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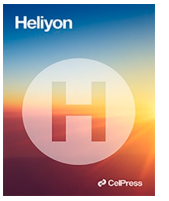

Research article

Application of phytosynthesized silver nanoparticles (SNPs) against *Erwinia amylovora* causing fire blight disease

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ABSTRACT

The bacterium *Erwinia amylovora* is responsible for the destructive disease known as fire blight in pear trees. This highly detrimental condition poses a significant threat to the health and vitality of these trees. The existing strategies for managing fire blight disease involve the regular use of copper compounds and streptomycin, particularly during periods when environmental factors are conducive to the spread of the infection. Silver nanoparticles, also known as SNPs, are tiny specks of silver ranging in size from 10 to 100 nm. These particles are created through various chemical and biological processes. Numerous studies have demonstrated their ability to exhibit antibacterial properties against a wide range of human and animal pathogens. In this investigation, the dimensions of SNPs were ascertained by employing aqueous extracts derived from apple, pear, and quince leaves. The average sizes of the SNPs were found to be approximately 30 nm, 38 nm, and 55 nm, apple, quince and pear respectively. The pear mature fruits successfully managed to control the rot caused by the disease-causing *E. amylovora*. This study shows the viability of utilizing leaves extract from apple, pear, and quince as a suitable medium for the production of silver nanoparticles. These nanoparticles hold potential for effectively managing fire blight disease.

1. Introduction

Erwinia amylovora, belonging to the *Erwiniaceae* family and the Enteriobacterales order, is a gram-negative bacterium that can function as a facultative anaerobe [1]. The bacterium is accountable for fire blight, an extremely damaging disease that impacts a range of host plants such as apples (*Malus* spp.), pears (*Pyrus* spp.), raspberries (*Rubus* spp.), quince (*Cydonia* spp.) and other crops within the *Rosaceae* family [2,3]. Iran, with its vast land area and favorable weather conditions, is highly suitable for apple cultivation [4]. The apples cultivated within this nation show a wide array of forms and shades, showcasing a sturdy pulp that can be observed in tones of crimson, ivory, or pale yellow [5]. The economic and agricultural implications of fire blight on these plants are substantial. Quince cultivation in Iran is limited to a relatively small area of approximately 6,100 ha, resulting in an annual production of 87,799 tonnes of quince fruit [6]. Consumers have become aware of the quince's exceptional antioxidant and anti-allergic properties. Among pome fruit trees, the pear is particularly susceptible to fire blight disease caused by *E. amylovora* [7]. The economic impact of fire blight control is substantial on a global scale, as the swift dissemination of *E. amylovora* is facilitated by precipitation, air currents, and various insects [8]. Iran, one of the world's major pear producers, is actively engaged in pear cultivation [9].

The fire blight disease first presents itself with symptoms appearing on flowers, leading to the development of water-soaked lesions

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that rapidly wilt [10]. The flowers that have been infected continue to exist on the plants. To address the fire blight disease, the current approach to chemical control includes the regular use of copper compounds and streptomycin. Nevertheless, the use of these methods is limited in various countries due to their harmful effects on the environment [11,12]. An area of research that shows promise is the application of nanotechnology in developing highly efficient materials at the nanometer scale. Silver nanoparticles have attracted considerable interest due to their wide-ranging antimicrobial properties, positioning them as a potential remedy for managing plant diseases [13]. Nanobiotechnology has the capability to transform numerous industries by creating novel materials and devices that exhibit enhanced properties and performance [14]. Nevertheless, to fully exploit the benefits and mitigate the drawbacks of nanomaterials, it is imperative to gain a deeper understanding of the fundamental mechanisms and optimize their design for specific applications. Nanobiotechnology has found significant applications in the field of nanomaterial biosynthesis [15]. Biomedical research, photocatalytic degradation of pollutants, and the use of SNPs as an anti-caries agent in dentistry are some potential areas where SNPs can be utilized [16]. Ongoing research is being conducted to gain a better understanding of the risks associated with SNPs. Various emerging fields of study have developed as a result of natural processes that produce inorganic compounds with nanoscale dimensions. These disciplines primarily focus on the biological synthesis of nanomaterials [17]. In an investigation concerning the antifungal properties of SNPs in lip balm cosmetic formulations, it was discovered that SNPs demonstrated commendable antifungal efficacy and compatibility with living organisms, thereby presenting themselves as promising substitutes for traditional preservatives [18]. The process of synthesizing SNPs in an environmentally friendly manner involves three primary stages: choosing an appropriate solution medium, utilizing a non-toxic reducing agent, and employing a safe material for stabilizing the nanoparticles [19]. In recent times, the distinctive physicochemical characteristics of SNPs have garnered considerable interest, owing to their potential applications in diverse domains such as medicine, catalysis, and environmental remediation [20]. These nanoparticles exhibit remarkable properties such as antimicrobial, anti-inflammatory, and wound-healing capabilities, which make them highly promising for biomedical purposes [21]. Despite concerns about their toxicity to humans and the environment, the high antimicrobial activity of SNPs at low concentrations and their low bacterial resistance have prompted researchers to consider their application [22]. By utilizing SNPs, the environmental risks associated with excessive use of antibiotics or pesticides can be reduced [23]. Plant extracts have been preferred for the synthesis of SNPs due to their safety for the environment [24]. In a recent study, SNPs were employed to facilitate easy synthesis of highly stable and environmentally friendly nanoparticles derived from aqueous extracts of the leaves and petals of *Tagetes erecta* L. The synthesized nanoparticles exhibited significant antibacterial activity against *E. amylovora* [25]. In another investigation, the antimicrobial efficacy of sesame essential oil and SNPs produced from sesame oil was assessed against *E. amylovora*. The biosynthesized SNPs demonstrated significant antibacterial activity against *E. amylovora* [26].

The primary objective of this study is to utilize the extract derived from the leaves of apple, pear, and quince to enhance the production of silver nanoparticles (SNPs) through plant-based procedures. This approach is not only cost-effective but also straightforward, making it an advantageous method for the biosynthesis of nanoparticles. The testing procedure involves a biological method conducted under standard temperature and pressure conditions, eliminating the need for chemical solvents as reducing and stabilizing agents.

The biosynthesized nanoparticles will be assessed using a range of techniques, including UV spectrophotometer, AFM, TEM, DLS, and XRD.

The study aims to regulate the underlying factors contributing to the occurrence of fire blight disease in pears. Our research focuses on examining the impact of inhibiting pathogenic bacteria through the use of biosynthesized silver nanoparticles (SNPs). In the upcoming experiments, we will investigate various minimum inhibitory concentrations (MIC) of these biosynthesized SNPs as a novel antibacterial agent, intending to inhibit the pathogenicity of fire blight in pears.

After conducting a thorough review of available literature, no evidence was found to support certain claims. Additionally, we will explore the potential of utilizing extracts from apple, pear, and quince leaves for the phytosynthesis of SNPs and subsequently employ them in combating fire blight.

Our extensive literature review indicated a lack of evidence regarding the comparative antimicrobial effects of three biosynthetic SNPs derived from the leaves of pome fruit trees against *E. amylovora*. This study represents the first report on the inhibitory properties of biosynthetic SNPs in mitigating cell death induced by the pathogen in pear leaves.

2. Materials and methods

2.1. Chemical substances, a specific type of bacteria, and the organic matter derived from plants

The study conducted by Tarighi et al. [7] utilized the *E. amylovora* (Burrill 1882) ATCC49946 strain. The pears (*Pyrus communis* cv. *Spadona*) were sourced from the Horticultural Science Research Institute (HSRI) in Karaj, Iran. The quince (*Cydonia oblonga* Miller), apple (*Malus domestica*), and pear leaves were collected from the botanical garden of Ferdowsi University of Mashhad during the autumn season. In cases where it was necessary, a concentration of 10 μg/mL of streptomycin antibiotic was employed.

2.2. The process of plant leaves extract

To produce the aqueous leaf extract of pear, quince, and apple, fresh leaves were collected from the respective plants and underwent a disinfection procedure involving 75 % alcohol for 5 min. Following this, the leaves were rinsed thrice with sterilized distilled water. A total of 10 g of cleaned leaves were then boiled in 200 mL of deionized water for a period of 10 min. Following this, the resulting suspension was subjected to centrifugation at a speed of 8000 rpm for 5 min, as described by Khatami et al. [24]. The filtrate

acquired from this procedure underwent filtration using Whatman No. 1 filter paper. Subsequently, these solutions were preserved at a temperature of 4 °C until they were prepared for application. It is important to highlight that the pH of the aqueous extract derived from the leaves remained constant at 6.5 during the entire duration of the experiments.

2.3. The eco-friendly reaction of synthesizing silver nanoparticles (SNPs)

In order to produce SNPs, a mixture was prepared by combining 10 mL of 0.001 M silver nitrate ($AgNO_3$) solution with 50 mL of leaf extract. Subsequently, the mixture was left to incubate at 28 °C for a period of 2 h. The synthesis of SNPs from the aqueous leaf extract was evaluated using an ultraviolet–visible (UV–Vis) spectrophotometer, with the absorption wavelength being recorded between 350 and 500 nm at room temperature.

2.4. The physicochemical properties of silver nanoparticles (SNPs) analysis

The determination of the morphological properties and dimensions of SNPs was achieved through the utilization of transmission electron microscopy (TEM) measurements, utilizing a Carl ZEISS transmission electron microscope from Germany. Additionally, surface examinations of SNPs were performed using atomic force microscopy (AFM).

The high resolution X-ray diffraction (XRD) technique was employed to examine the progression of compounds, assess their quality, identify their phase, and analyze the crystalline metallic SNPs. The examination process involved investigating the 2θ range from 30° to 80°, using Cu Kα radiation with a wavelength of 1.541874 nm.

Elemental composition investigations were carried out by employing energy dispersive X-ray (EDX) spectroscopy.

The DLS measurements for the recently synthesized SNPs were conducted by Cordouan Technologies Company in France. These measurements were performed under specific conditions, which included a temperature of 25 °C, laser power set at 50 %, DTC position in the upward direction, and a wavelength of 657.00. All measurements were carried out in triplicates. The DLS technique yielded several parameters, such as the zeta-average and the polydispersity index (PDI). Among these parameters, the z-average is regarded as the primary and most reliable parameter generated by the DLS spectrometer.

2.5. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The method for microdilution, as modified by Akhlaghi et al. [27], was employed to ascertain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SNPs. In this investigation, 100 μL of SNPs were mixed with 100 μL of mueller hinton broth medium, resulting in a concentration of 500 μg/mL of SNPs in the initial well of each row in 96-well microtiter plates. Subsequently, the SNPs were serially diluted by a factor of 2 in the 96-well plates, generating a spectrum of concentrations ranging from 500 to 0.48 μg/mL, with a total volume of 200 μL. Following this, 10 μL of an overnight culture of *E. amylovora* with a concentration of 1×10^8 CFU/mL was introduced into each well of the 96-well plate. The MIC was defined as the lowest concentration of SNPs at which no discernible growth (increase in turbidity) was detected. To ascertain the minimum bactericidal concentration (MBC), a 10 μL volume was extracted from every well of a 96-well plate that contained concentrations higher than the minimum inhibitory concentration (MIC). Subsequently, these samples were cultured on NA medium for a duration of 24 h at a temperature of 28 °C. The MBC was determined as the concentration at which no bacterial growth was detected on the NA medium. To ensure precision and consistency of outcomes, this procedure was repeated thrice.

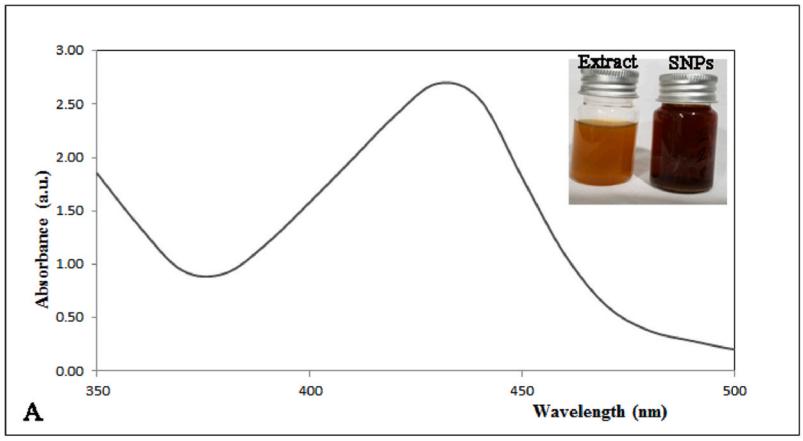
2.6. SNPs in vivo test for their effectiveness in reducing soft rot disease on pear fruit

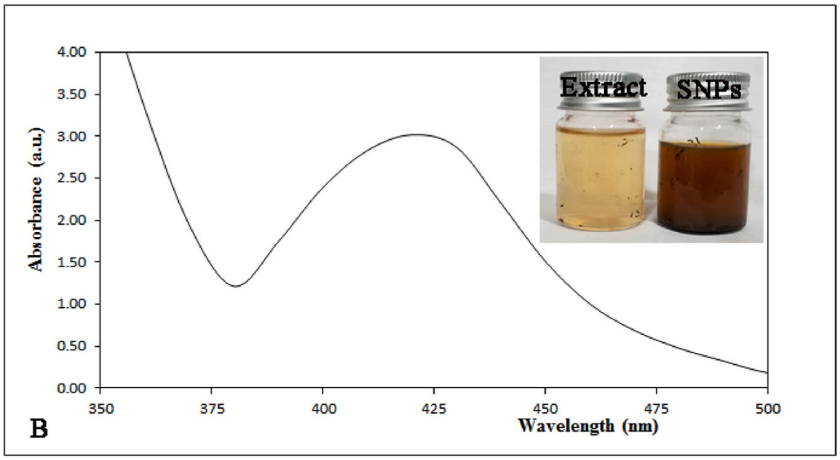
The pear fruits sourced from the gardens of Ferdowsi University in Mashhad, Iran were utilized. Only healthy fruits of consistent size and free from any mechanical damage were chosen for the purpose of this study. The soft rot virulence test was conducted in order to investigate the impact of SNPs on diminishing symptom expression in fruit tissues. The fruits underwent a thorough washing process using distilled water, which was repeated three times. Subsequently, they were subjected to surface disinfection by immersing them in a solution of 5 % sodium hypochlorite. After disinfection, the fruits were dried. To obtain overnight bacterial cultures, the cultures were harvested through centrifugation. These cultures were then suspended in double sterile distilled water, which was adjusted to a turbidity of 0.3 at 600 nm, equivalent to a concentration of 1×10^6 CFU mL^{-1}. Following the introduction of SNPs at MIC concentration diluted in one percent Tween 20 to the suspension, 150 μL of the suspension was injected using insulin needles into the vegetables tissues at a depth of 1 mm. The infected tissues were then maintained at a temperature of 28 °C in humidified plastic containers. The initial weight of the fruits (IW) was documented, and 48 h later, the decayed tissue (DW) was removed from the tubers, weighed, and separated. The percentage of macerated tissue was determined by applying formula 1:

The percentage of decay was calculated using the formula Decay (%) = (DW/IW) × 100. The findings were then contrasted with those of the control group, which consisted of fruits that were solely inoculated with *E. amylovora* suspensions [28].

2.7. Measurement of cell death

The pear leaves were subjected to a minimum inhibitory concentration (MIC) of SNPs prior to the introduction of bacteria. The pear pathogens were then introduced at different time intervals, namely 0, 6, 12, 24, and 48 h after inoculation (hpi). The effect of SNPs on cell death resulting from the invasion of *E. amylovora* was examined using the methodology described by Daroodi et al. [29]. The





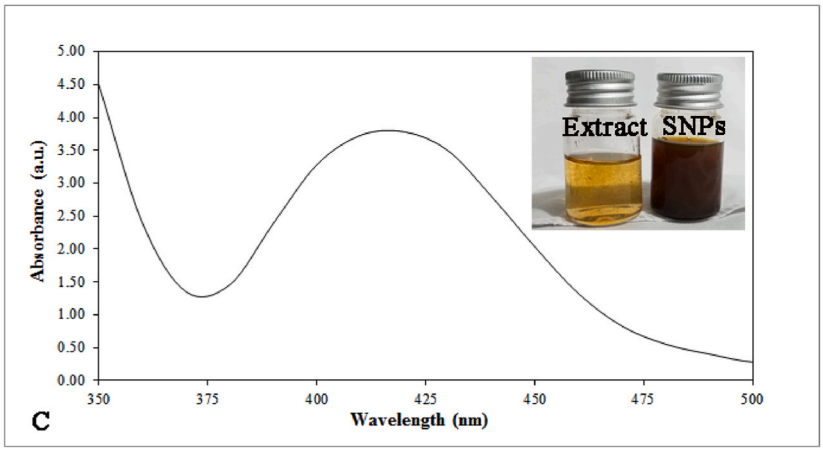


Fig. 1. The color alteration of the leaf extract solution with the inclusion of silver nitrate ($AgNO_3$) is depicted, where in a prominent peak at 430 nm is observed. (A), (B) and (C) are reaction of apple, quince, and pear respectively.

experiment was conducted with three replications and three iterations.

2.8. Statistical analysis

The analysis of the collected data was performed utilizing SAS software, specifically SAS Institute's version 9, located in Cary, NC, USA.

3. Results

3.1. Investigation the utilization of visual observation and UV–Vis spectroscopy techniques

The eco-friendly synthesis of SNPs was successfully carried out by utilizing leaf extracts obtained from pear, quince, and apple as a sustainable and natural source. The process involved the reduction of silver nitrate ions through an overnight exposure to the leaf extract, leading to the production of SNPs. This was confirmed by the emergence of a distinct brown coloration after a 24-h period, as illustrated in Fig. 1(A–C). Intriguingly, the introduction of silver ions as an electron acceptor to leaves extract, which acted as an electron donor, resulted in a noticeable color transformation to a vivid red hue. This color change signified the reduction of (Ag^{+}) to (Ag^{0}) and the subsequent formation of SNPs.

3.2. X-ray diffraction technique observation

The X-ray diffraction analysis indicated the existence of four separate peaks across the 2 theta range of 30–80, as illustrated in Fig. 2 (A–C). The crystalline structure of the SNPs was validated through the identification of XRD peaks at 111, 200, 220, and 311 planes, situated at 38°, 44°, 64°, and 77° angles, respectively.

3.3. EDX analysis

The elemental analysis of silver (Ag) and the determination of the elemental composition of the samples were carried out using the

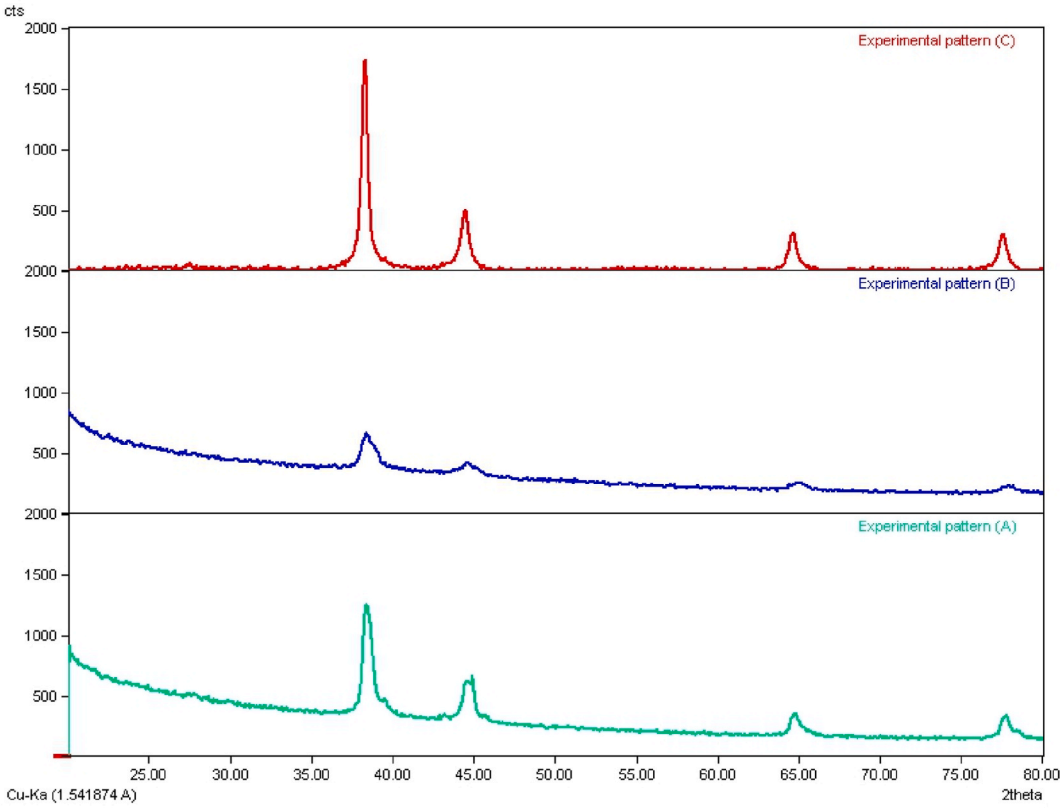


Fig. 2. Three XRD patterns of silver nanoparticles (SNPs) biosynthesized from leaves aqueous extract. (A), (B) and (C) are SNPs of apple, pear and quince respectively. The notable peaks align with distinct 2theta diffraction angles of 38°, 44°, 64°, and 77°, which correspond to the Bragg reflections originating from the (111), (200), (220), and (311) crystal planes, respectively.

EDX spectroscopy technique. The energy dispersive X-ray (EDX) spectroscopy pattern of the SNPs synthesized by the leaf extract of apple, quince, and pear were observed Supplementary Figs. 1(A–C), and the outcome verified the existence of elemental silver in the solution that were prepared.

3.4. TEM and AFM studies

TEM analysis was utilized to determine the shapes of SNPs. The TEM images presented in Fig. 3 reveal that the majority of the synthesized SNPs were spherical and hexagonal, Fig. 3(A–F). In order to examine the surface characteristics of the synthesized SNPs, AFM was employed to capture both one-dimensional (1D) images Fig. 4(B–D, F) and three-dimensional (3D) images Fig. 4(A–C, E). The analysis of AFM images further confirmed the existence of spherical SNPs with particle sizes that varied.

3.5. Dynamic light scattering (DLS) analysis

Using dynamic light scattering (DLS) research, the size of silver nanoparticles (AgNPs) were measured. Supplementary Fig. 2 (A-C) depicts the Zaverage and the particle number within the 10–100 nm size range was indicated by the particle size distribution

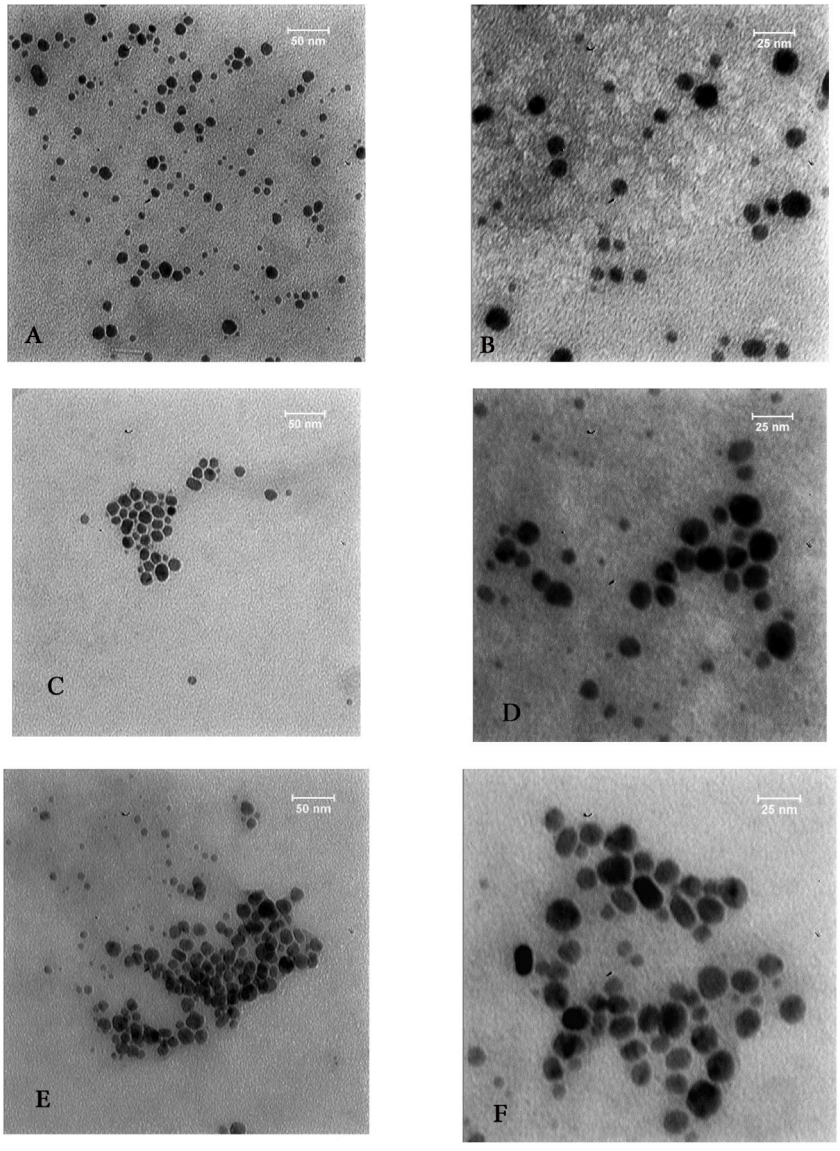


Fig. 3. Various images of SNPs were captured from a liquid solution that had been incubated with leaves extract containing silver ions for a duration of 24 h, utilizing two different scales of 50 and 25 nm for SNPs-apple (A, B), SNPs-pear (C, D), and SNPs-quince (E, F). The transmission electron microscopy (TEM) findings revealed the presence of both spherical and hexagonal structures of silver nanoparticles.

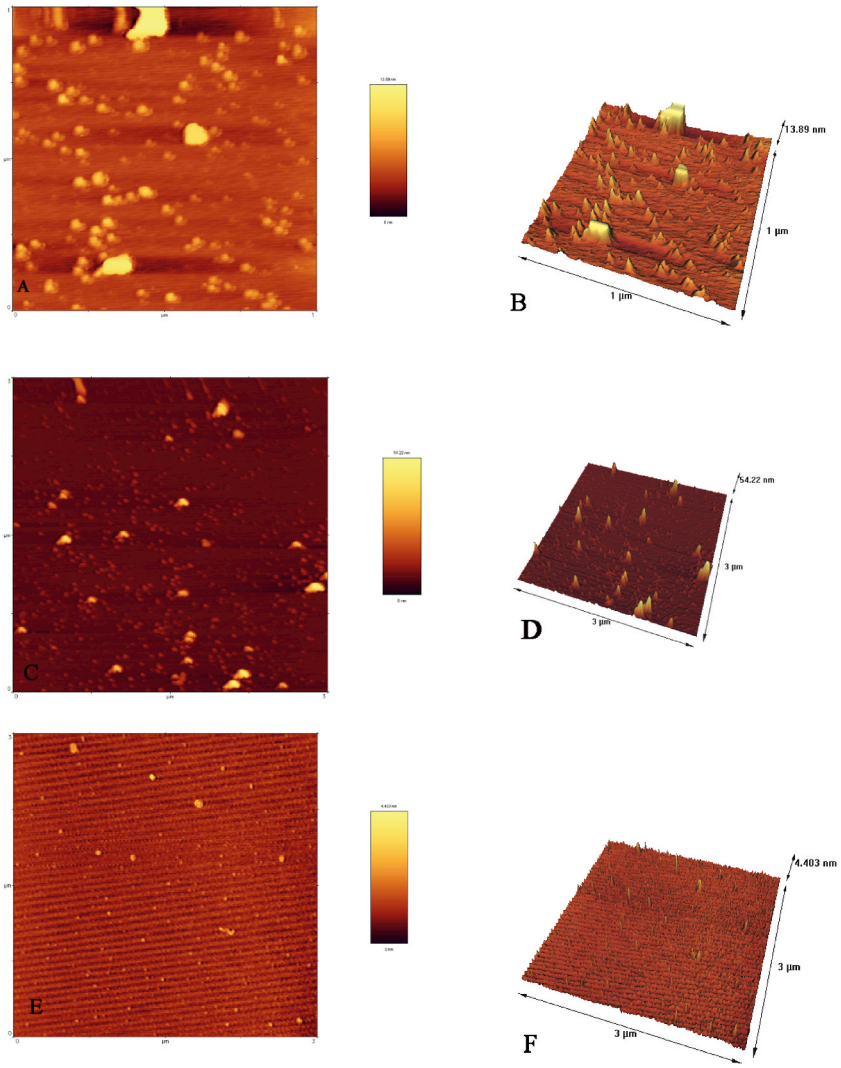


Fig. 4. Atomic force microscopy (AFM) images depicting spherical and hexagonal silver nanoparticles (SNPs) synthesized from three distinct plant leaves, apple (A, B), quince (C, D), and pear (E, F) were acquired in both one-dimensional and three-dimensional formats.

Table 1
Shows size dispersion number, PDI and Zaverage for SNPs of apple, quince and pear.

SNPs	Zaverage (nm)	PDI	Dmean number
Apple	100.51	0.1911	30.47
Quince	152.18	0.2040	38.42
Pear	161.01	0.2300	55.72

measurement Supplementary Fig. 2 (D-F), and PDI, Table 1. Supplementary Fig. 2 (B, C, D) showed mobility of SNPs.

3.6. MIC and MBC measurement

The utilization of green synthesized SNPs enabled the determination of the minimum inhibitory concentration (MIC) for SNPs-pear and SNPs-quince, which were found to be 25 µg/mL. The MIC for SNPs-apple was determined to be 6.25 µg/mL. The MBC measured for SNPs-apple is 12.5 and for SNPs-pear and SNPs-quince were 50 µg/mL. These results are presented in Table 2.

Table 2
Results of SNPs inhibitory properties against *E. amylovora*.

Concentration (μg/ml)	Growth (OD = 600 nm)				
	Control	Streptomycin	SNPs		
			Pear	Apple	Quince
100	1.98 ± 0.63^{a}	0^{d}	0^{d}	0^{d}	0^{d}
50	1.95 ± 0.55^{a}	0^{d}	0^{d}	0^{d}	0^{d}
25	1.93 ± 0.57^{a}	0^{d}	0.12 ± 0.05^{d}	0^{d}	0.19 ± 0.07^{d}
12.5	1.95 ± 0.76^{a}	0^{d}	0.38 ± 0.07^{c}	0^{d}	0.31 ± 0.15^{c}
6.25	1.95 ± 0.58^{a}	0^{d}	0.91 ± 0.19^{b}	0.11 ± 0.08^{d}	0.95 ± 0.12^{b}
3.12	1.93 ± 0.51^{a}	0^{d}	1.12 ± 0.49^{a}	0.31 ± 0.15^{c}	1.81 ± 0.41^{a}
1.62	1.91 ± 0.54^{a}	0.34 ± 0.09^{c}	1.91 ± 0.51^{a}	1.87 ± 0.17^{a}	1.83 ± 0.45^{a}

* The mean inhibitory concentration (MIC) and (MBC) progression presented in our study represents the average outcome of three separate trials conducted for each treatment, with the analysis utilizing a variance of $n = 5$, a commonly utilized method. Variations in traits were indicated by the use of different letters, signifying significant differences at a significance level of $p \leq 0.05$, as determined by Duncan's Multiple Range Test. The control group consisted of a mixture of three leaves extract, while Streptomycin served as the positive control.

3.7. SNPs antibacterial effects on the pear mature fruits

The SNPs exhibited a decrease in symptoms on individual pear fruits when compared to the positive control group, which consisted of fruits inoculated with *E. amylovora* and displayed the highest percentage of decay, Fig. 5(A–E). Our findings highlight the effectiveness of SNPs treatment in mitigating bacterial disease development in fruits, as evidenced by a comparison with control samples in an *in vitro* assay, Fig. 5(A–E). The utilization of SNPs has demonstrated a significant reduction in fruit rot. Specifically, SNPs-apple, SNPs-quince, and SNPs-pear resulted in a decrease of 14.5 %, 22.6 %, and 34.5 % in rot, respectively, Fig. 6.

3.8. Measurement of cell death

Plant cell death in response to pathogen treatments exhibited an initial increase within the first 6 h after infection, followed by a subsequent decline until 12 h post-infection. Following this, there was a revival in cellular demise up to 48 h after the infection occurred. The utilization of *E. amylovora* + SNPs treatments led to a rise in cellular mortality until 12 h post-infection (hpi), followed by a subsequent decline until 48 hpi. Conversely, the leaves subjected to SNPs treatment displayed a minor elevation in cell death. The results of the experiment demonstrated that pre-inoculating pear leaves with SNPs prior to pathogen exposure led to a notable decrease in cell death when compared to leaves that were only infected with pathogens, Fig. 7. Additionally, both the control group and the group of leaves treated with SNPs but not exposed to pathogens maintained a stable condition with minimal cell death. The findings suggest that SNPs may play a crucial role in protecting pear leaves against cell death caused by pathogens.

4. Discussion

Currently, the biogenic production of silver nanoparticles (SNPs) utilizing plant materials has emerged as a novel technique that has captured the interest of numerous researchers due to its straightforward one-step procedure that does not generate toxins [30,31]. Therefore, this method is a sustainable approach that minimizes the presence of harmful residues in both soil and water, making it environmentally friendly. The SNPs were produced using various components of plants, including leaves, flowers, fruits, roots, rhizomes, and so forth [32,33].

Plant diseases are a major contributor to yearly financial setbacks in the farming sector. In order to combat this urgent problem, nanotechnology has surfaced as a hopeful method to boost and maintain plant well-being [34,35]. The domain of nanotechnology, specifically in the area of nano-agriculture, provides various possibilities that have the capability to introduce innovative strategies to crop cultivation and plant safeguarding. Through streamlining the use of nanomaterials, nano-agriculture introduces a fresh approach to enhancing plant health and lessening the consequences of plant diseases [36,37].

Fire blight has inflicted harm upon fruit trees across the globe, including Iran. Consequently, scientists worldwide have endeavored to discover the most effective methods for managing this disease, employing various chemicals, primarily antibiotics, to combat fire blight. Nevertheless, the utilization of these substances is restricted due to the uncertain adverse effects caused by copper compounds. Moreover, the application of copper compounds can potentially induce toxicity in plants [38,39].

This study was designed to explore the role of silver nanoparticles (SNPs) synthesized using the leaves of the most vulnerable host of *E. amylovora*, in response to numerous reports highlighting the antimicrobial activity of these SNPs. The leaf extracts derived from apple, quince, and pear were employed in the production of SNPs following treatment with silver nitrate at various proportions. A shift to a brown color was observed due to the consecutive development of SNPs, a change that was further supported by ultraviolet–visible spectroscopy. The green-synthesized silver nanoparticles exhibited a prominent absorption band at 450 nm, which can be attributed to their surface plasmon resonance (SPR) characteristics [40,41]. The degree of color change, transitioning from a yellowish hue to a deep red shade, is directly influenced by both the amount of extract used and the duration of the incubation period. This phenomenon may be attributed to the activation of longitudinal plasmon vibrations and the reduction of $AgNO_3$. The X-ray diffraction (XRD) analysis

Fig. 5. *In vitro* pear fruit assay conducted to evaluate the inhibitory effects of SNPs against the rot ability caused by *E. amylovora*. The findings demonstrate a noteworthy reduction in symptoms on fruits treated with SNPs compared to the control group. Moreover, the symptoms resulting from the infection by bacteria alone were evidently suppressed. (A), (B), (C), (D) and (E) were control, pathogen, SNPs-pear, SNPs-quince and SNPs-apple respectively.

revealed four distinct peaks, each exhibiting unique spectral characteristics. These prominent peaks correspond to specific 2theta diffraction angles of 38°, 44°, 64°, and 77°, which are associated with the Bragg reflections from the (111), (200), (220), and (311) crystal planes, respectively. The results of the X-ray diffraction study unequivocally demonstrated that SNPs were synthesized using three different plant sources. Furthermore, the sharpness of the peaks indicates that these SNPs possess a polyhedral morphology defined by their crystalline planes [42,43]. Energy Dispersive X-ray Spectroscopy (EDX) serves as a robust analytical method for the characterization of SNPs. This technique facilitates the assessment of elemental composition while also offering valuable information regarding the morphology and crystallographic structure of the nanoparticles. In a research that employed olive fruit extract for the purpose of biosynthesis, Energy Dispersive X-ray (EDX) analysis confirmed the presence of silver and demonstrated that phytochemicals effectively capped the particles, thereby improving their stability against aggregation [44]. A new study examining SNPs biosynthesized from the leaf extract of *Trigonella foenum-graecum* revealed that energy-dispersive X-ray (EDX) analysis not only validated the presence of silver but also offered valuable information regarding the efficacy of these nanoparticles in catalytic applications [45].

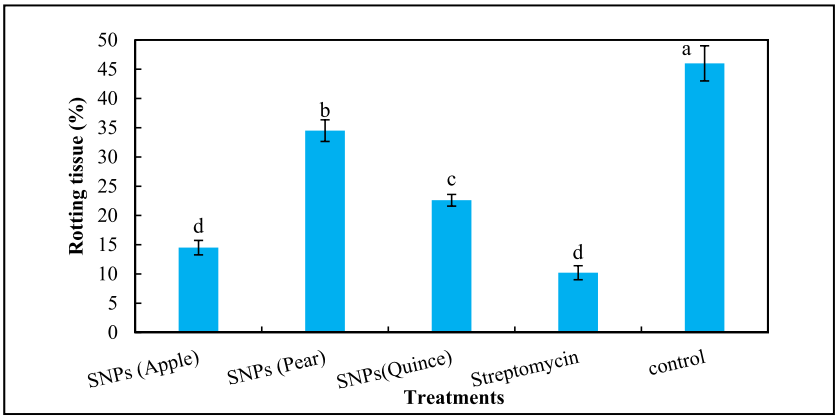


Fig. 6. The inhibitory impact of SNPs on the ability of *E. amylovora* to cause tissue maceration in pear fruits after 48 h was compared to control treatments, where fruits were inoculated with a suspension of bacteria alone. The bars in the graph represent the standard errors of the means from three replicates. Different letters are used to indicate significant changes.

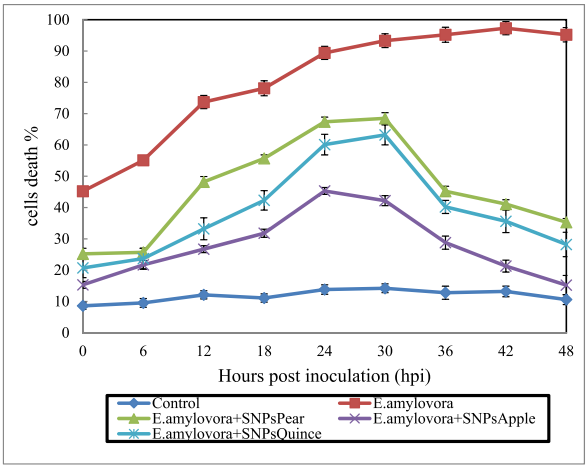


Fig. 7. The effects of cell death occurring 48 h post-inoculation with *E. amylovora*, along with the treatments utilizing SNPs, were investigated.

The TEM and AFM images revealed that the majority of the synthesized SNPs exhibited a spherical morphology and were found to be crystalline in nature. The antibacterial activity of the SNPs synthesized by the leaves extract was demonstrated through *in vitro* experiments, specifically against *E. amylovora*. Furthermore, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the synthesized SNPs against *E. amylovora* were determined. The *in vivo* experiments demonstrated that the application of biosynthesized SNPs to pear fruits resulted in a decrease in disease symptoms when compared to fruits that were solely inoculated with the pathogen.

Various mechanisms of action of SNPs have been identified thus far. These mechanisms encompass protein disruption, reactive oxygen species (ROS) generation, antioxidant degradation, and cell membrane dysfunction (e.g., disruption and permeabilization) [46]. Furthermore, SNPs have been shown to trigger mutagenesis and reduce the expression of transfer protein genes. SNPs possess the capacity to trigger cell denaturation through their assault on cell membranes and structures [47,48]. Furthermore, these SNPs have the capability to disturb transport systems, such as ion currents, resulting in the swift aggregation of silver ions. SNPs, characterized by a significant surface-to-volume ratio, exhibit potent antimicrobial effects by virtue of their enhanced ability to engage with cellular membranes, leading to disruption of the cell wall structure [49]. Consequently, they impact key cellular processes such as the respiratory chain, cell division, DNA replication, and protein synthesis in microorganisms [50]. To date, numerous studies have documented the application of different nanoparticles in combating plant pathogens. One particular study demonstrated the effective use of three types of nanomaterials, specifically C_{60}, CuO and TiO_2, in addressing sweet potato Rhizopus soft rot disease [51]. The fungus *Botrytis cinerea* is responsible for gray mold disease in both tomatoes and plums. Research has indicated that SNPs with a particle size of less than 100 nm can significantly suppress the mycelial growth of *B. cinerea*, demonstrating a markedly superior inhibitory effect compared to zinc oxide nanoparticles (ZnO NPs) and copper oxide nanoparticles (CuO NPs) [52]. Nanoparticles induce systemic acquired resistance in host plants, thereby enhancing their protection against diseases. The interaction between plants and nanoparticles can be optimized to promote the enrichment of specific microbiota within the phyllosphere, rhizosphere, and root

endosphere. This interaction may yield additive benefits through the induction of systemic resistance, the synthesis of antibiotics or other valuable biomolecules, the release of volatile organic compounds, and the formation of biofilms [53]. Research has indicated that SNPs can be released into the environment under specific conditions, such as exposure to simulated perspiration fluids. The observed toxicity of these particles may stem from a combination of the inherent properties of SNPs and the ions they generate. Investigations into the feasibility and challenges of performing a human health risk assessment for nano-silver, based on existing literature, have revealed significant gaps in the available data concerning both exposure levels and potential hazards [54]. Consequently, additional studies are required to assess the possible risks linked to the utilization of SNPs.

The current research findings could serve as a foundation for upcoming investigations focused on the commercialization of biosynthesized SNPs products for sustainable agriculture. We believe that this approach has the potential to effectively manage various plant bacterial diseases, ultimately contributing to the promotion of sustainable agricultural practices.

5. Conclusion

In the past few years, various components of plants have been employed to minimize and stabilize nanoparticles, replacing harmful chemicals due to their safety and cost-effective nature. Through a sustainable synthesis process, these nanoparticles (SNPs) have been effectively produced, exhibiting hexagonal and spherical shapes with particle sizes ranging from 30 nm to 55 nm. To assess their potential, the antibacterial efficacy of these biofabricated nanoparticles was examined against *E. amylovora* under controlled in *in vitro* conditions. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were associated with the silver nanoparticles biosynthesized from the apple plant, suggesting that the antibacterial properties of these nanoparticles are superior to those derived from the other two plant sources.

CRediT authorship contribution statement

Saeed Tarighi: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Meysam Soltani Nejad:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval and consent to participate

Not applicable.

Data availability

Available data is presented in the supplemental files.

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Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42567.

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