edition 2.0, Cornell University, Geneva, NY.

Gurunathan, S., Han, J., Park, J. H., Kim, J.-H., 2014, "A Green Chemistry Approach for Synthesizing Biocompatible Gold Nanoparticles", *Nanoscale Research Letters*, Vol. 9, No. 1, pp. 248.

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., Danquah, M. K., 2018, " Review on Nanoparticles and Nanostructured Materials: History, Sources, Toxicity and Regulations", *Beilstein Journal of Nanotechnology*, Vol. 9, No. 1, pp. 1050–1074.

Klute, A., 1986, "Water Retention: Laboratory Methods", In A. Klute (Ed.), Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods (Second Edi,), Madison, Wisconsin USA: American Society of Agronomy, Inc. Soil Science Society of America, Inc. 635–662.

Lavanya, K., Kalaimurugan, D., Shivakumar, M. S., Venkatesan, S., 2020, "Gelatin Stabilized Silver Nanoparticle Provides Higher Antimicrobial Efficiency as Against Chemically Synthesized Silver Nanoparticle", *Journal of Cluster Science*, Vol. 31, No. 1, pp. 265–275.

Lee, C., Zhang*, P., 2013, "Facile Synthesis of Gelatin-Protected Silver Nanoparticles for SERS Applications", *Journal of Raman Spectroscopy*, Vol. 44, No.6, pp.823–826.

Mahmoud, K. H., Abbo, M,. 2013, "Synthesis, Characterization and Optical Properties of Gelatin Doped with Silver Nanoparticles", *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, Vol. 116, pp. 610–615.

McLean, E. O., 1983, "Soil pH and Lime Requirement". Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 199–224.

Mishra, P., Xue, Y., Eivazi, F., Afrasiabi, Z., 2021, "Size, Concentration, Coating, and Exposure Time Effects of Silver Nanoparticles on the Activities of Selected Soil Enzymes", *Geoderma*, Vol. 381, pp. 114682.

Moebius-Clune, B.N., Moebius-Clune, D.J., Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., Ristow, A.J., van Es, H.M., Thies, J.E., Shayler, H.A., McBride, M.B., Kurtz, K.S.M., Wolfe, D.W., Abawi, G.S., 2016, Comprehensive Assessment of Soil Health – The Cornell Framework, Edition 3.2, Cornell University, Geneva, NY.

Morones-Ramirez, J. R., Winkler, J. A., Spina, C. S., Collins, J. J., 2013, "Silver Enhances Antibiotic Activity Against Gram-Negative Bacteria", *Science Translational Medicine*, Vol. 5, No.190, pp. 190ra81-190ra81.

Nagarajan, M., Benjakul, S., Prodpran, T., Songtipya, P., Kishimura, H., 2012, "Characteristics and Functional Properties of Gelatin from Splendid Squid (*Loligo formosana*) Skin as Affected by Extraction Temperatures", *Food Hydrocolloids*, Vol. 29, No. 2, pp. 389–397.

Nannipieri, P., Kandeler, E., Ruggiero, P., Burns, R. G., Dick, R. P., 2002, "Enzymes in the Environment: Activity, Ecology, and Applications", Enzyme Activities and Microbiological and Biochemical Processes in Soil. New York (NY): Marcel Dekker, 1–33.

Nasiriboroumand, M., Montazer, M., Barani, H., 2018, "Preparation and Characterization of Biocompatible Silver Nanoparticles Using Pomegranate Peel Extract", *Journal of Photochemistry and Photobiology B: Biology*, Vol. 179, pp. 98–104.

Nelson, D. W., Sommers, L., 1982, "Total Carbon, Organic Carbon, and Organic Matter 1". Methods Of Soil Analysis. Part 2. Chemical and Microbiological Properties, (methodsofsoilan2), 539–579.

Perugini Biasi-Garbin, R., Saori Otaguiri, E., Morey, A. T., Fernandes da Silva, M., Belotto Morguette, A. E., Armando Contreras Lancheros, C., Kian, D., Perugini, MR., Nakazato, G., Durán, N.,

Nakamura, C. V., Yamauchi, L. M., Yamada-Ogatta, S. F., 2015. "Effect of Eugenol against *Streptococcus agalactiae* and Synergistic Interaction with Biologically Produced Silver Nanoparticles", *Evidence-Based Complementary and Alternative Medicine*, Vol. 2015, pp861497.

Peyrot, C., Wilkinson, K. J., Desrosiers, M., Sauvé, S., 2014, "Effects of Silver Nanoparticles on Soil Enzyme Activities with and Without Added Organic Matter", *Environmental Toxicology and Cchemistry*, Vol. 33, No. 1, pp. 115–125.

Pourjavadi, A., Soleyman, R., 2011, "Silver Nanoparticles with Gelatin Nanoshells: Photochemical Facile Green Synthesis and Their Antimicrobial Activity", *Journal of Nanoparticle Research*, Vol. 13, No. 10, pp. 4647–4658.

Prabhu, S., Poulose, E. K., 2012, "Silver Nanoparticles: Mechanism of Antimicrobial Action, Synthesis, Medical Applications, and Toxicity Effects", International Nano Letters, Vol. 2, No. 1, pp. 32.

Rahmatpour, S., Shirvani, M., Mosaddeghi, M. R., Nourbakhsh, F., Bazarganipour, M., 2017, "Dose–Response Effects of Silver Nanoparticles and Silver Nitrate on Microbial and Enzyme Activities in Calcareous Soils", *Geoderma,* Vol. 285, pp. 313–322.

Rath, G., Hussain, T., Chauhan, G., Garg, T., Kumar Goyal, A., 2016, "Fabrication and Characterization of Cefazolin-Loaded Nanofibrous Mats for the Recovery of Post-Surgical Wound", *Artificial Cells, Nanomedicine, and Biotechnology*, Vol. 44, No. 8, pp. 1783–1792.

Rhoades, J. D., 1982, "in Methods of Soil Analysis. Part2, Chemical and Microbiological Properties". Soluble salts, 167–179.

Scandorieiro, S., de Camargo, L. C., Lancheros, C. A. C., Yamada-Ogatta, S. F., Nakamura, C. v, de Oliveira, A. G., Andrade C. G. T. J., Duran, N., Nakazato, G., Kobayashi, R. K. T., 2016, "Synergistic and Additive Effect of Oregano Essential Oil and Biological Silver Nanoparticles against Multidrug-Resistant Bacterial Strains ", *Frontiers in Microbiology*, Vol. 7, pp. 760.

Singh, J., Dutta, T., Kim, K.-H., Rawat, M., Samddar, P., Kumar, P., 2018, "'Green' Synthesis of Metals and Their Oxide Nanoparticles: Applications for Environmental Remediation", *Journal of Nanobiotechnology*, Vol. 16, No. 1, pp. 84.

Song, W., Ge, S., 2019, "Application of Antimicrobial Nanoparticles in Dentistry", *Molecules*, Vol. 24, No. 6, pp. 1033.

Su, C. H., Velusamy, P., Kumar, G. V., Adhikary, S., Pandian, K., Anbu, P., 2017, "Studies of Antibacterial Efficacy of Different Biopolymer Protected Silver Nanoparticles Synthesized under Reflux Condition", *Journal of Molecular Structure*, Vol. 1128, pp. 718–723.

Tabatabai, M. A., Bremner, J. M., 1969, "Use of P-Nitrophenyl Phosphate for Assay of Soil Phosphatase Activity", *Soil Biology and Biochemistry*, Vol. 1, No. 4, pp. 301–307.

Thalmann, A., 1968, "Zur Methodik der Bestimmung der DehydrogenaseaktivitAt im Boden mittels triphenytetrazoliumchlorid (TTC)", *Landwirtsch Forsch*, Vol. 21, pp. 249–258.

Wijnhoven, S. W. P., Peijnenburg, W. J. G. M., Herberts, C. A., Hagens, W. I., Oomen, A. G., Heugens, E. H. W., Roszek, B., Bisschops, J., Gosens, I., Van De Meent, D., Dekkers, S., De Jong,W. H., van Zijverden, M., Sips, A. J.A.M., Geertsma, R. E., 2009, " Nano-Silver – a Review of Available Data and Knowledge Gaps in Human and Environmental Risk Assessment", *Nanotoxicology*, Vol. 3, No. 2, pp. 109–138.

Wright, A. F., Bailey, J. S., 2001, "Organic Carbon, Total Carbon, and Total Nitrogen Determinations in Soils of Variable Calcium Carbonate Contents Using a Leco CN-2000 Dry Combustion Analyzer", *Communications in Soil Science and Plant Analysis*, Vol. 32, No. 19–20, pp. 3243–58.

Yılmaz Öztürk, B., Yenice Gürsu, B., Dağ, İ., 2020, "Antibiofilm and Antimicrobial Activities of Green Synthesized Silver Nanoparticles Using Marine Red Algae *Gelidium corneum*", *Process Biochemistry*, Vol. 89, pp. 208–219.

Yiwei, A., Yunxia, Y., Shuanglong, Y., Lihua, D., Guorong, C., 2007, "Preparation of Spherical Silver Particles for Solar Cell Electronic Paste with Gelatin Protection", *Materials Chemistry and Physics*, Vol. 104, No. 1, pp. 158–161.

Yun'an Qing, L. C., Li, R., Liu, G., Zhang, Y., Tang, X., Wang, J., Liu, H., Qin, Y., 2018, " Potential Antibacterial Mechanism of Silver Nanoparticles and the Optimization of Orthopedic Implants by Advanced Modification Technologies", *International Journal of Nanomedicine*, Vol. 13, p. 3311.

Zhang, C., Hu, Z., Deng, B., 2016, "Silver Nanoparticles in Aquatic Environments: Physiochemical Behavior and Antimicrobial Mechanisms", *Water Research*, Vol. 88, pp. 403–427.