PLANT ECOPHYSIOLGY
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PLANT ECOPHYSIOLGY
Plant Ecophysiology 3(2024) 8-24

# Application of Biosynthesis of silver nanoparticles using *Trachyspermum ammi* extract in controlling of Onion bacterial rot

**Z. Fotoohiyan *[a]**

[a]Department of Plant pathology, Jiroft Branch ,Islamic Azad University, Jiroft, Iran

E-mail: (corresponding author): Jfotoohiyan@yahoo.com

https://doi.org/10.5281/zenodo.10462990

**Abstract**

Today, the use of metal nanoparticles, as a suitable alternative to chemical poisons reduces their risk to the environment and consumers. Therefore, the aim of this study is the biosynthesis of silver nanoparticles using the aqueous extract of native Ajowan seeds and its effect against Onion bacterial rot. In this research, first, isolation and identification of pathogenic bacteria from different areas of Jiroft city was done. Then the biosynthesis of Ag NPs was carried out by aqueous extract of Ajowan seeds and the their effects against *Pectobacterium* were investigated in laboratory and warehouse conditions. A total of 12 bacterial isolates, N3 isolate from Anbarabad region had the highest pathogenicity. The biosynthesis of Ag NPs was confirmed by observing the date color after 15 minutes in the dark at room temperature and the ultraviolet spectrometer showed that the surface plasmon resonance is at 420 nm. The growth inhibition rate of biosynthesized Ag NPs and Mancozeb poison each alone and their combination against *Pectobacterium in vitro* were 62.45, 100 and 88.67%, respectively. Also, Ag NPs and Mancozeb each alone within three months and combining them together within two months caused 100% shelf life of onions in storage. However, the combination of biosynthesized Ag NPs and Mancozeb together with *Pectobacterium* had 100% inhibition in the first and second month and 85% in the third month against bacterial rot disease. Therefore, the results showed that biosynthesized Ag NPs were effective in controlling pathogenic bacteria and can replace agricultural toxins.

***Keywords:*** Onion, Soft rot, Biosynthesis, Aqueous Extract of Ajowan and silver nanoparticles

## Introduction

Most of the agricultural products after harvesting are exposed to threats caused by plant pathogens that reduce crop life. One of the most important pathogens of onions is *Pectobacterium carotovorum* subsp. *carotovorum*, which causes disease and economic damage in a large number of plant species (Schaad *et al*., 2001). One of the important characteristics of this bacterium is the production of a large number of extracellular enzymes including pectinase, cellulase, protease and pectin lyase, which cause deterioration of plant cell walls and soft rot of plant tissues (Hayward, 2003). The use of chemical pesticides to reduce postharvest pollution has devastating effects on the environment and human health (Behdad *et al*., 2013). Therefore, in line with the goals of comprehensive disease management, biological control has a significant impact on the preservation of biodiversity, ecosystem sustainability and food health (Karakaş *et al.*,

2023). The use of biosynthesized silver nanoparticles (Ag NPs) by plants is one of the biocontrol methods. (Darbahania *et al*., 2015).

Silver has long been known as an antimicrobial agent against a wide range of bacteria, fungi, or viruses (Nowak *et al*., 2011). Today, with the reduction of the size of silver metallic particles in nano-size, its antimicrobial properties have increased, which is due to the increase in the surface area to the volume of particles (Bayda *et al*., 2020). The toxicity induced by Ag NPs is due to oxidative stress, which accumulates in the cytoplasm and nucleus of the cell and leads to the production of free radicals (Khatoon *et al*., 2015). Also, by binding to the bacterial cell membrane and penetrating into the cell, Ag NPs prevent the proliferation of bacteria and deactivate their function. Of course, the amount of use of nanoparticles should be in a range that destroys microorganisms and foreign agents and is ineffective on plant cells (Ajitha *et al*., 2016).

The synthesis method of Ag NPs is also one of the important points in the use of these materials, because Ag NPs are also synthesized biologically by plants in addition to physical-chemical methods (Baladi *et al*., 2019). The advantages of biosynthesis are lower cost, environmentally friendly and easy production on a large scale. In these methods, there is no need to use high temperature and pressure. For this reason, today plants are widely used as renewable and cheap sources without environmental damage in the synthesis of nanoparticles (Kavosi and Yaghoubi, 2017). Plants have important chemical compounds such as alkaloids, flavonoids, tannins and phenols in their various organs and tissues that have the ability to reduce silver to a metal element and biosynthesize silver in nano-size (Vidyasagar *et al*., 2023). Flavonoids present in aqueous extract of plants act as reducing agent and convert silver ions in reaction solution into Ag NPs (Ali *et al*., 2016). In a similar study, the production of Ag NPs was carried out using a single stage biological method and using oak fruit extract and its antibacterial activity was investigated. The color change from clear to dark brown in the solution was observed. The antimicrobial activity of Ag NPs was proven against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* (Chahar Doli and Khodadadi, 2013).

*Trachyspermum ammi* is a herbaceous plant that grows in Iran, India, Pakistan and Egypt (Sharafzadeh and Alizadeh, 2012). The fruit of this plant is used in traditional Iranian medicine as an anti-flatulent, anti-nausea and diuretic agent. There are approximately 16 compounds in Ajowan extract, but thymol is the most important and main phenolic compound in Ajowan that makes up at least 40-50% of the extract constitutes at least 40-50% of the extract (Gerchach and Reddy, 2002). Also, considering the position of Jiroft city as the hub of agricultural production in the country, especially in the production of vegetables such as onions (Adly Sardoyi *et al*., 2012) and the need to use appropriate methods in order to continue the ability to produce and preserve the environment, the aim of this study was to investigate the biosynthesis of Ag NPs by the local Ajowan and its effect on *Pectobacterium* causing bacterial decay of onions in laboratory and warehouse conditions. Also, the effect of biosynthesized Ag NPs is compared with the common chemical poisons in controlling this disease, which has not been done in this regard so far. The introduction of nanotechnology into the agricultural and food industry ensures an increase in production and quality, while preserving the environment and resources of the planet (Haji Rostamiloo *et al*., 2019).

## Materials and Methods

***Isolation of bacteria causing onion rot***

First, onions with signs of decay were randomly collected from three warehouses in Jiroft city (Islamabad, Aliabad and Anbarabad) and each was placed in a separate bag and transported to the laboratory. Then the contaminated samples were washed with sterile distilled water to remove external factors and disinfected in 2% javel water for one minute and washed with sterile distilled water. Then, from the distance between infected tissue and healthy tissue, half-centimeter samples were placed in sterile physiological serum solution on a stirrer at 120 rpm for 20 minutes. Then, the resulting suspension was cultured in Nutrient Agar medium for 5 days at 30°C. Morphologically grown colonies were studied and purified and finally encoded (Mavandadi *et al*., 2015).

***Biochemical identification of isolates isolated from infected onions***

Identification of pathogenic bacterial isolates was done based on biochemical tests including gram staining, catalase and oxidase test, sensitivity to erythromycin, negative indole production, negative sugar reduction from sucrose, positive simon citrate and motility. Each isolated bacterium from the infected onion tissue was subjected to these tests and the bacteria that responded to all the tests according to the growth characteristicsof *Pectobacterium* wereselected for further research (Mansfield *et al*., 2012).

***Pathogenicity test of bacterial isolates causing rots***

First, a few healthy onions of the same size were selected and the external surface of each was disinfected and scratched with a scalpel, and then a block of 5 mm was removed from the young edge of each of the isolates and inoculated into the scratches. Then, the onion samples were placed inside a plastic box between two layers of wet, sterile Watt-Man paper in an incubator at 30 °C for one week. Each test was performed in three repetitions and a control sample (sterile onion without inoculation) was also placed in the mentioned conditions. Onion samples were evaluated daily for symptoms and rotting (Tasveli and Asgharzadeh, 2009). Then, one of the isolates with more pathogenicity was selected for further tests.

***Collecting and preparing aqueous extract of Ajowan plant***

The collection of the seeds of native plants of Jiroft region was done in 1401. Then the samples were sent to the Faculty of Pharmacy of Kerman province for accurate identification. The seeds were washed with sterile distilled water and dried in the shade. The dried seeds were turned into powder using a mill. To prepare aqueous extract by maceration method, first, 100g of powdered seeds of Ajowan plant were mixed with 500 ml of double distilled water and boiled for 10 minutes. Then, the aqueous extract was filtered using Whatman number one filter paper and sterilized by a 0.45 micron filter (Kaviani and Asfoori, 2017). The sterilized extract was stored at 4 °C for the next steps

***Biosynthesis and production evaluation of Ag NPs***

First, molarities of 10, 15 and 20 mM were prepared from silver nitrate salt. 90 ml of silver nitrate with molarities of 10, 15 and 20 mM were mixed with 10 ml of aqueous extract of Ajowan seeds and placed at room temperature in dark conditions for 30 minutes. For negative control, only silver nitrate molarity and only aqueous extract of Ajowan were placed in one treatment (Heidari *et al*., 2015).

In order to evaluate the production of Ag NPs by the aqueous extract of Ajowan, two methods of colorimetic and photoabsorption reading were used by spectrophotometer and FTIR analysis (Mittal *et al*., 2012). The first sign of the production of Ag NPs is the color change of the solution towards orange, which, of course, should be checked with the negative control treatments (Aqueous Ajowan extract, Ag nitrate molarity) to evaluate the color change. Then, the optical absorption spectrum of Ag NPs was read at wavelengths of 380, 400, 420, 440, 480 and 500 nm and the resulting graph was drawn. After completing the biosynthesis process of Ag NPs, the resulting nano solution was centrifuged at 12000 rpm for 20 minutes to concentrate the nano-soluble particles and the supernatant was discarded. Then, in order to wash and disperse the settled nanoparticles by adding double ionized water, the centrifugation process was repeated three times (Kaviani and Asfoori, 2017). Among other important points related to the synthesized sample of Ag NPs, it is not oxidized and its color does not change to gray because in this case Ag NPs lose their properties (Mittal *et al*., 2012).

***Investigating the antioxidant properties of biosynthesized Ag NPs***

First, a 0.1 mM solution was prepared from DPHH. Then 0.5 ml of it was mixed in 95% ethanol with 100 µl of silver nanoparticle solution biosynthesized by the aqueous extract of Ajowan. The resulting suspension was placed in the dark at 38°C for 31 minutes, and then its absorption was read at 518 nm. In order to compare the activity of biosynthesized Ag NPs, the standard composition of reducing glutathione (GSH) was used as a standard antioxidant. To determine the value of IC50 for Ag NPs produced in five different dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$) and the standard composition of glutathione was performed. Each test was performed in three times and the mean values were used as the calculation criteria. The percentage of de-radicalization activity was calculated using the following equation (Haji Rostamiloo *et al*., 2019).

Percentage of radical absorption=100× (reaction adsorption-blank adsorption)÷ blank adsorption

In this regard, Blank adsorption indicates the adsorption of the control solution, which contains 0.5 mM DPPH solution of 0.1 mM and 100 µl of 95% ethanol instead of the Ag NPs solution and reaction adsorption is the adsorption of the sample solution of Ag NPs.

***The effect of biosynthesized Ag NPs and Mancozeb on pathogenic isolates in vitro***

The effect of Ag NPs on pathogenic isolates was measured by disk diffusion method. Initially, $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$were prepared by adding one, 0.5, 0.25, 0.125 and 0.625 ml of Ag NPs, respectively. Then, 15 µl of each dilution was poured on the sterile blank disk and given time to dry the nanoparticles on the disc. Then, after making Mueller Hinton agar medium, first 0.5 McFarland of *Pectobacterium* isolated from infected onions was prepared in sterile physiological serum and 0.2 µl of suspension was poured on Muller medium under aseptic conditions. Then they were cultured completely uniformly with glass spread. In the next step, blank disks of five different

dilutions of nanoparticles were placed on each culture medium at regular intervals from each other and also at a distance from the edge of the plate. Then the plates were incubated at 30 °C for 24 hours and were examined for the formation or non-formation of inhibition zone. In the investigation of the effect of Mancozeb on the pathogenic isolate, first different concentrations of the poison were prepared according to the instructions written on the back cover and successive dilutions (0.125, 0.25, 0.5, 1 and 2 ppm) and then according to the above, 15 µl of each of the dilutions were prepared. It was poured on a sterile blank disk and was given time to dry. In the next step, blank discs containing five different poison dilutions were placed on each culture medium inoculated with bacteria. Then the plates were incubated at 30 °C for 24 hours and evaluated for the formation or absence of a growth zone. At the end, the most effective dilution of the synthesized Ag NPs and Mancozeb, which had the highest inhibition *in vitro*, was selected for storage experiments.

***The effect of Ag NPs and Mancozeb on pathogenic bacteria in storage conditions***

First, healthy onions of the same size were selected and after being transferred to the laboratory, they were disinfected with 3% Javel water. Then the disinfected onions were placed separately for 5 minutes in a $10^{-1}$ dilution of biosynthesized Ag NPs and Mancozeb (2 ppm) and then dried on a wire net for 2 hours. This test was done in 6 treatments and in three random blocks. The treatments performed in this test include 1) immersing the samples in Ag NPs for 5 minutes, 2) immersing the samples in a concentration of 2 ppm of Mancozeb for 5 minutes, 3) inoculating the samples with pathogenic isolates, 4) immersing the samples in a mixture of nanoparticles Silver and pathogenic isolates, 5) Immersing the samples in a mixture of Mancozeb and pathogenic isolates. 6) Immersing the samples in a mixture of Ag NPs, Mancozeb and pathogenic isolate. Onion samples were placed in plastic bags individually and stored for three months in storage conditions (26°C) and the results were checked (Nirmala *et al*., 2023).

***Statistical analyses***

Graphs were drawn with Excel 2018 software and data analysis was done using SPSS statistical software version 26 and the mean comparisons were done by Duncan multi-domain test at 5% level.

**Results**

***Isolation of bacteria causing onion rot***

A total of 12 bacterial isolates with *Pectobacterium* appearance characteristics including white, rounded colonies with smooth and convex edges were isolated from three warehouses in Jiroft city, Islamabad codes (S1, S2, S3, S4), Aliabad codes (A1, A2, A3, A4) and Anbarabad codes (N1, N2, N3, N4) and biochemical tests were performed to identify them (Table 1).

**Table 1.** Biochemical identification of onion rot bacterial from warehouses in Jiroft city.

| Isolate code | Gram | Microscopic Morphology | SIM | Catalase | Oxidase | TSI | Simon Citrate | MR-VP |
|---|---|---|---|---|---|---|---|---|
| S1 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| S2 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| S3 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| S4 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| A1 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| A2 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| A3 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | negative | alkali/alkali | positive | -/- |
| A4 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | negative | alkali/alkali | positive | -/- |
| N1 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | منفی | alkali/alkali | positive | -/- |
| N2 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| N3 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |
| N4 | negative | coccobacillus | movement positive/endole negative/H2S negative | positive | positive | alkali/alkali | positive | -/- |

***The results of the pathogenicity test of bacterial isolates***

The pathogenicity test on healthy onion samples showed that all 12 isolates had the ability to cause rotting disease on the onions as discoloration and bad odor and the N3 isolate collected from Anbarabad region with more severe symptoms were selected for other tests.

***Collection of Ajowan plants and biosynthesis of Ag NPs***

Trachyspermum ammi, which is shown in Fig. 1, was introduced after being identified by the Faculty of Pharmacy of Kerman province. The biosynthesis of Ag NPs happened by adding 90 ml of 20 mM silver nitrate to 10 ml of the aqueous extract of Ajowan seeds, and the date color appeared from the initial minutes, which became more intense after 15 minutes (Fig. 2). In 10 and 15 mM concentrations of silver nitrate, with a longer duration (three to four hours) and less color intensity, a color change was observed towards tan. Therefore, the concentration of 20 mM was selected for continuing the work.

**Fig. 1.** *Trachyspermum ammi* plant and seeds native to Jiroft region

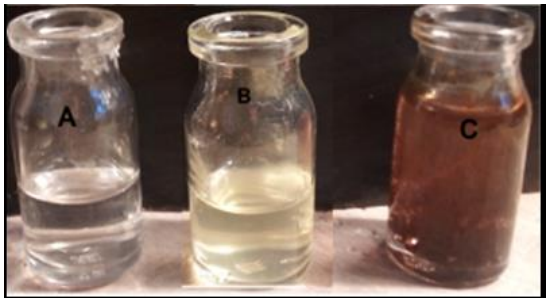


**Fig 2.** A: 20 mM silver nitrate B: Aqueous extract of Ajowan seeds C: Biosynthesis of Ag NPs by observing the purple color after 15 minutes

***Evaluation of biosynthesized Ag NPs in terms of optical absorption and FTIR***

The optical absorption of Ag NPs biosynthesized by the aqueous extract of Ajowan seed was read at wavelengths of 380, 400, 420, 440, 460, 480 and 500 nm, and the highest absorption at the wavelength of 420 nm is equal to 3.759. The optical absorption peak of Ag NPs at wavelengths of 400 to 440 nm shows that surface plasmon resonance has occurred in this peak. Fig. 3 shows the optical absorption curves of biosynthesized Ag NPs and the extract of Ajowan seeds (control). Also, The results of infrared spectrometer (FTIR) are given in Fig. 4.

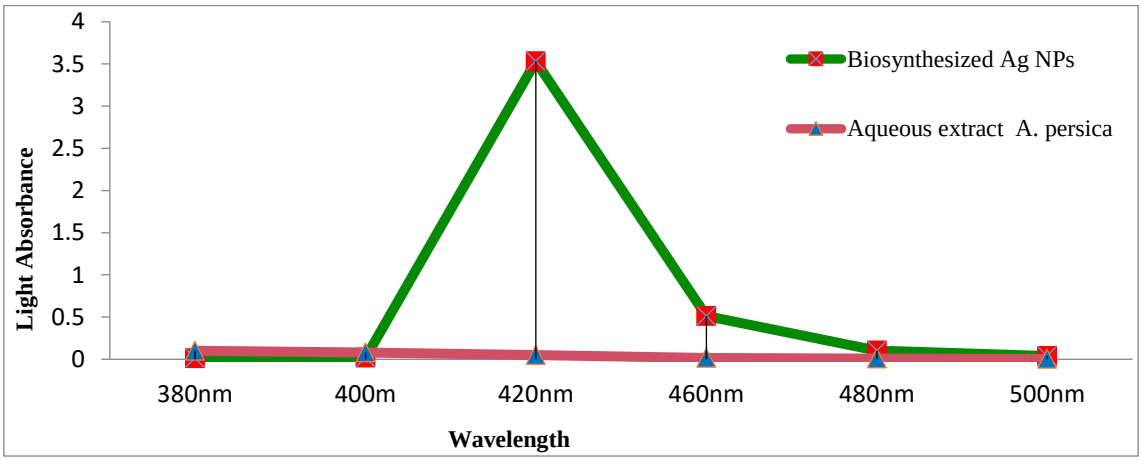


**Fig. 3.**The curve related to the optical absorption of biosynthesized Ag NPs and the aqueous extract of Ajowan seeds

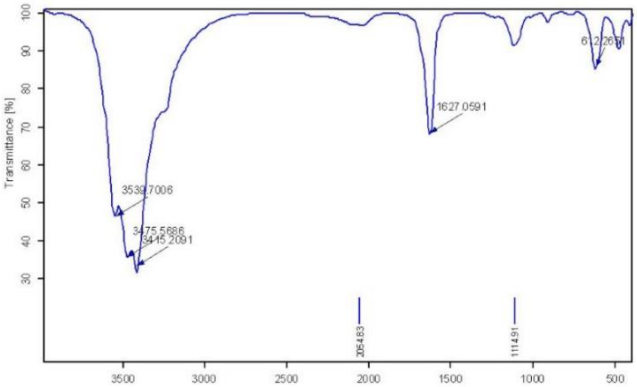


**Fig. 4.** Results of infrared spectrometer (FTIR) of biosynthesized Ag NPs

**Antioxidant properties of Ag NPs biosynthesized by aqueous extract of Ajowan seeds**

The results of DPPH radical adsorption activity of Ag NPs biosynthesized by aqueous extract of Ajowan seeds showed that the removal of glutathione radicals by DPPH method was higher in the higher dilution of Ag NPs and decreased with dilution reduction, which is due to the antioxidant properties of Ag NPs (Table 2). The comparison of the average percentage of DPPH radical absorption is given in Diagram 1. According to Duncan's comparison test, there is a significant difference between different dilutions of Ag NPs and glutathione standard.

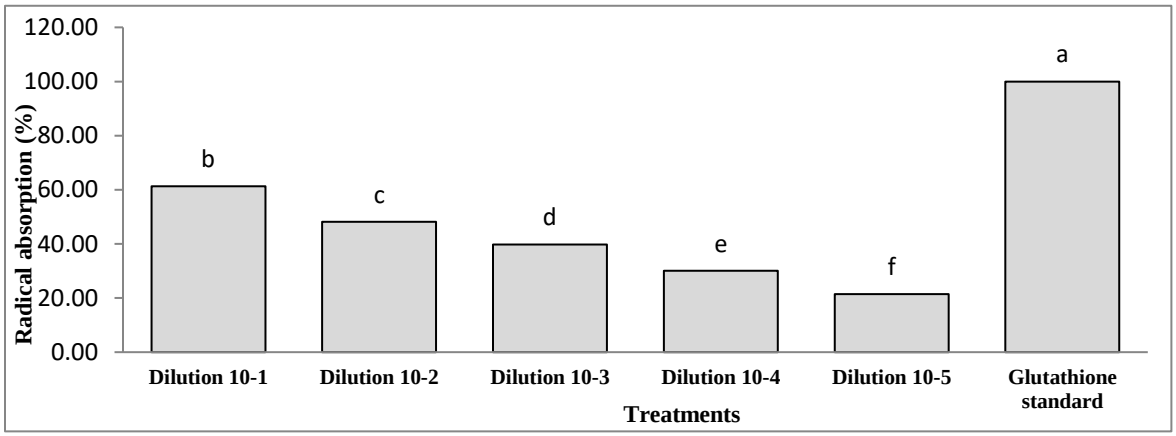


**Diagram 1.** Comparison of the average percentage of DPPH radicals adsorption by Ag NPs biosynthesized by aqueous extract of Ajowan seeds

***The effect of biosynthesized Ag NPs and Mancozeb against Pectobacterium in vitro***

The inhibition percentage of the growth of each of the treatments of biosynthesized Ag NPs (1-10 dilution), 20 mM silver nitrate and aqueous extract of Ajowan seeds on *Pectobacterium* was calculated after five days. The mean percentage of inhibition of growth against *Pectobacterium* isolates was investigated by Duncan's test and it is shown in Diagram 2 at the 5% probability level. There was a significant difference between the inhibition percentage of the growth of synthesized Ag NPs and 20 mM silver nitrate, as well as the aqueous extract of Ajowan seeds, and the Ag NPs biosynthesized by the aqueous extract of Ajowan showed 62.45% inhibition of the growth of *Pectobacterium*.

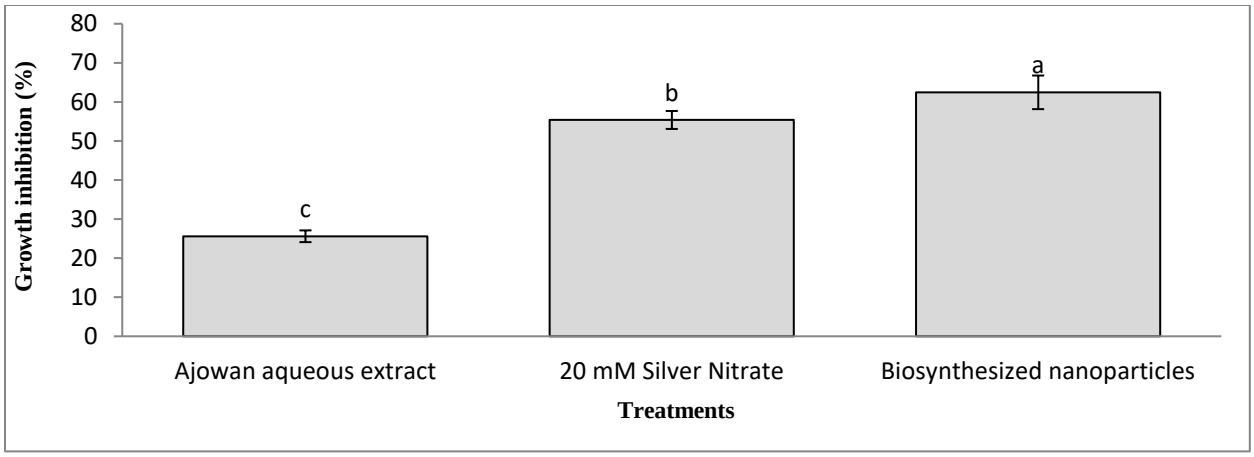


**Diagram 2.** The mean inhibitory percentage of Ag NPs against *Pectobacterium* was determined *in vitro*

***The effect of Mancozeb against Pectobacterium in vitro***

The inhibition percentage of the growth of different concentrations of Mancozeb (2, 1, 0.5, 0.25 ppm) was determined. The comparison of the average inhibition percentage of the growth of Mancozeb against the Pectobacterium causing onion rot was calculated and its results are shown in Diagram 3. Comparison of growth inhibition percentage showed that there is a significant difference between all poison concentrations at the 5% probability level, and the highest growth inhibition percentage is related to the 2 ppm concentration, which was estimated as 100%. Therefore, this dilution was selected for storage experiments.

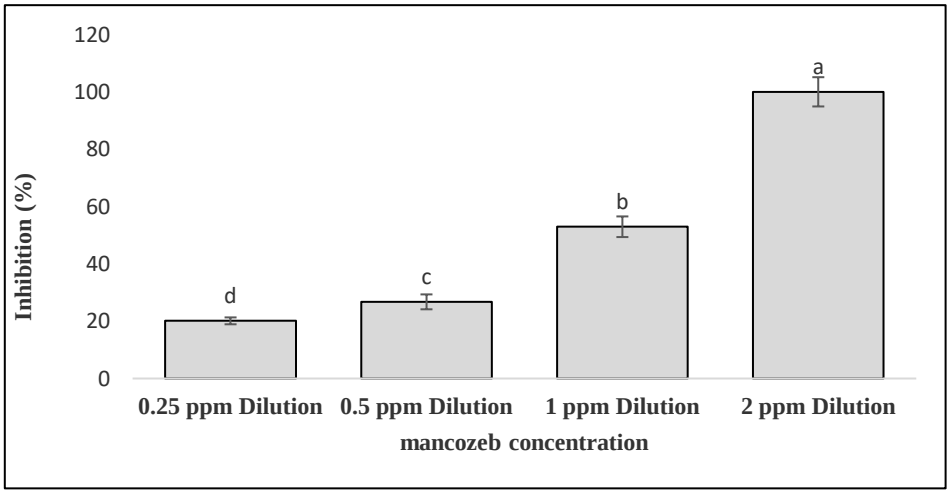


**Diagram 3.** Percentage inhibition of the growth of *Pectobacterium* by different concentrations of Mancozeb

***The effect of combining synthesized Ag NPs and Mancozeb against Pectobacterium in vitro***

The results of the combination of Mancozeb (2 ppm) and Ag NPs biosynthesized by the aqueous extract of Ajowan seeds at $10^{-1}$ dilution with each other and their effect on *Pectobacterium*, which causes onion rot, and the graph of comparing the averages by Duncan's test are shown in Diagram 4.

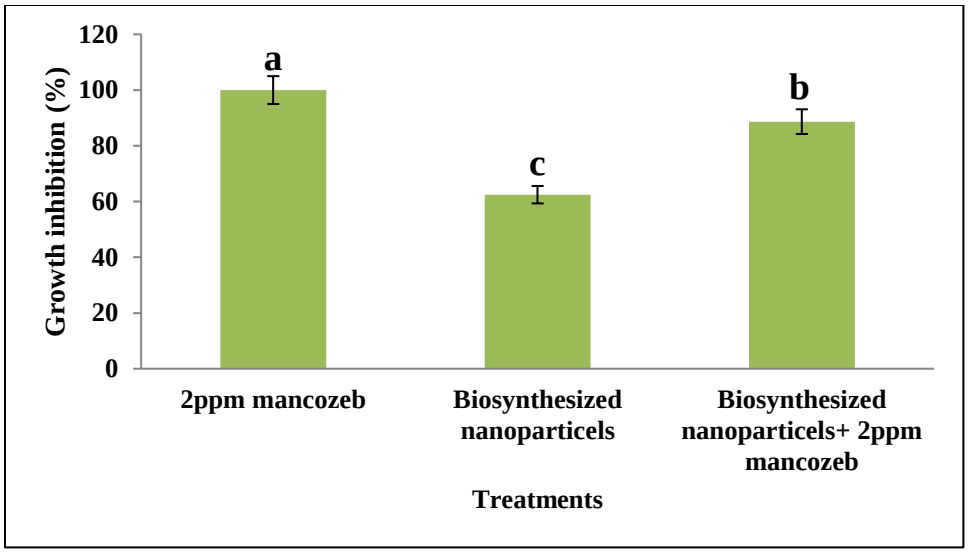


**Diagram 4.** Percentage of inhibiting the growth of the combination of Mancozeb and biosynthesized Ag NPs on *Pectobacterium*.

***The effect of biosynthesized Ag NPs and Mancozeb on Pectobacterium in storage conditions***

The inhibition percentage of each treatment compared to the control during three months of onion storage is shown in Diagram 5. In this section, the control treatments including biosynthesized Ag NPs with $10^{-1}$ dilution, 20 mM silver nitrate and Mancozeb (2 ppm) caused 100% shelf life of onions for three months in storage, and there was no change in onions. Also, the aqueous extract of Ajowan seeds in the first, second, and third month, caused 75%, 50%, and 50%, respectively shelf life of onion in stock against rot disease. However, in each of these treatments, the inhibition rate decreased along with the pathogenic bacteria. In addition, the combination of biosynthesized Ag NPs and Mancozeb together with *Pectobacterium* had 100% inhibition in the first and second month and 85% in the third month against onion rot disease.

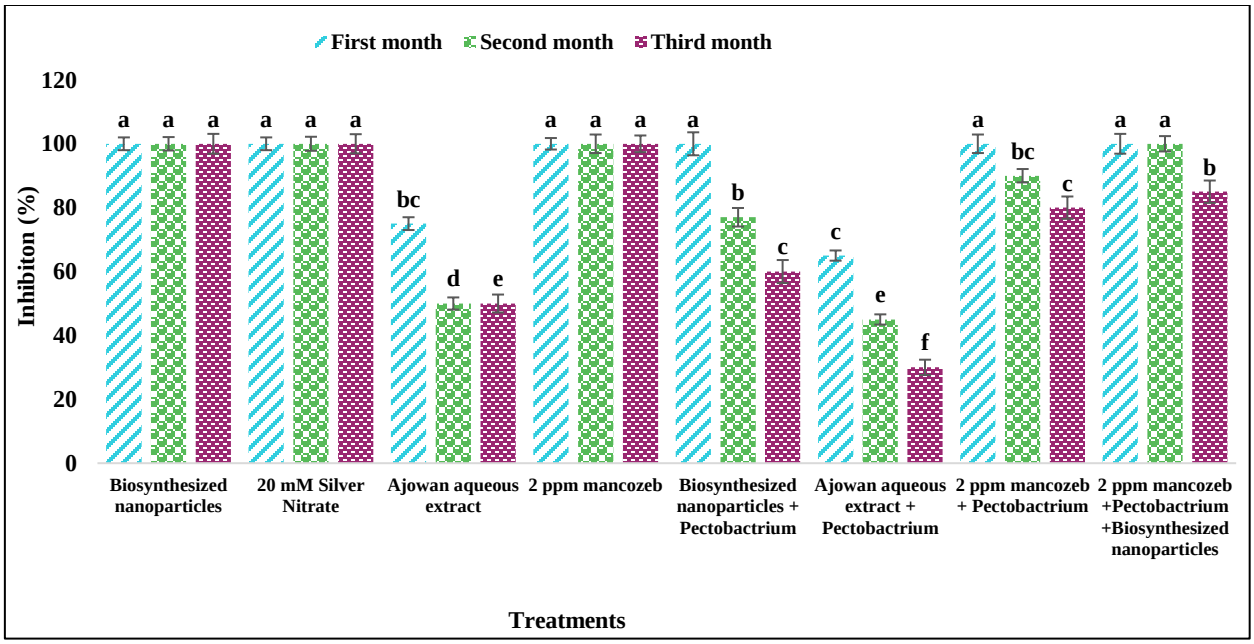


**Diagram 5.** The inhibition percentage of different treatments on *Pectobacterium* in warehouse conditions during three months

## Discussion

Bacterial rot is one of the most important diseases of agricultural products, including onions, in the field and warehouse (Zherebilo *et al*., 2001). *Pectobacterium carotovorum* causes diseases and economic losses in a large number of plant species (Schaad *et al*., 2001). One of the important features of this bacterium is the production of a large number of extracellular enzymes, including pectinase, cellulase, protease and pectin lyase, which can destroy plant cell walls and cause soft decay of plant tissues (Hayward, 2003). The use of chemical poisons is common among farmers, but the damage caused by poisons to the farmer, the environment, and the consumer is irreparable (Behdad *et al*., 2013). Therefore, biological control methods should be used for control. One of the ways of post-harvest control is the use of non-toxic technologies such as Ag NPs and biological control using bacteria, fungi, yeasts (Darvish Nia *et al*., 2015). The use of nanotechnology means that nano particles are synthesized using biological method and then its effect on pathogens is investigated. The results show that engineered nanoparticles (1 to 100 nm) have inhibitory activity of plant pathogens and play a role in the management of plant diseases as bactericides and fungicides as well as nanofertilizers in promoting plant health. Some nanoparticles act directly as an antimicrobial agent (Venat *et al*., 2018)/

Due to the human need to increase the production of agricultural products, climate change, the management of plant diseases and pathogens is important (Elmer *et al*., 2018). The synthesis of nanoparticles by chemical and physical methods has disadvantages due to the use of hazardous chemical substances, radiation, carrying out reactions in special conditions (temperature and pressure), expensive materials and creating potential risks for the environment. But in the biological method of nanoparticles, micro-organisms (bacteria, fungi) and plants can be used. So far, the biological production of nanoparticles has been carried out by

various plants such as *European marjoram* (Kavosi and Yaghoubi, 2017), thyme (Ekhtiari *et al*., 2017), Yarrow (Baladi *et al*., 2019) and *Artemisia absinthium* (Ali *et al*., 2016). One of the research aspects of medicinal plants is their use in the production of nanoparticles, which is a faster and cheaper method than chemical methods and has less risk for humans and the environment (Dehghan Neiri *et al*., 2017). In the green synthesis method, metal ions are converted into nanoparticles using plant compounds without special conditions and other stabilizer agents in a simple reaction. Bioactive substances and compounds in plant extracts, including flavonoids and other active metabolites soluble in water, can be used for the reduction of metal ions to nanoparticles at room temperature (Kavosi and Yaghoubi, 2017).

In this research, firstly, the bacteria causing onion rot was isolated from infected onions with the signs of crushed tissue, changed color to brown and black, with a bad odor and watery, from the warehouses of Jiroft areas (Islamabad, Aliabad and Anbarabad) in Kerman province. According to the biochemical identification of isolates isolated from onion samples, were Gram-negative bacterium, *Pectobacterium carotovorum* pv *carotovorum*. During the pathogenicity test, among the 12 bacterial isolates, the code N3 collected from Anbarabad region caused more severe disease on healthy onion after five days, so this isolate was used to continue the work. Also, in this research, the extract of Ajowan seeds was used to produce Ag NPs. The extracts of plants contain reductive compounds that have the ability to regenerate silver nitrate. Therefore, the native plants of Jiroft region were collected and after identification, its aqueous extract was prepared and sterilized by passing it through 0.45 micron filter to prevent any microbial contamination. In the following, the aqueous extract of Ajowan seed was used for the biosynthesis of Ag NPs. For the biosynthesis of Ag NPs, first, different molarities (10, 15, 20 mM) were prepared of silver nitrate. Then, silver nitrate molarities were mixed in different ratios with aqueous extract of Ajowan seed and the reaction was evaluated in dark conditions at room temperature for 30 minutes. The creation of a dark brown color is the first sign of the biosynthesis of Ag NPs. In reaction that 90 mL silver nitrate 20 mM was combined with 10 ml aqueous extract of Ajowan seed. The color change was observed quickly towards tan and after 15 minutes the intensity of color increased, which indicating the higher reduction of silver nitrate by the compounds in aqueous extract of Ajwain seeds. Also, in this study, the antioxidant properties of biosynthesized Ag NPs were evaluated and it was found that Ag NPs at higher concentrations have higher antioxidant properties which this process was evaluated by the absorption activity of DPPH radicals by Ag NPs. In the research of Mittal *et al*., in 2012, also they investigated the antioxidant properties of Ag NPs synthesized from the flower extract of *Rhododendron Daricum*.

Ag NPs were evaluated in terms of synthesis, size distribution and microscopic evaluation using ultraviolet spectrometer, and the antioxidant activity of Ag NPs by plant phenolic substances was investigated by DPPH evaluation and it was found that the biosynthesized nanoparticles have antioxidant properties. Similarly, in 2017, Rasheed *et al*., investigated the antioxidant activities of Ag NPs biosynthesized from the extracts of leaves of four species of Terminalia (Arjun). The results showed that the antioxidant activity of Ag NPs is dependent on the dose of Ag NPs. In the next step, the optical absorption of biosynthesized Ag NPs was read and the highest absorption peak was at 420 nm, which is the surface plasmon resonance of Ag NPs in this peak. One of the properties of Ag NPs is the optical

absorption in the range of 420 to 480 nm, which normally has the highest absorption at 420 or 440 nm. Similarly, in the research of Kaviani and Asfoor in 2017, the formation of Ag NPs synthesized by aqueous extract of *Artemisia artemisia* plant was characterized by the absorption peak at a wavelength of about 490 nm, and the size of Ag NPs ranged from 27 to 65 nm. In another study, the formation of Ag NPs by *Achillea millefolium* was confirmed by spectrophotometer at 450 nm wavelength (Karimi and Mohsenzadeh, 2013). In the research of Mohan *et al*., the biosynthesis of Ag NPs was performed using *Canthium coromandelicum* leaf extract. In their research, they was used silver nitrate solution (1 mol). The results of UV absorption spectroscopy showed that there was a strong resonance on the surface of Ag NPs at 430 nm (Mohan *et al*., 2014). Also, in the research of Khalili and Baghbani Arani in 2016, the green synthesis of Ag NPs was investigated using the extract of the Cinnamon plant and ultraviolet-visible spectroscopic analysis, and the existence of a peak was confirmed at 234 nm. In the research of Ali *et al*. in 2016, the biosynthesis of Ag NPs by aqueous extract of *Artemisia absinthium* was the best result related to silver nitrate (2 mM) and the aqueous extract of the plant (10 mg/ml), when combined with different ratios, affected the size, stability and production of Ag NPs. Alsamaria *et al*., also performed the biosynthesis of Ag NPs using the aqueous extract of turmeric powder and ultraviolet spectroscopy. The maximum absorbance of UV-VIS spectra was at 432 nm and the biosynthesized Ag NPs showed high antimicrobial activities and efficacy against food pathogens. TEM and scanning electron microscopy images show that there is severe shrinkage and damage of the bacterial cell wall and leakage or loss of bacterial intracellular content (Alsammarraie *et al*., 2018). The difference in the absorption increase time is probably due to the different laboratory conditions during the production of nanoparticles, for example, in the case of the production of Ag NPs by *Aloe vera*, this time is 24 hours and the peak of the spectrophotometric absorption increase is also reported to be uncertain (Shankar *et al*., 2004).

The specific size and morphology of Ag NPs allows them to show high antimicrobial activity by damaging and destroying the bacterial membrane (Morones *et al*., 2005). Also, the activity of Ag NPs depends on the concentration and was more significant for Gram-negative bacteria than Gram-positive bacteria. Studies have stated that the difference between Gram-negative and Gram-positive bacteria in front of Ag NPs is related to their cell wall structure. Gram-negative bacteria have a thinner cell wall that has little strength, and on the other hand, there is a layer of lipopolysaccharide on their outer surface that has a negative charge. The existence of a negative charge on the surface of the bacterial cell makes the interaction between the Ag NPs, which have a weak positive charge, easier with the bacterial cell. This interaction initially creates a hole in the cell wall, and then when the nanoparticles enter the bacterial cell, it interferes with the growth of the bacterial cell and finally causes the death of the bacteria (Guzman *et al*., 2012). In a similar study by Heydari *et al*. (2016), rosemary extract containing Ag NPs had a lethal effect on *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli bacteria.* In the study of Nikprest *et al*., (2018), the effect of Ag NPs synthesized using the leaf extract of Amaranthus on the plant pathogens *Pseudomonas syringii* and *Xanthamonas oryzae* was studied and compared with the ciprofloxacin antibiotic. And it was found that the Amaranth plant is a suitable option for producing Ag NPs and the nanoparticles produced in this way had antibacterial properties against these

pathogens. Also, in the study of producing Ag NPs by a single-step biological method and using oak fruit extract and investigating its antibacterial activity, a change in color from clear to dark brown was observed in the solution, the amount of absorption also increased, and the highest absorption was observed at 420 nm. The antimicrobial activity of silver nanoparticle was proven against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* (Chahar Douli and Khodadadi, 2013). Chemical poisons cause a lot of damage to people and the environment due to their improper use and dangers. Therefore, the use of safe or low-risk substances is very important in controlling plant pathogens. One of these plant pathogens is *Pectobacterium* bacterium which causes bacterial rot in various crops such as onions. This bacterium has a global spread. Contamination of products by this bacterium occurs mostly in storage conditions. In the present study, the antibacterial effect of Ag NPs biosynthesized by aqueous extract of Ajowan seeds on the bacteria causing soft rot of onion was investigated in laboratory and warehouse conditions. Also, the effect of Mancozeb on bacteria and finally the combined effect of Mancozeb and biosynthesized Ag NPs were also investigated in laboratory and warehouse conditions. The results showed that the growth inhibition rate of biosynthesized Ag NPs and Mancozeb each alone and their combination against *Pectobacterium* in laboratory conditions were 62.45, 100 and 88.67% respectively, and this rate was 55.38% for silver nitrate 20 mM and 55.38% for aqueous extract of Ajowan. The seed of female plant was equal to 25.61%. Since silver has antimicrobial properties, according to the results of this research, it was found that Ag NPs had a higher inhibitory percentage than the extract of Ajowan seeds, which shows the greater effect of nanoparticles. Also, Ag NPs and Mancozeb each alone within three months and combining them together within two months caused 100% shelf life of onions in storage. However, the combination of biosynthesized Ag NPs and Mancozeb together with *Pectobacterium* had 100% inhibition in the first and second month and 85% in the third month against bacterial rot disease. Therefore, the use of biosynthesized Ag NPs can be an alternative to chemical poisons in the control of plant pathogens.

## Conclusion

The antimicrobial properties of silver metal have been known for a long time and it has been used in the treatment of many microbial infections. In general, the results of this research showed the successful production of Ag NPs using the aqueous extract of Ajowan seeds as a fast and low-cost biological method. And due to the proof of the antimicrobial activity of these nanoparticles, its use alone and in combination with chemical agents in various fields is suggested to control post-harvest diseases of fruits and vegetables during storage.

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