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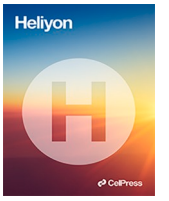

Research article

Green synthesis of silver nanoparticles by sweet cherry and its application against cherry spot disease

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ABSTRACT

Asia has a rich history of cultivating sweet cherries, a practice that has been carried out since ancient times. However, the effective management of Alternaria disease in sweet cherry crops has presented a formidable challenge, resulting in notable decreases in yield. Various attempts have been made to employ both chemical and biological treatments; however, their effectiveness has been restricted. In order to tackle this problem, an investigation was carried out, with the primary objective of isolating and identifying *Alternaria* isolates that are accountable for the occurrence of sweet cherry soft spot rot. Out of the twelve isolates examined, the CHM-4 isolate was found to be the most pathogenic. Its identification was achieved through the use of the ITS genomic region (ITS1 and ITS4), and the BLAST results revealed a 95 % similarity with *Alternaria alternata* (MG744381.1). The objective of the research was to explore the potential of silver nanoparticles (SNPs) synthesized by phytosynthesis as a novel antifungal agent to combat sweet cherry soft spot pathogenicity. The biosynthesis of SNPs was carried out using sweet cherry fruits kernel exudate, which served as an environmentally friendly source. The exudates exhibited the ability to produce nanoparticles with an average size of 24.97 nm. Analysis conducted using a transmission electron microscope (TEM) revealed the multifaceted structure of these nanoparticles. Furthermore, when tested at concentrations of 5, 10, 20, and 40 μg/ml, these biosynthetic nanoparticles demonstrated the capability to inhibit the growth of Alternaria fungi and effectively destroy fungal hyphae. It is advisable to utilize diverse components of sweet cherry for the synthesis of various nanoparticles owing to their compatibility with the surrounding environment.

1. Introduction

Nanobiotechnology, an interdisciplinary domain, amalgamates the realms of nanotechnology and biology to fabricate and employ substances and apparatuses at the nanoscale [1]. This cutting-edge field holds immense promise for diverse sectors such as agriculture,

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medicine, and the production of sustainable energy [2]. This involves utilizing biological systems like microorganisms and plants to produce nanoparticles under mild and environmentally friendly conditions [3]. Referred to as green synthesis, this approach offers numerous advantages, including its eco-friendliness, cost-effectiveness, and potential for large-scale production [4]. These synthesized nanomaterials have been extensively evaluated for their potential applications in diverse fields like pharmaceuticals, food, environment, agriculture, energy, and biomedical treatment [5,6]. To fully harness the potential of green biosynthesis in producing a wide range of high-quality nanomaterials, further investment and research are imperative [7]. SNPs (silver nanoparticles) is a term used to describe silver particles that are extremely small, typically measuring less than 100 nm in size [8]. The unique characteristics SNPs, such as its high surface-to-volume ratio, have garnered significant attention and have found applications in various fields [9]. SNPs can be synthesized using various techniques, one of which is green synthesis utilizing plant extracts. However, the use of SNPs also raises concerns regarding its potential impact on the environment and human health [10]. The green approach for preparing nanomaterials is often preferred due to its numerous advantages over physiochemical techniques. This approach involves the use of benign compounds, ensuring long-term sustainability, facilitating easy manufacturing, promoting biologic compatibility, expediting the process, and reducing energy requirements [11–14]. When plant extracts are used for the phytosynthesis of SNPs, it is typically carried out under low-temperature conditions to ensure a slow reaction rate and effective conversion of Ag^{2+} ions [15]. In the selection of plant species for the production of SNPs, it is important to consider plants that possess bioactive compounds capable of functioning as reducing and stabilizing agents [16]. The size, shape, and properties of the nanoparticles produced can be influenced by the selection of plant species. To achieve the desired characteristics in the SNPs, it is essential to choose plant species that have known bioactive compounds suitable for nanoparticle synthesis [17].

Soltani Nejad et al. conducted a study wherein they synthesized SNPs using Paulownia fruit exudates. The average diameter of these nanoparticles was measured to be 46 nm. Interestingly, the antifungal properties against the disease-causing fungus that affects harvested bananas were observed by the researchers to be exhibited by nanoparticles of different concentrations [18]. In another investigation, the petals of the quince were employed to synthesize SNPs through the phytosynthesis technique. The resultant SNPs exhibited remarkable antibacterial properties against the pear fire blight disease [19]. In a separate study, researchers utilized *Mangifera indica* leaf extract to produce SNPs. The resulting biosynthetic nanoparticles exhibited notable antimicrobial properties, effectively impeding the growth of both Gram-positive and Gram-negative bacteria, as well as fungi, with an inhibition rate exceeding 85 % [20]. The utilization of nanomaterial in the field of agriculture has emerged as a promising strategy to improve plant productivity while simultaneously addressing concerns related to soil and water pollution [21]. Additionally, it offers a means to protect crops from a wide range of pests and SNPs has been extensively researched due to its potential uses in the field of plant pathology, specifically in relation to its antifungal and antibacterial properties [22,23]. SNPs have been extensively researched for its potential applications in plant pathology, particularly in the areas of antifungal and antibacterial activities. However, the use of SNPs in plant pathology poses several challenges. One of the primary challenges is the potential environmental impact of SNPs, as it can accumulate in soil and water and have adverse effects on non-target organisms [24,25]. Furthermore, the long-term effects of SNPs on plant growth and development are not yet fully understood, and further research is required to comprehend the implications and potential risks associated with the use of SNPs in plant pathology [26]. Additionally, the use of SNPs in plant pathology may raise concerns about the safety of food products, as SNPs can potentially accumulate in edible plant tissues [27]. Therefore, while SNPs shows promise for various applications in plant pathology, it is crucial to conduct more research to fully understand its potential risks and benefits.

The sweet cherry (*Prunus avium*) is a succulent stone fruit that does not undergo climacteric ripening. It is predominantly cultivated in nations with moderate climates, such as Iran [28]. This species is believed to have its roots in a region encompassing Asia Minor, Iran, Iraq, and Syria. Within Iran, there are twenty three significant cultivars of sweet cherry that have been gathered from various provinces [29].

Postharvest fruit rot in sweet cherry (*Prunus avium*) has been attributed to *Alternaria* spp., specifically *Alternaria alternata* [30]. Cherries in the Central Chile region were observed to have been impacted by a type of rot that caused the fruit surface to develop irregular lesions ranging in color from pale to dark brown. The affected cherries exhibited a firm texture, which is a characteristic feature of this type of rot. The presence of green to light brown fungal growth resembling an *Alternaria*-like infection was also observed [31].

In Iran, *Alternaria alternata* has been found to be the culprit behind leaf spot diseases in a variety of plants [32]. In the peppermint fields of Kerman, located in the southeast region of the country, leaf spot symptoms were observed and the fungus was identified as *A. alternata* [33]. Brown spot symptoms were also observed on tangerine hybrid cultivars in the same province, and the fungus was once again identified as *A. alternata* [34]. Moreover, research has been carried out to examine the pathogenicity and genetic diversity of *A. alternata* isolates from India in connection with the occurrence of leaf spot disease in vegetable crops [35]. These findings underscore the prevalence of *A. alternata* and its impact on various crops in the world. The primary objective of this study is to utilize the exudates derived from the inner core of sweet cherry fruit in order to facilitate the production of SNPs. The biosynthesis of these nanoparticles will be accomplished by utilizing plant-based processes, which provide a cost-effective and uncomplicated approach. The testing procedure entails a biological method conducted at standard temperature and pressure conditions, thereby eliminating the need for chemical solvents as reducing and stabilizing agents. To evaluate the biosynthesized nanoparticles, a variety of techniques will be employed, such as spectrophotometer UV, AFM, TEM, XRD, EDX, and FTIR. The aim of this study is to identify the root cause of the postharvest spot disease in cherry fruits caused by *A. alternata* and determine its morphological and molecular characteristics. Additionally, the study will investigate the effects of inhibiting the fungus mycelium using biosynthesized SNPs. The novel antifungal agent will be tested at various concentrations to determine its effectiveness in inhibiting the pathogenicity of *A. alternata* in sweet cherry. The novelty of this study is utilization of sweet cherry (*Prunus avium*) kernel for synthesizing SNPs and using them to control postharvest spot disease in sweet cherry fruits. The primary objectives of research in this domain are to optimize the biosynthesis

process, understand the underlying mechanisms, and address concerns related to scalability and purity.

2. Materials and methods

2.1. Chemicals

Silver nitrate ($AgNO_3$), Sodium hypochlorite, and potato dextrose agar (PDA) were supplied by Merck Company, Germany. The polymerase chain reaction (PCR) chemicals were purchased from CinnaGen Company, Iran.

2.2. Sweet cherry kernel exudates

Fresh cherry fruits were utilized to produce exudates of sweet cherry (*Prunus avium*) kernel. The process involved separating the core from the fruits and crushing its outer cover. The cores were then disinfected with sterile water and incubated for three days at 28 ± 1 °C. Tiny, pristine fragments with a weight of 10 g were carefully positioned within an Erlenmeyer flask; 100 ml of deionized water was added to it. After soaking, the kernel pieces were filtered through Whatman No. 1 filter paper and then stored at a temperature of 4 °C until they were needed for further use. The pH of the exudate was consistently maintained at 6.3 throughout the entire study [36].

2.3. Phytofabrications of SNPs

The synthesis of SNPs from sweet cherry kernels exudates was carried out using a 15 ml volume of 10^{-3} M of $AgNO_3$ solution. The main goal was to utilize the bioreduction approach. The experimental procedure involved maintaining the sample at ambient temperature for approximately 180 min without any stirring or movement. Following a lapse of approximately 180 min, the mixtures were carefully examined to determine the extent of Ag^+ bioreduction and the generation of SNPs [37,38].The control tests, which contained exudates without included silver nitrate, were utilized to compare and differentiate the watched color alter within the treated tests. The prepared samples were created by mixing exudate with a solution of $AgNO_3$ with a concentration of 0.001 M. Additionally, cherry kernel exudates were applied as control samples in the instrumental analysis of the SNPs that were synthesized by biological means. The assessment of SNPs production from exudates obtained from sweet cherry kernels involved the application of various spectrophotometric techniques.

2.4. Evolution of UV–Visible and the solution color changes

Three distinct experiments were conducted at 28 ± 1 °C using a UV–Visible spectrophotometer to validate the phytosynthesis of SNPs from the exudates of sweet cherry kernels. The absorption wavelength was determined within the 350–600 nm range after reaction time 24h.

2.5. The utilization of TEM and AFM analysis

A suspension comprising phytosynthesized SNPs as the cherry kernel exudate underwent ultrasound treatment for a duration of 4min, followed by the addition of a specific quantity of zinc to the resulting suspension. The suspension was then meticulously applied onto a carbon film-containing grid, and this entire procedure was performed in the presence of ambient air at 28 ± 1 °C. Notably, the suspension was dried on the grid without the application of heat. Electron microscopy was utilized to evaluate the dimensions, morphology, and spatial arrangement of the SNPs [39]. Conversely, the quantification of SNPs levels was achieved by employing atomic force microscopy (AFM) [40,41].

2.6. X-ray diffraction and energy-dispersive (XRD) and X-ray spectroscopy (EDX) analysis

To explore the potential of exudates of sweet cherry kernel in the formation of crystals, an experiment was conducted using a biosynthesized of silver sample and X-ray exposure. In order to simplify the examination of the powder specimen, a suspension containing SNPs was produced with precision at a temperature of 29 °C. The suspension was subsequently centrifuged at a velocity of 12,000 revolutions per minute for a period of 10 min. After the centrifugation step, the liquid portion above the sediment was cautiously extracted, and the remaining mixture was thinned down by adding deionized water until it reached a total volume of 33 ml. This dilution process was carried out thrice. Afterward, the resulting blend underwent a drying process at a temperature of 63 °C for a period of 24h. The dried powder obtained from this process was then utilized for X-ray diffraction analysis, with the angle of interest being represented by 2θ [42]. In order to delve deeper into the elemental makeup of the specimen, the utilization of energy dispersive X-ray (EDX) spectroscopy method was implemented [43].

2.7. Analysis conducted through the utilization of FTIR spectroscopy

A FTIR analysis was carried out to determine the molecules and biological functional groups responsible for the biosynthesis of SNPs. To facilitate the analysis, a powder sample was prepared, a suspension of cherry fruit inner core exudates containing phytosynthesized SNPs was centrifuged at a speed of 6000 rpm for 15min, and the upper phase was decanted. The remaining treatment

samples were then kept at 60 °C for 72 h and subsequently dried. Upon completion of the process, the powder was subjected to analysis through a German-manufactured Fourier transform infrared spectrometer model 27 Tensor [18,44].

2.8. Isolating and identifying diseases that affect sweet cherry

To identify the disease agent responsible for soft spot rot in sweet cherry fruits, the specimens were gathered from plantations situated in Mashhad, Chenaran, and Ortokand within the Razavi Khorasan region of Iran. The collected fruits underwent a sterilization process, which involved removing the surface parts and treating them with Sodium hypochlorite 1 %. After being rinsed twice with sterile distilled water, fruit chips were acquired from both healthy and infected border tissue. These chips were subsequently positioned onto potato dextrose agar (PDA) and incubated at a temperature of 28 °C. Pathogenic fungi purification was isolated and fresh potato carrot agar (PCA, containing 10 g/L of potato and carrot) culture medium was utilized to transfer it for further incubation. Morphological identification was done using the morphological key to Alternaria Simmons [45].

2.9. Molecular identification of cherry sweet fungus

In order to ascertain the pure culture of fungi using molecular techniques, the isolates were meticulously prepared as individual spores and subjected to genomic DNA extraction using the CTAB method. ITS1 and ITS4 primers were used to amplify ITS1, 5.8S, and ITS2-rRNA from DNA, and the resulting PCR products were electrophoresed on a 1 % agarose gel containing ethidium bromide [46]. The PCR products were purified, sequenced, and analyzed using the Clustal W tool to identify the molecular identification of the sweet cherry disease agent.

2.10. SNPs inhibitory properties on the mycelium growth

To evaluate the effectiveness of biosynthetic SNPs in combating *Alternaria alternata*, four different concentrations of SNPs suspension (5, 10, 20, and 40 μg/ml) were incorporated into potato dextrose agar medium (PDA) prior to pouring the plates. Subsequently, 2 mm diameter agar plugs fungal mycelia were introduced into the central region of each SNP-containing Petri dish. Subsequently, the Petri dishes were placed in an incubator set at a temperature of 28 °C for a duration of two weeks. In order to guarantee precision and dependability, all trials were performed three times. The inhibition rate was assessed utilizing the procedure elucidated by Soltani Nejad et al., [22]. Control was exudates of sweet cherry kernel.

2.11. Examining the impact of SNPs on the physical characteristics of the mycelia in Alternaria alternata

The main objective of this investigation was to examine the alterations in the structure of hyphae in pathogenic fungi when subjected to unilateral culture conditions. In order to achieve this, Petri dishes were prepared with different concentrations (5, 10, 20, and 40 μg/ml) of SNPs added in PDA medium. Furthermore, control Petri dishes were employed, consisting solely of sweet cherry kernel exudates, where the pathogenic fungi were cultured. In order to carry out the experiment, a minute amount of the pathogen was extracted from the outer edge and underwent unidirectional cultivation through the utilization of SNPs in the well diffusion technique.

2.12. Effects of SNPs on Alternaria alternata spore germination

The study aimed to investigate the impact of SNPs on Alternaria spore germination. The method employed was based on the one described by Sharma et al. [47] with some modifications. The SNPs were prepared beforehand at concentrations ranging from 5 to 40 μg/mL, containing Tween 20 (0.1 % v/v). The spores were obtained from fungi cultivated for 15 days and added to water agar Petri dishes containing biosynthetic SNPs. A negative control Petri dish without nanoparticles containing Tween 20 (0.1 % v/v) plus exudates of sweet cherry kernel was used. The Petri dishes were kept at 28 °C ± 1 and observed under a light microscope (OLYMPUS DP12, Japan) after 12–24 h, depending on spore germination in the control group. One hundred spores were counted for each treatment, and the percentage of spore germination was calculated.

2.13. Statistical analyses method

The recorded data underwent statistical analysis using SAS software (SAS Institute, version 9, Cary, NC, USA). The Duncan Multiple Board test was employed for the analysis.

3. Results

3.1. Analysis via visual observation and UV–visible spectroscopy

The eco-friendly synthesis of SNPs was achieved using cherry kernel exudates obtained from *Prunus avium*. The process involved the separation of the cherry inner core from the fruits, as depicted in Fig. 1(a–c). After 72 h, the cherry fruit inner core exudates were collected, as shown in Fig. 1d. During the exudation exposure, the silver nitrate ions were reduced, leading to the formation of SNPs. This reaction resulted in a brown color, which was observed after 24 h, as illustrated in Fig. 2. The successful formation of SNPs was

confirmed by the presence of absorbance peaks between 400 and 450 nm, which served as the criterion for identifying the nanoparticles. A significant alteration in color, the move from colorless $AgNO_3$ to deep brown has presented compelling evidence for the potential conversion of Ag^{+} to Ag^{0}. However, the initial hypothesis was confirmed through the utilization by UV–Vis spectroscopy [38].

3.2. Analysis of the high-definition transmission electron microscope (TEM) and atomic force microscopy (AFM)

The spherical formation of SNPs resulting from colloidal Phytosynthesis has been confirmed through electro micrograph results, as illustrated in Fig. 3a and b. Furthermore, the TEM electro micrograph in Fig. 3c shows biosynthesized SNPs utilizing sweet cherry (*Prunus avium*) kernel exudates, with an average size of 24.97 nm.

The AFM technique was employed to analyze the surfaces of the synthesized SNPs, and the findings were illustrated through both one-dimensional (1D) and three-dimensional (3D) images. The AFM images provided valuable insights into the morphology of the SNPs, showcasing distinct spherical particles with diverse sizes. This observation is visually depicted in Fig. 4(a–b).

3.3. XRD analysis and EDX

Upon conducting an analysis of the X-ray diffraction (XRD) pattern of SNPs that were biosynthesized by sweet cherry kernel exudates, it was discovered that there were four distinct and intense peaks present, Fig. 5. These peaks serve as an indication of the crystalline nature of the phytosynthesis of SNPs.

The utilization of sweet cherry kernel exudates in the synthesis of SNPs was analyzed through EDX spectroscopy, as shown in Fig. 6. This technique enabled the elemental analysis of the silver content present in the sample. The results of the analysis confirmed the presence of elemental silver in the nanoparticles that were prepared, leaving no doubt about its existence.

3.4. FTIR studies

Fig. 7 shows the spectrum obtained from the Fourier Transform Infrared (FTIR) analysis of a powder containing SNPs. These nanoparticles were produced using sweet cherry kernel exudates. The FTIR analysis demonstrates the involvement of carboxyl (-C═O), hydroxyl (O–H), and amine (N–H) functional groups found in the exudates in the reduction of Ag^{+} ions to Ag^{0} nanoparticles.

3.5. Isolation of sweet cherry pathogen from sweet cherry fruits and pathogenicity evolution

Numerous *Alternaria* isolates were isolated from different parts of sweet cherry fruits that displayed symptoms of soft spots. A total of twelve *Alternaria* sp. isolates were collected from the fruit tissue, as shown in Table 1 and Fig. 8(a–c). The virulence capacity of these isolates was evaluated on sweet cherry fruits using the Ahmad et al. method, and it was found that CHM-4 exhibited the most potent virulence capacity on sweet fruits [30]. Molecular identification and subsequent tests were conducted on CHM-4.

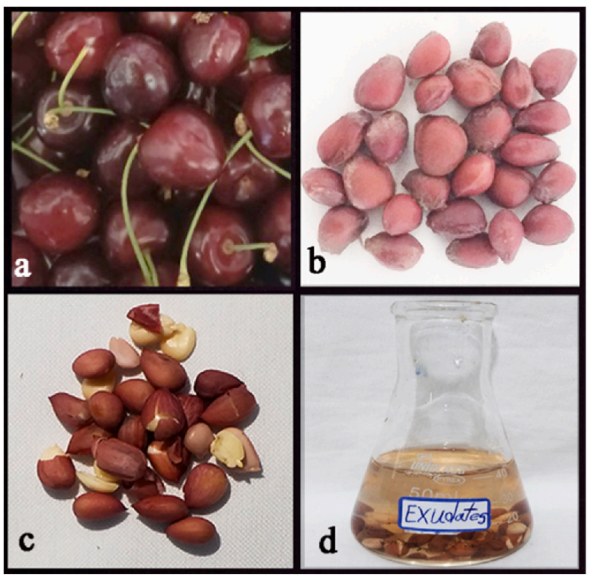

Fig. 1. (a), (b) and (c) depict the sequential stages involved in the exudates of cherry inner core from the fruits. (d) demonstrates the exudates found in the inner core of the cherry fruit after a duration of 72 h.

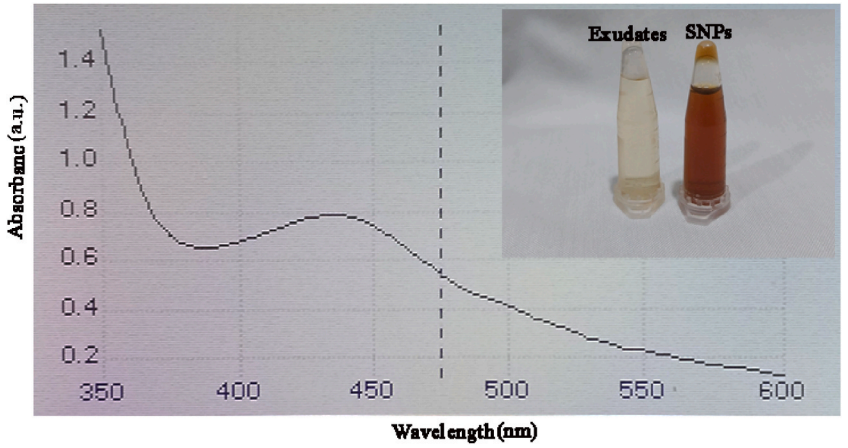

Fig. 2. The color change of the exudate and the UV–Vis spectra of SNPs synthesized ecofriendly were observed after being exposed to $AgNO_3$ after 24 h, while the control sample was solely the exudate.

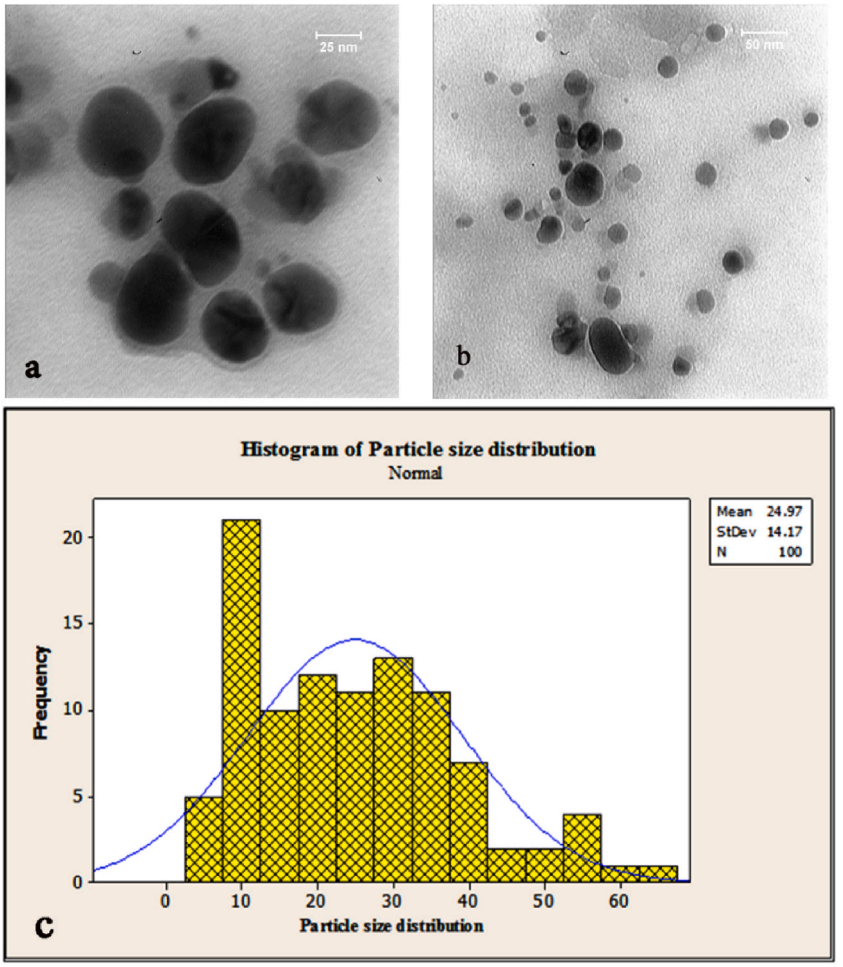

Fig. 3. The TEM findings of SNPs that were synthesized from sweet cherry kernel exudates are presented in this study. The spherical shape of SNPs at 25, and 50 nm is depicted in (a) and (b), respectively. Additionally, the average particle size distribution is illustrated in the histogram (d).

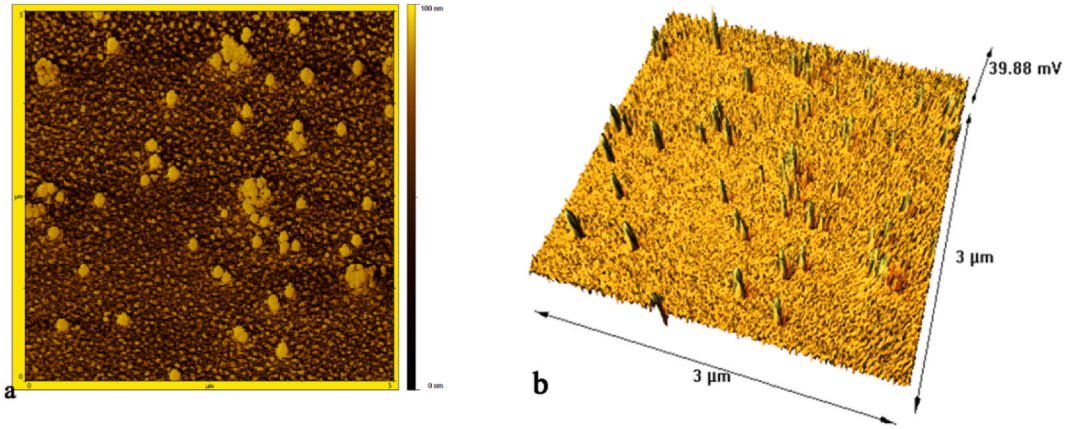

Fig. 4. The utilization of sweet cherry kernel exudates in the green synthesis of SNPs was analyzed through atomic force microscopy (AFM) imaging. The obtained results reveal the characteristic existence of spherical SNPs, which is evident in both the 1D (a) and 3D (b) images.

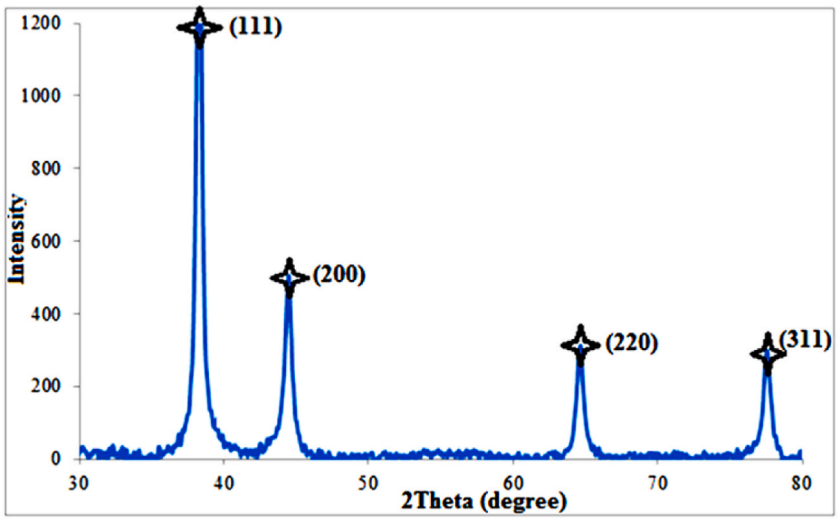

Fig. 5. The analysis of SNPs biosynthesized by sweet cherry kernel exudates using X-ray diffraction (XRD) pattern reveals the existence of four distinct and strong peaks, which suggest the crystalline nature of the ecofriendly synthesis of SNPs.

3.6. Molecular characterization

The polymerase chain reaction (PCR) was utilized to amplify the rDNA-ITS (Internal Transcribed Spacer) gene, as depicted in Fig. 9a and Supplementary Fig. 1. Subsequently, the amplified gene sequences were compared to all available sequences in the GenBank database using the BLAST sequence search program. Among these sequences, those that displayed the highest similarity to *Alternaria alternata* were identified, with an E value of 0.0 and a maximum identity of 95 %. The length of the sequenced fragments of the ITS gene was determined by referencing the accession number OR878473 in GenBank. Through phylogenetic analysis based on the ITS sequence, it was determined that our isolated Alternaria, named as CHM-4, belongs to *Alternaria alternata* (MG744381.1), as illustrated in Fig. 9b.

3.7. Control of fungus mycelium growth

The findings derived from the study on inhibition reveal that the impact of various concentrations of SNPs (5, 10, 20, and 40 µg/ml) in conjunction with the fungal culture medium, responsible for hindering the growth of mycelium, indicate that an increase in the concentration of green synthesized SNPs results in a decrease in the level of inhibition of mycelium extension, Fig. 10(a–e) and Fig. 11.

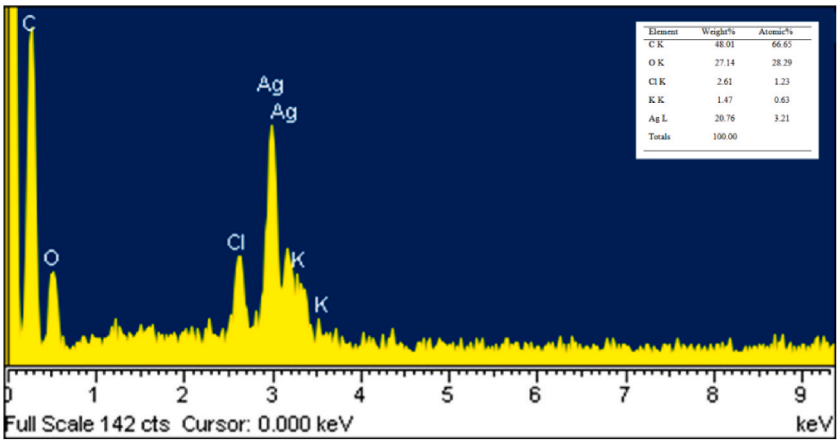

Fig. 6. Upon analyzing the EDX patterns of SNPs obtained from the exudates of sweet cherry kernels, it has been observed that there are unique signals present, indicating the existence of silver within the sample.

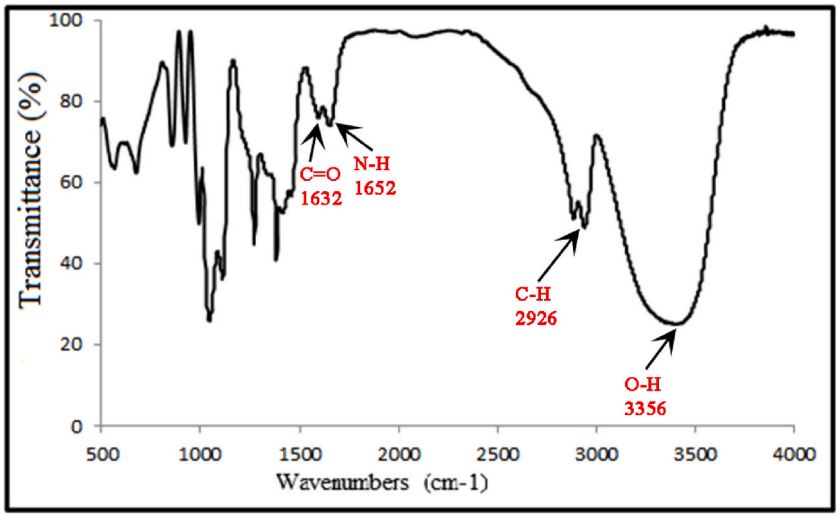

Fig. 7. The utilization of Fourier Transform Infrared (FTIR) spectroscopy was implemented to examine the SNPs that were synthesized through phytosynthesis, with the assistance of sweet cherry kernel exudates.

Table 1
The pathogenicity test results for the diameter of rot on sweet cherry fruits were compared at 3, 6, and 9 days post-inoculation with *Alternaria* spp.

	Day of recording the rot diameter (mm)			
Isolates	Region	3rd	6th	9th
CHC-1	Chenaran	4.92 ± 0.87^{a}	7.71 ± 1.54^{b}	11.88 ± 2.77^{c}
CHC-2	Chenaran	4.08 ± 0.51^{a}	7.22 ± 1.32^{b}	11.53 ± 2.61^{c}
CHC-3	Chenaran	4.85 ± 0.79^{a}	7.37 ± 1.19^{b}	11.87 ± 2.28^{c}
CHC-4	Chenaran	4.79 ± 0.61^{a}	7.35 ± 1.26^{b}	11.75 ± 2.19^{c}
CHM-1	Mashhad	4.95 ± 0.91^{a}	8.94 ± 1.86^{bc}	12.21 ± 2.87^{c}
CHM-2	Mashhad	4.81 ± 0.83^{a}	7.82 ± 1.79^{b}	11.92 ± 2.91^{c}
CHM-3	Mashhad	4.98 ± 0.91^{a}	8.52 ± 1.63^{bc}	11.84 ± 2.31^{c}
CHM-4	Mashhad	7.23 ± 2.78^{b}	12.41 ± 3.47^{c}	16.44 ± 3.14^{d}
CHO-1	Ortokand	4.13 ± 0.54^{a}	7.28 ± 1.35^{b}	11.61 ± 2.66^{c}
CHO-2	Ortokand	4.54 ± 0.73^{a}	7.17 ± 1.11^{b}	11.21 ± 2.24^{c}
CHO-3	Ortokand	4.74 ± 0.78^{a}	7.32 ± 1.24^{b}	11.81 ± 2.52^{c}
CHO-4	Ortokand	4.05 ± 0.72^{a}	7.11 ± 1.13^{b}	11.13 ± 2.12^{c}

The statistical analysis demonstrated significant differences between the means of the data sets, as evidenced by the similarity of letters in the columns ($p \leq 0.5$), which were assessed using Duncan's multiple range tests. The trials were conducted thrice.

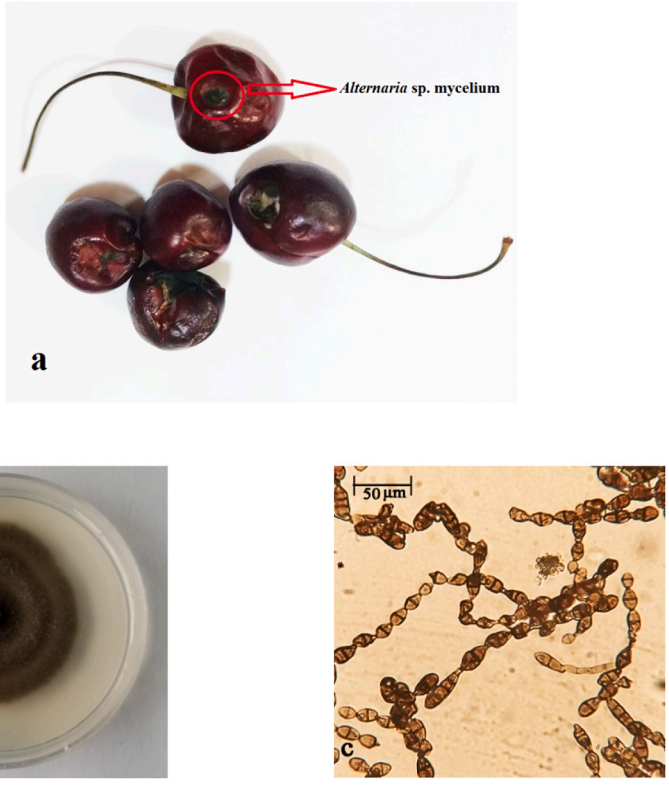

Fig. 8. The *Alternaria* sp. was isolated through the identification of soft spots on sweet cherry fruits (a). A pure culture of *Alternaria alternata* CHM-4 was then developed and grown on PCA culture medium for a period of two weeks (b). The resulting isolates of CHM-4 exhibited spore chains (c).

3.8. Effect of destruction of SNPs on mycelium causing the disease

The inhibition zone was visualized through the utilization of light microscopy, revealing the inhibitory effects against *Alternaria alternata* isolate CHM-4. Various concentrations of SNPs (5, 10, 20, and 40 µg/ml) were employed on the mycelium, resulting in the observed impairment of fungal hyphae, Fig. 12(a–e).

3.9. Spore germination investigation

The growth of *Alternaria alternata* isolate CHM-4 spores was greatly hindered when exposed to the tested SNPs, as depicted in Fig. 13. The data obtained clearly demonstrated that the application of different concentrations of SNPs significantly reduced the germination of the fungus spores. Moreover, when the SNPs were applied at 40 concentrations, the spore germination was completely inhibited.

4. Discussion

The employment of plant materials for the phytosynthesis of SNPs is a novel approach that has captured the interest of researchers in recent times [48,49]. This approach is distinguished by its simplicity, as it encompasses a solitary procedure and does not necessitate the utilization of any hazardous substances. Consequently, this methodology is regarded as sustainable as it generates insignificant levels of harmful remnants in both the soil and water, making it an environmentally friendly approach [50,51]. SNPs have been ecofriendly synthesis produced by utilizing the various constituents of a plant encompass its leaves, flowers, fruits, exudates, roots, rhizomes., and other plant parts [52,53].

The agricultural industry suffers significant economic losses every year due to plant pathogens. Nanotechnology has emerged as a viable solution to address this concern, offering promising prospects for enhancing and maintaining the well-being of plants [54,55]. The realm of nano-agriculture, specifically, presents a wide array of possibilities that can revolutionize the methods employed in crop cultivation and safeguarding plants. Nano-agriculture offers a groundbreaking approach to improving plant health and minimizing the impact of plant pathogens by optimizing the utilization of nanomaterials [56]. The fungus *Alternaria alternata* is a latent necrotrophic microorganism that is responsible for postharvest diseases in a variety of fruits, such as bell pepper, apples, and sweet cherries [57,58]. In sweet cherries postharvest fruits, it manifests as black spots on the surface of ripening fruits, the lesions have the potential to progress into smooth and slightly sunken areas, varying in color from dark brown to black. These firm lesions can expand to a diameter

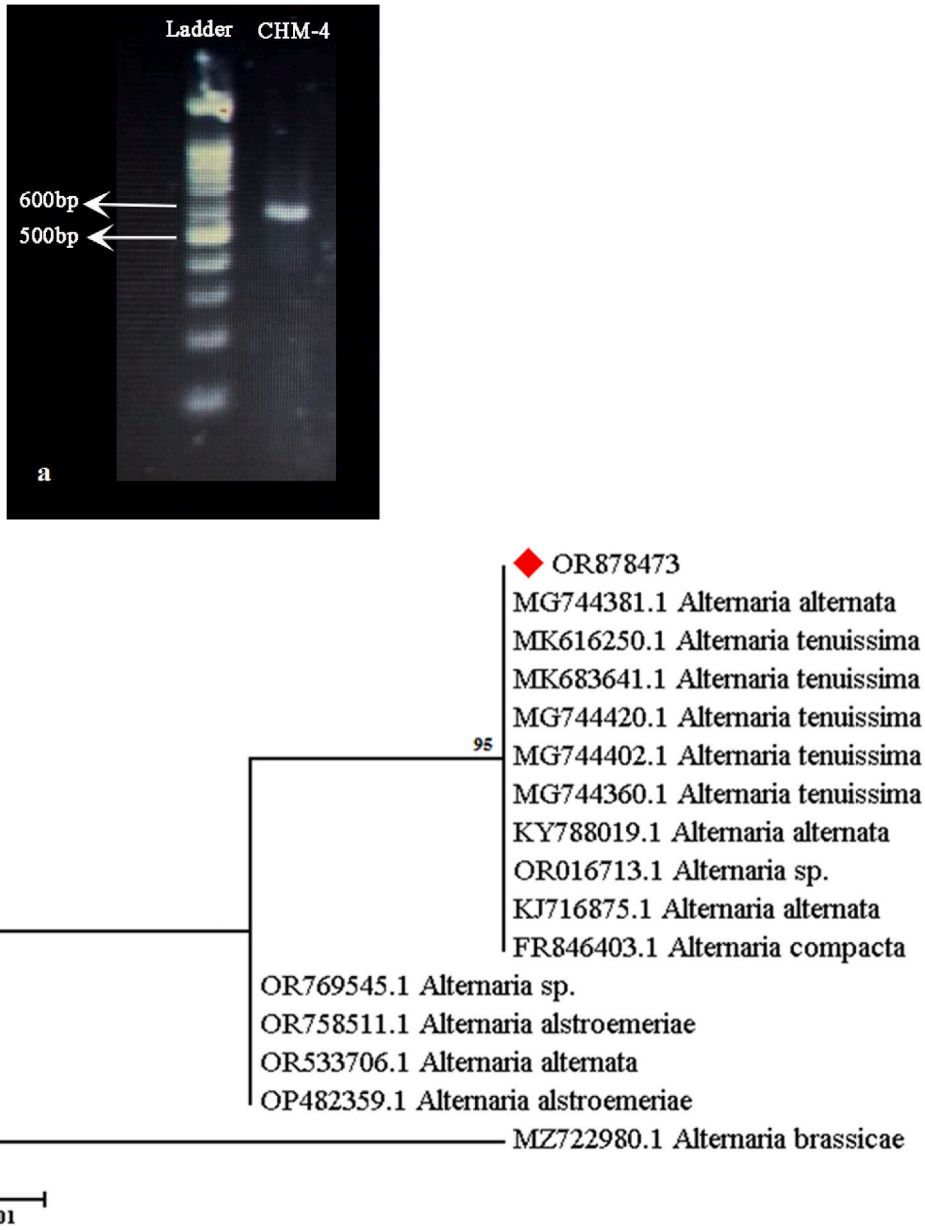

Fig. 9. The amplified PCR product obtained from the ITS region in *Alternaria* sp. L-100bp ladder demonstrates a significant amplification of the expected band size throughout. The construction of a phylogenetic tree, utilizing ITS sequence data, serves as a valuable tool for predicting and evaluating the similarity and evolutionary relationships among different *Alternaria* isolates. This analysis was conducted using the MEGA 7.0 software, employing the Maximum Likelihood method in conjunction with 1000 bootstrap replicates. In this study, *Alternaria brassicae* (MZ722980.1) was used as the outgroup, and it was observed that isolate CHM-4 exhibits the highest similarity to *Alternaria alternata* (MG744381.1).

of several centimeters [45]. In sweet cherries, it causes soft rot with a disease incidence of 30 % in each box containing fruit. To manage rot caused by *Alternaria alternata* in fruits, various methods are employed, including the use of synthetic fungicides to control its growth and sporulation [59]. In order to effectively control and reduce the detrimental effects of fungal pathogens on sweet cherries during long-term storage, the application of fungicides has become a widely adopted preventive measure [60]. Nevertheless, the utilization of fungicides in sweet cherry cultivation has given rise to environmental challenges and raised concerns regarding human safety, the emergence of pest-resistant strains, and the persistence of toxic residues [58]. This study aimed to explore the potential antimicrobial properties of SNPs synthesized using secretions from the inner core of sweet cherry fruit kernels. The SNPs were synthesized by exposing the sweet cherry inner core exudates to silver nitrate at various time intervals. The formation of SNPs was confirmed by the observation of a change in color to dark brown, which was further supported by UV–Visible spectroscopy analysis. Given the numerous

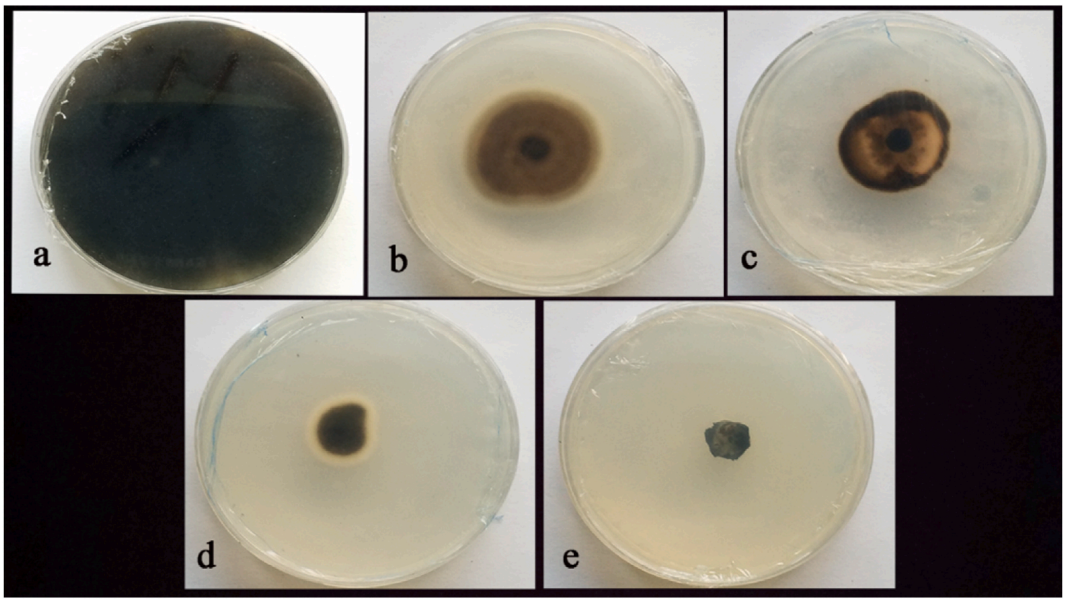

Fig. 10. The mycelium extension of *Alternaria alternata* isolate CHM-4 was examined for inhibitory effects caused by SNPs derived from cherry kernel exudates. The concentrations tested were as follows: (a) control, (b) 5 μg/ml, (c) 10 μg/ml, (d) 20 μg/ml, and (e) 40 μg/ml.

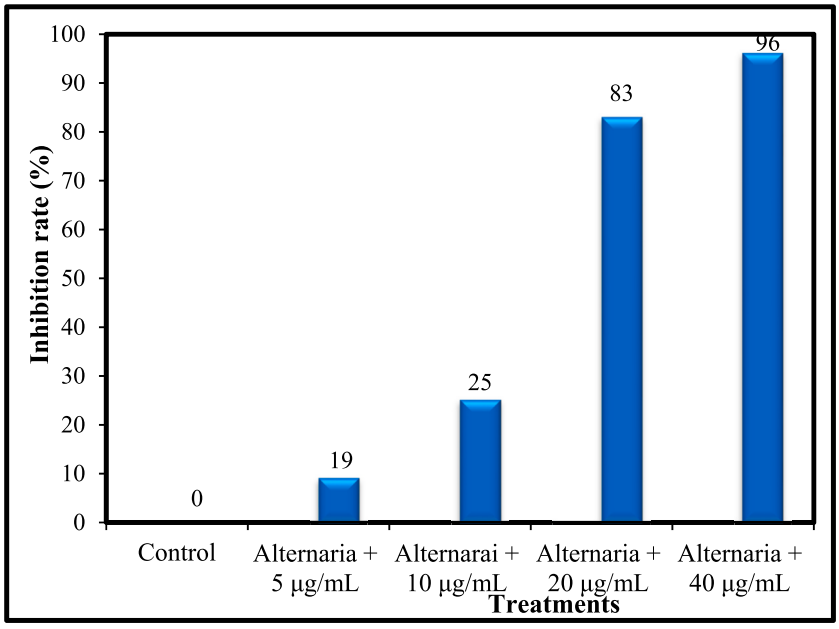

Fig. 11. The mycelium extension of *Alternaria alternata* isolate CHM-4 was influenced by the effects of sweet cherry kernel exudates on the *in vitro* release of SNPs and RH% calculated.

reports on the antimicrobial activity of SNPs, this investigation sought to contribute to the existing knowledge in this field. Green-synthesized SNPs exhibited a prominent absorption band at 440 nm, which can be attributed to their surface plasmon resonance (SRP) characteristics [36].

Upon examination of the TEM and AFM images of the synthesized SNPs, it was observed that the majority of the particles exhibited a spherical shape and were crystalline in nature. The presence of silver element in the biosynthesized SNPs was confirmed by the EDX signals, which also indicated a peak optical absorption range. The emergence of EDX peaks originating from C, O, and Cl could be attributed to either the carbon-coated copper grid or the emission of X-rays from the proteins and enzymes present in the sweet cherry exudates [61].

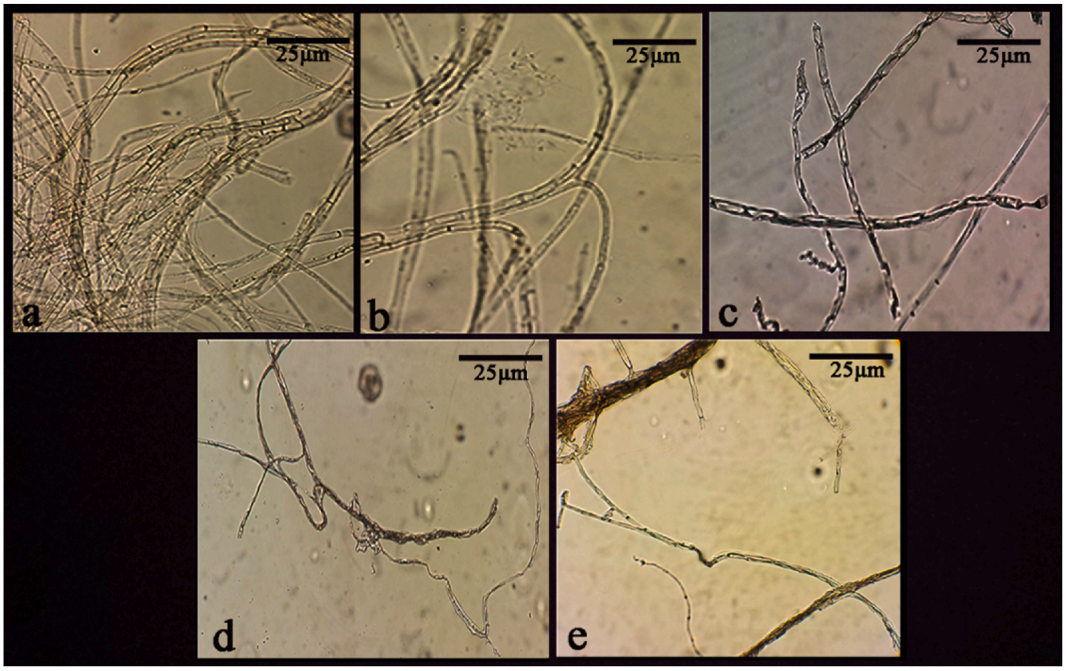

Fig. 12. The impact of different concentrations of SNPs derived from sweet cherry kernel exudates on the mycelium of *Alternaria alternata* isolate CHM-4 was investigated. The concentrations tested were as follows: (a) control, (b) 5, (c) 10, (d) 20 and (e) 40 µg/ml. The study aimed to assess the damaging effects of these SNPs on the growth and development of the fungal mycelium.

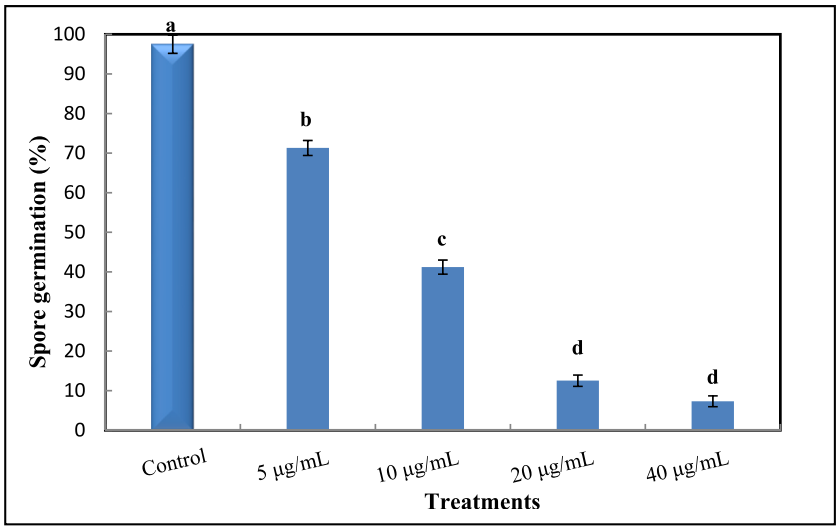

Fig. 13. The graph illustrates the impact of various concentrations of biosynthetic nanoparticles derived from sweet cherry kernel exudates on the inhibition of *Alternaria alternata* isolate CHM-4 spore germination.

The degree of color transformation, ranging from a yellowish hue to a deep brown shade, is directly proportional to both the amount of the exudates used and the duration of the incubation period. This phenomenon can be attributed to the activation of longitudinal plasmon vibrations and the reduction of $AgNO_3$ [62].The FTIR spectra exhibited a variety of absorption bands spanning from 530 to 3400 cm^{-1}, indicating the potential existence of biomolecules responsible for the reduction and stabilization of silver ions into SNPs within the sweet cherry exudates [63]. The XRD results displayed distinct peaks at different two theta values, indicating the presence of SNPs with diverse face-centered cubic silver planes [64]. Among these peaks, the sharpest one was observed at a two theta value of 38°, corresponding to the (111) plane. Consequently, it can be inferred that the SNPs exhibit similarities to the surface of the supporting substratum [65]. Different factors of nanomaterials such as their shape, size, dissolution, agglomeration state, chemical composition, specific surface area, crystal structure, surface morphology, surface energy, surface coating, and surface charge have a

significant influence on biological interactions, fate, and the intended or unintended consequences of nanomaterials [66,67].

Various mechanisms of action of SNPs have been identified thus far. These encompass the interference with protein function, the generation of reactive oxygen species (ROS), the breakdown of antioxidants, and the impairment of cell membrane integrity (such as disruption and permeabilization) SNPs have been discovered to stimulate the generation of reactive oxygen species (ROS) in diverse organisms. For example, a research study observed an elevation in ROS production in *Candida albicans* subsequent to exposure to SNPs, resulting in a delay in the cell cycle and a synergistic cytotoxicity when combined with 3-bromopyruvate [68]. Another review emphasized the augmented formation of ROS within cells due to the heightened chemical reactivity of SNPs, which can lead to oxidative stress and cytotoxic effects [69]. Furthermore, a model based on charging and discharging has been proposed to elucidate the interactions between SNPs and ROS. This model involves the charging of SNPs through superoxide-mediated mechanisms, followed by discharge through reactions with oxygen and Ag^{+}.Furthermore, SNPs have been discovered to elicit mutagenesis and reduce the expression of transfer protein genes [70]. The application of SNPs can bring about significant morphological changes in cell membranes, which can lead to disruptions in membrane fluidity and metabolic activities. This, in turn, can cause depolarization of the membranes and damage to cell components, which may ultimately result in cell death [71]. The impact of SNPs on cellular membranes and structures can cause denaturation, leading to the disruption of transport systems, including ion currents. This can result in the rapid accumulation of silver ions, which can further exacerbate the damage caused by SNPs [72].The presence of silver ions in high quantities causes a buildup that ultimately hinders vital cellular functions like respiration and metabolism due to their reactive interactions with different molecules [70]. In our study, the use of SNPs for treating fungi led to the severe deterioration of hyphal walls and subsequent plasmolysis of hyphae. These observations highlight the substantial effects of silver ions on cellular processes. The dynamic antifungal properties of SNPs, characterized by their significant surface-to-volume ratio, stem from their enhanced ability to interact with cellular layers and disrupt the structure of the cell membrane [73]. Consequently, SNPs exert an influence on various aspects of a microorganism, including the respiratory chain, cell division, DNA, and proteins [74,75]. Numerous studies have demonstrated the promising antifungal properties of SNPs. SNPs have proven to be effective against a range of fungal pathogens, including *Candida albicans*, *Aspergillus species*, and phytopathogenic fungi [76–78]. Furthermore, SNPs have shown efficacy against *Naegleria fowleri*, a free-living amoeba responsible for primary amoebic meningoencephalitis (PAM). The antifungal activity of SNPs has been observed to vary based on concentration and size. In conclusion, these studies highlight the potential of SNPs as efficient antifungal agents against various fungal pathogens. The potential risks associated with the utilization of SNPs have sparked concerns regarding their impact on human health and the environment. Although several studies have demonstrated the robust antifungal properties of SNPs, their applications in diverse fields such as food packaging, seed preservation, biofertilizers, cosmetics, and pharmaceuticals, the precise influence of nanoparticle size and morphology on their antifungal activity remains unclear based on the available search results.

5. Conclusion

The research findings suggest that SNPs derived from sweet cherry fruit kernel secretions have significant antifungal capabilities, making them a suitable alternative to chemical-based methods in combating post-harvest diseases. SNPs, nanoemulsions, and nanoformulations exhibit characteristics that enhance their effectiveness as nanopesticides, resulting in increased solubility. The SNPs have the ability to specifically target post-harvest diseases with greater efficiency, thereby minimizing their effects on non-target areas. This investigation represents the first report demonstrating the characteristics of biosynthesized SNPs in the management of Alternaria, the causal agent of cherry spot disease. The effectiveness of the bio-SNPs in combating different Alternaria diseases is suggested to be influenced by their size and dosage, as observed in the study. To gain a deeper understanding of their efficacy, further investigations are advised.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Meysam Soltani Nejad: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Neda Samandari Najafabadi:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Sonia Aghighi:** Writing – review & editing, Validation, Supervision. **Meisam Zargar:** Validation, Supervision. **Maryam Bayat:** Writing – review & editing, Software, Formal analysis. **Elena Pakina:** Writing – review & editing, Validation, Methodology.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31508.

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