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ORIGINAL PAPER

Ziziphus spina-christi extract-stabilized novel silver nanoparticle synthesis for combating *Fusarium oxysporum*-causing pepper wilt disease: in vitro and in vivo studies

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Abstract

The novelty of the present study is studying the ability of aqueous *Ziziphus spina-christi* leaves' extract (ZSCE) to produce eco-friendly and cost-effective silver nanoparticles (Ag NPs) against *Fusarium* wilt disease. Phytochemical screening of ZSCE by HPLC showed that they contain important antimicrobial substances such as Rutin, Naringin, Myricetin, Quercetin, Kaempferol, Hesperidin, Syringeic, Eugenol, Pyrogallol, Gallic and Ferulic. Characterization methods reveal a stable Ag NPs with a crystalline structure, spherical in shape with average particle size about 11.25 nm. ZSCE and Ag NPs showed antifungal potential against *F. oxysporum* at different concentrations with MIC of Ag NPs as 0.125 mM. Ag NPs treatment was the most effective, as it gave the least disease severity (20.8%) and the highest protection rate (75%). The application of ZSCE or Ag NPs showed a clear recovery, and its effectiveness was not limited for improving growth and metabolic characteristics only, but also inducing substances responsible for defense against pathogens and activating plant immunity (such as increasing phenols and strong expression of peroxidase and polyphenol oxidase as well as isozymes). Owing to beneficial properties such as antifungal activity, and the eco-friendly approach of cost and safety, they can be applied in agricultural field as novel therapeutic nutrients.

Keywords *Fusarium oxysporum* · Immunity · Silver nanoparticles · Pepper plant · *Ziziphus spina-christi* extract

Introduction

There are some important and active compounds in plant extracts (active metabolites) used as antimicrobials, antitumor, and antioxidants agents, in the food additives, and cosmetics (Parham et al. 2020; Salehi et al. 2020; Abdel-Rahman et al. 2022). In addition, the plant extracts were used for controlling prevalent plant diseases which serves as one of the most effective ways and eco-friendly approach (Gonelimali et al. 2018). *Ziziphus spina-christi* is one of those plants whose extracts are used as active component against various plant pathogens which infected some other important crops (Hafiz et al. 2019; Alotibi et al. 2020), where the aqueous extract of *Ziziphus spina-christi* leaves contains many phenolic compounds and flavonoids (Purnamayati et al. 2021; Zandiehvakili and Khadivi 2021) which make it special in the fight against pathogenic bacteria and fungi (El Maaiden et al. 2019; Hassan et al. 2021a; Mulyani et al. 2021).

Nanotechnology is one of the important sciences that have been used in the fight against fungal, viral and bacterial

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infections (El-Waseif et al. 2019; Abdelaziz et al. 2021; Hashem et al. 2021). One of these methods is the use of plant extracts (leaves, fruits, seeds, bark, roots) to prepare nanoparticles for some minerals in what is known as the green nano-metal method (Kuppusamy et al. 2016; Nasrollahzadeh et al. 2019; El Shafey 2020; Abdel-Rahman et al. 2022), and there were numerous researches for the synthesis of nanomaterials for iron, zinc, copper, titanium and others, in which silver salts occupied a wide position in this researches (Behravan et al. 2019; Sánchez-López et al. 2020). Ag NPs has a distinctive effect against bacteria and fungi that cause plant diseases (Alkhattaf 2021) and the green method for Ag NPs synthesis is safe, low-cost, eco-friendly and effective in combating plant pathogens due to its small size and high surface area (Mollick et al. 2019; Acharya et al. 2020; Castillo-Henríquez et al. 2020).

Pepper (*Capsicum annuum L.*) is one of the important agricultural crops rich in high nutritional value because it contains many vitamins, polyphenols, carotenoids, antioxidants, and minerals such as calcium, phosphorous, magnesium and potassium (Olatunji and Afolayan 2018; Baenas et al. 2019; Hernández-Pérez et al. 2020). The pepper plant is thus one of the most important vegetables widely cultivated worldwide (Gniffke et al. 2013; Lin et al. 2013).

As the total cultivated area in Egypt is 41,047 hectares and produces 623,221 tons annually, pepper plants are exposed to many biotic and abiotic risks and challenges (Abdelaziz et al. 2021). Unfortunately, these risks increase with the current climate disturbances all over the world, which helps the attack of many pathogens (Lindner et al. 2010). The pepper crop is under attack by many fungi, the most famous of which is the soil-transmitted disease Fusarium, which causes wilt disease, which is responsible for major plant death and consequently great losses in yield and quality in Egypt and in many countries of the world (El-Hamidi and Zaher 2018; Johnston-Monje et al. 2021; Pawaskar and Kerkar 2021). Pepper plant is highly susceptible to Fusarium wilt disease caused by *Fusarium oxysporum f. s*. Capsaicin and symptoms of the disease are wilting, yellowing of leaves, and destruction of the plant (Ahmed 2013; Mahfouz and Mohamed 2019). To reduce the infection from Fusarium wilt, fungicides are used. We should not fail to note that the excessive use of pesticides has led to more serious problems than the disease itself, as it has negatively affected humans, animals, the environment and beneficial microbial communities for soil and plants (Bhandari 2014; Rani et al. 2021).

Plant resistance means preventing or limiting the progression of damage to it, whether (biotic or abiotic) (Rausher 2001). Systemic plant resistance can be induced by either biological or chemical means. Inducers of resistance affect anatomical structures, morphology, or the production of certain chemical compounds that inhibit the pathogen or reduce the severity of stress (Witzell and Martín 2008; Vargas-Hernandez et al. 2020). Any of the structural or chemical weapons may already be present in the plant regardless of whether or not a pathogen is attacked or a particular stress, or any of these weapons may arise in response to an attack on the plant by a pathogen or stress (Ab Rahman et al. 2018). To overcome this problem, several techniques have been developed, among them is the use of nanomaterials. To reduce the use of fungicides, many techniques have been used, including plant extracts and biosynthetic nanomaterials from plant extracts.

Materials and methods

Materials

Silver nitrate (Ag NO_3) was obtained from El-Gamhouria Trading Chemicals and Drugs Company, Egypt with purity ≥ 90.0%, based on trace metal analysis.

Plant extract

The *Ziziphus spina-christi* (ZSC) was obtained from the Research Farm, Faculty of Agriculture, Al-Azhar University, Sadat City, Menoufia, Egypt. The aqueous ZSC leaves' extract (ZSCE) was prepared according to published papers (Ramamoorthy et al. 2001; Farrag et al. 2017) and stored at 4 °C until used.

Phytochemical screening

Phytochemical assays for the screening and identification of bioactive chemical components in aqueous ZSCE was investigated by method described according to recent publications (Harborne 1998; Ahmad et al. 2014; Sharaf et al. 2021).

HPLC analysis

The chemical contents of the phenolic and flavonoid compounds in ZSCE were investigated with the HPLC technique as described in the following references (Kuntić et al. 2007; Lin et al. 2013). Chromatographic analysis was performed by HPLC (Agilent 1100) which was composed of pump, UV/Vis. detector, C-18 column (125 mm × 4.60 mm, and 5 µm particle size).

Green synthesis of silver nanoparticle (Ag NPs) using aqueous ZSCE

Ziziphus spina-christi leaves were obtained fresh and washed in double-distilled water. Leaves were cut into small pieces, and 15 g were weighed and added to 250 mL distilled

water, which was then boiled to 100 °C, filtered through filter paper, and kept at 4 °C as aqueous ZSC leaves' extract (ZSCE).

We prepared a stock solution of Ag^+ ions by weighing out 170 mg of Ag NO_3 and dissolved it to 1 L distilled water to prepare a final concentration of 1 mM. With continual stirring, 20 mL of ZSCE was added to 80 mL of 1 mM Ag NO_3 in this experiment, and within 20 min, the color of the mixture had changed from pale green to brown, indicating the formation of biogenic Ag NPs (Abdel-Rahman et al. 2022).

Characterization of the synthesized Ag NPs

The crystallinity and the crystallite size and/or lattice of the synthesized Ag NPs were estimated by the XRD-6000 lists, Shimadzu apparatus, SSI, Japan. The intensity of the diffracted X-rays was tested as diffracted angle 2θ. The most predominate Ag NPs size and their distribution was defined by Dynamic Light Scattering (DLS-PSS-NICOMP 380-USA). In addition, the microstructure, mean particle size and the shape of the synthesized Ag NPs were evaluated using high-resolution transmission electron microscope (HRTEM, JEM2100, Jeol, Japan).

The surface morphology and the grain size of Ag NPs were investigated by SEM, ZEISS, EVO-MA10, Germany. In addition, EDX analysis (BRUKER, Nano GmbH, D-12489, 410-M, Germany) was used to estimate the elemental structure, purity and the percentage of each metal presented in our samples.

Finally, FTIR spectral analysis was a vital target that gives information regarding the chemical functional groups presented in the plant extract. The experiments were carried out using a JASCO FTIR 3600 Infra-Red spectrometer after conducting KBr pellet technique. It was determined at a wave number range from 400 to 4000 cm^{-1}.

Source of pathogen *(F. oxysporum)*

F. oxysporum f. sp. Lycopersici RCMB008001 was obtained from Mycology Lab. (Faculty of Science, Botany and Microbiology Dep., Al-Azhar University Cairo, Egypt). It was confirmed by pathogenicity test according to Aldinary et al. (2021). The inoculum of the pathogenic fungus *F. oxysporum f. sp. Lycopersici* was prepared according to the recent paper (Hashem et al. 2021).

In vitro antifungal activity

Using agar diffusion well, the antifungal activity of ZSCE and the biogenic Ag NPs was evaluated (Attia et al. 2021). It was performed by making wells filled with different concentration of ZSCE (2%, 4%, 6%, 8% and 10%) and different Ag NPs concentrations (1, 0.5, 0.25, 0.125, 0.0625 and 0.031 mM), and the antifungal activity was assessed after 5 days of incubation at room temperature (Khalil et al. 2021).

Pot experiment

Healthy and analogous 3-week-old pepper seedlings were selected from the Agricultural Research Center of the Ministry of Agriculture, Egypt. The seedlings were planted in plastic pots (20 cm in diameter) containing 2 kg a mixture of sandy clay soil (1: 3 wt/wt), at botanical garden of Botany and Microbiology Department, Faculty of Science, Al-Azhar University.

The experiment was designed as follows: 1—control healthy, 2—*Fusarium*-infected control, 3—healthy plants treated with plant extract, 4—infected plants and treated with plant extract, and 5—healthy plants treated with the biogenic Ag NPs, and 6—infected plants treated with the biogenic Ag NPs. The experiment was followed up and the symptoms of infection were recorded after 15 days of infection. Samples were taken to estimate the morphological and biochemical characteristics after 50 days of planting (Attia et al. 2021).

Disease symptoms and disease index

Disease symptoms were assessed 50 days after inoculation, while the disease index and percent protection caused by ZSCE or ZSCE-mediated Ag NPs biosynthesis were evaluated according to the published paper (Farrag et al. 2017).

Plant resistance metabolic indicators

Fresh leaf samples were taken from all treatments to estimate the photosynthetic pigments according to the method described by Vernon and Seely (2014). In addition, the content of osmolytes were measured in the dry leaves as the total protein content according to the method described by Vernon and Seely (Vernon and Seely 2014), the total carbohydrate content according to the method described by Irigoyen et al. (Irigoyen et al. 1992) and the free proline content the method described by Bates et al. (Bates et al. 1973). The phenol content of the leaves was also estimated according to the method described by Diaz and Martin (Diaz and Martin 1972). The enzymatic activity of the peroxidase enzymes was determined by the method described by Srivastava (Srivastava 1987) and polyphenol oxidase by the method described by Matta and Dimond (Matta and Dimond 1963).

Isozyme electrophoresis

The procedure was used to evaluate the peroxidase (POD) isozyme and was described in details according to Barcelo et al. (Barceló et al. 1987). The isozyme polyphenol oxidase (PPO) was calculated according to the methods determined by Thipyapong et al. (Thipyapong et al. 1995).

Statistical analysis

One-way analysis of variance (ANOVA) was applied to the results. Least significant difference (LSD test) using CoStat (CoHort, Monterey, CA, USA) was used to demonstrate statistically relevant differences between the treatments at $p \leq 0.05$. Results are shown as mean ± standard errors ($n = 3$).

Results and discussion

Phytochemical screening of aqueous ZSCE

The results tabulated in Table 1 indicate the qualitative detection of Flavonoids, Tannins, Saponosides, Terpenes, Polyphenols and Alkaloids active compounds in crude aqueous ZSCE and the results were all positive for the aqueous ZSCE, which was compatible with the results obtained in both published papers (Ads et al. 2018; Hussein and Hamad 2021).

HPLC analysis

Table 2 and Fig. S1 show that phenolic and flavonoid compounds obtained using the HPLC analysis which are estimated as Rutin, Naringin, Myricetin, Quercetin, Kaempferol, Hesperidin, Syringeic, Eugenol, Pyrogallol, Gallic and Ferulic are important compounds contained in the extracts of the ZSCE (Roghini and Vijayalakshmi 2018), and it was observed with the presence of Rutin (3.0%), Naringin (4.8%), Myricetin (6.0%), Quercetin (7.0%), Kampferol (8.0%), Hesperidin (10.0%), Syringeic (5.2%), Eugenol (7.0%), Pyrogallol (9.0%), Gallic (10.0%) and Ferulic (11.0%). Other published paper (Abdulla et al. 2016) reported that Pyrogallol (12.86 mg/100 g), Ferulic (5.38 mg/100 g), Gallic (0.16 mg/100 g), Hesperidin (3.4 mg/100 g), Rutin (1.52 mg/ 100 g), Naringin (0.39 mg/100 g), Kaempferol (0.22 mg/100 g) and Quercetin (8.48 mg/100 g) were found in the tested extract.

Table 1 Qualitative detection of some active compounds in crude aqueous ZSCE

Active compounds	Inference
Flavonoids	+
Tannins	+
Saponosides	+
Terpenes	+
Polyphenols	+
Alkaloids	+

Table 2 Chemical composition analysis of phenolic and flavonoid compounds of water extract from aqueous ZSCE by HPLC

Compound	Retention time (minutes)	Concentration (mg/ml)
Rutin	3.0	5.76
Naringin	4.8	4.88
Myricetin	6.0	5.33
Quercetin	7.0	2.14
Kaempferol	8.0	3.26
Hesperidin	10.0	6.05
Syringeic	5.2	23.66
Eugenol	7.0	5.23
Pyrogallol	9.0	17.39
Gallic	10.0	4.26
Ferulic	11.0	5.09

Synthesis of biogenic Ag NPs

After mixing the extract solution (ZSCE) and silver nitrate solution, the color change after a short period (20 min) from color green to dark brown confirmed the formation of biogenic Ag NPs. In addition, the process of changing the optical color in solution resulted from the components of the aqueous extract such as polyphenols and flavones which are considered as a reducing factor (Rodríguez-León et al. 2013; Hamouda et al. 2019; Ijaz et al. 2020).

Characterization of green Ag NPs

Crystal design and the moderate crystal size of the biogenic Ag NPs were checked by XRD analysis; it tested the state of the experimental samples (Ashour et al. 2018; Maksoud et al. 2018; Abdel Maksoud et al. 2019; Maksoud et al. 2019; Pal et al. 2019).

XRD for the synthesized green Ag NPs is displayed in Fig. 1 and represents the presence of the crystal and amorphous peaks for the synthesized Ag NPs, and the filtrate ZSCE, respectively. First, the XRD result for ZSCE shows the primary amorphous peak at $2\theta = 22.24°$. On the other hand, the XRD result of the synthesized Ag NPs showed the diffraction characteristics peaks; 2ɵ at 38.18°, 44.01°, 46.57°, 77.67°, and 81.74° and described the Bragg's reflections at (111), (200), (220), (311) and (222), respectively.

The detected peaks were identical to the JCPDS of Ag NPs with a definitive card named JCPDS File No 04-0784

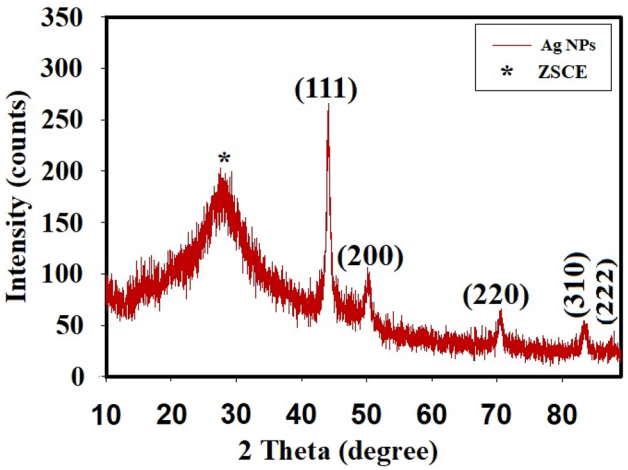

Fig. 1 XRD analysis of Ag NPs synthesized by ZSCE

(Cheng et al. 2015), suggesting that the green Ag NPs were crystal and had the face-centered cubic crystalline design. In addition, one amorphous peak is detected at 22.24° and is for ZSCE.

On the other hand, the average crystallite size of the synthesized Ag NPs is determined by the Williamson–Hall (W–H) equation (Belavi et al. 2012; Ashour et al. 2018; Maksoud et al. 2018; Pal et al. 2018; Maksoud et al. 2019), and is calculated to be 12.25 nm for Ag NPs synthesized by ZSCE according to Eq. 1:

$$\beta \cos\theta = \frac{k\lambda}{D_{W-H}} + 4\varepsilon \sin\theta \quad (1)$$

where $D_{W\text{-}H}$ is the average crystallite size, β is the full-width at half maximum, λ is the X-ray wavelength and θ is the Bragg's angle, k is a constant and ε is the strain of the samples.

EDX spectrophotometer analysis determined the presence of Ag element indicative of Ag NPs. The EDX analysis detected a powerful signal from Ag area of Ag NPs with (ZSCE) in Fig. 2. The elemental study of the synthesized ZSCE-Ag NPs was investigated by EDX analysis and confirmed the Ag NPs forming. Metallic Ag NPs usually show a standard optical absorption peak almost between at 3 and 4 keV approximately and the average concentration of elemental silver was 93.88%. Elemental analysis also showed that the content of silver was the highest, followed by C, O, N, and Si. The peaks of these biomolecules bind to the superficial Ag NPs.

TEM analysis is one of the generality important techniques to study the shape and size of the nanoparticles (Rauwel et al. 2015). HRTEM images are shown in Fig. 3 where typical size of green Ag NPs synthesized from ZSCE was mostly less than 100 nm as it is shown that distribution of the size Ag NPs is between 10.96 nm and 12.94 nm, and there is no significant difference in size of green Ag NPs, as TEM analysis showed and the analysis indicates that the biosynthesized Ag NPs are mostly spherical in shape and these analysis data agree with previously published articles (Shukla et al. 2012; El-Ansary et al. 2018; Alahmad et al. 2021).

The common particle size dispersion was determined by the DLS system and was defined as 23.8 nm in the Ag NPs produced by ZSCE as illustrated in Fig. 4. It was stated that DLS size range of the synthesized Ag NPs was noted to be greater than the HRTEM size. The reason was due to DLS analysis estimated the hydrodynamic size of the synthesized Ag NPs and were enclosed by water molecules and may be confirmed the large size of the capped Ag NPs (El-Batal et al. 2014; El-Batal et al. 2016; Baraka et al. 2017).

In Fig. 5, a 31.6 mV value refers the zeta potential of the biogenic Ag NPs synthesized using ZSCE; it is a negative value, and indicates great and long-term stabilization (Chakraborty et al. 2021) and further zeta potential value compatible with the values obtained by each of the published papers (Khorrami et al. 2020; Biswal et al. 2021), and suggested that this value and result for zeta potential causes the formation of NPs with highly stable colloidal properties.

SEM imaging is used to detect and confirm the surface morphology and uniformity of the synthesized Ag NPs. SEM image of green Ag NPs biosynthesized by ZSCE is shown in Fig. 6 with differing sizes and matches the spherical formation. The SEM results show a uniform NPs exterior, and the Ag NPs texture formation is cleared. Ag NPs were normally located with ZSCE and appeared as a bright NP combined with the stabilizer ZSCE.

FTIR spectrum is performed to determine the relations among the synthesized Ag NPs and ZSCE and the place of combination. FTIR spectrum of ZSCE has the main bands at 3314.86 and 1636.29 cm^{-1}, while the bands for the green Ag NPs (ZSCE-Ag NPs) are noticed at 3312.24, 1636.15, and 623.17 cm^{-1} as shown in Fig. 7.

The detected peak at 3314.86 cm^{-1} corresponded to O–H stretching regarding the hydroxyl group, and the peak at 1636.29 cm^{-1} is identified as the carbonyl stretch, which is matched to the amide I bond in the plant extract. FTIR results of ZSCE-Ag NPs exhibit a peak at 3312.24 cm^{-1} and is matched to O–H stretching (OH group), and the peak at 1636.15 cm^{-1} was identified to the carbonyl stretch. Distinctly, a peak located at 623.17 cm^{-1} was noticed in the FTIR of Ag NPs alone, which may be due to the combination of green Ag NPs with the hydroxyl group (as Ag–O) (Kumar et al. 2012; El-Batal et al. 2017; Ashour et al. 2018; El-Sayyad et al. 2018). FTIR results in the present study were similar to the literatures (de Matos et al. 2012; Kumar et al. 2012; Zarabi et al. 2014).

It is concluded that the T% of all peaks is decreased in the FTIR spectrum of green Ag NPs, which may be due to

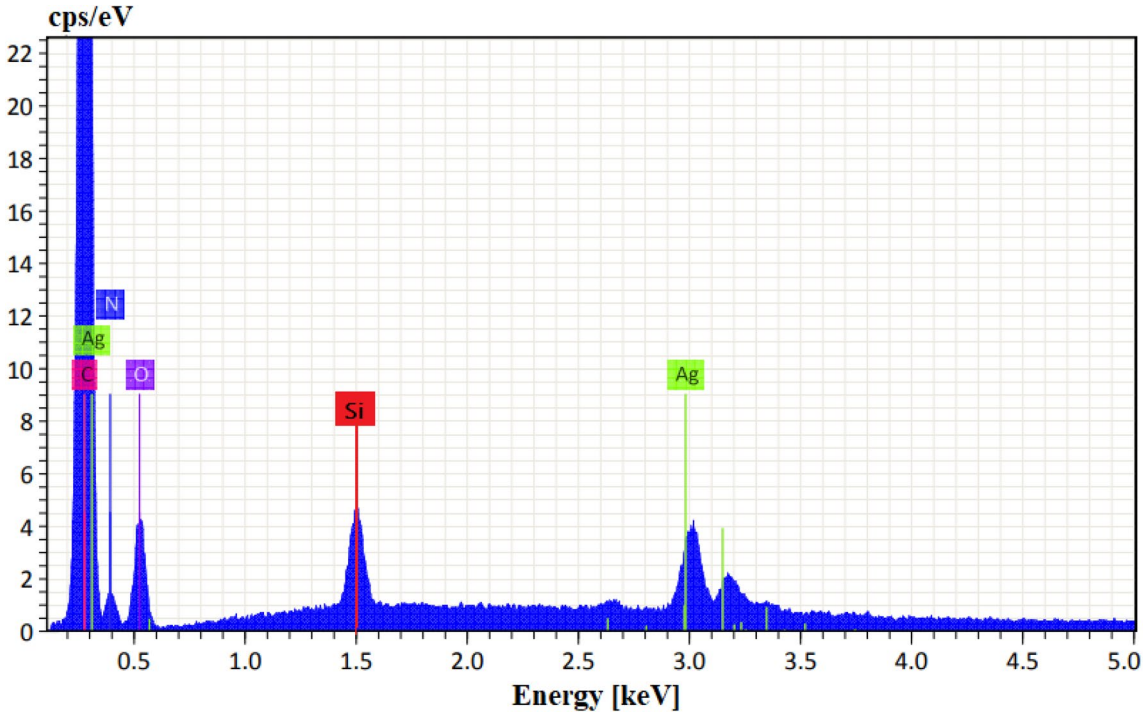

Fig. 2 EDX spectrum of Ag NPs synthesized by ZSCE

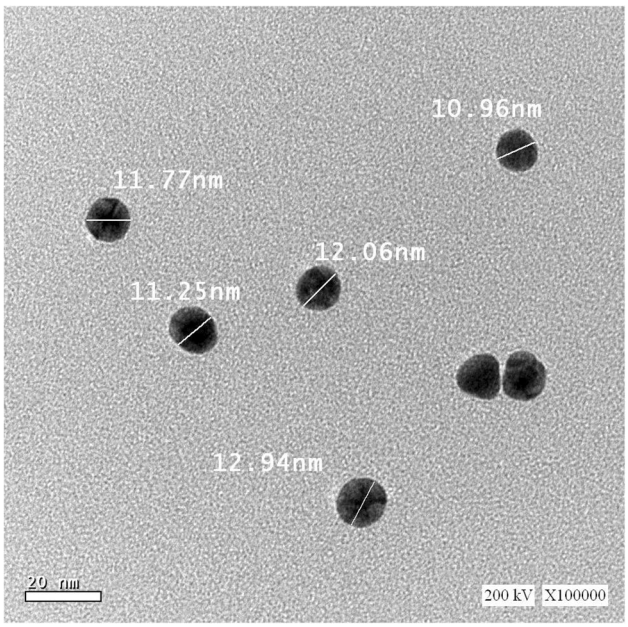

Fig. 3 HRTEM images of green Ag NPs synthesized using ZSCE

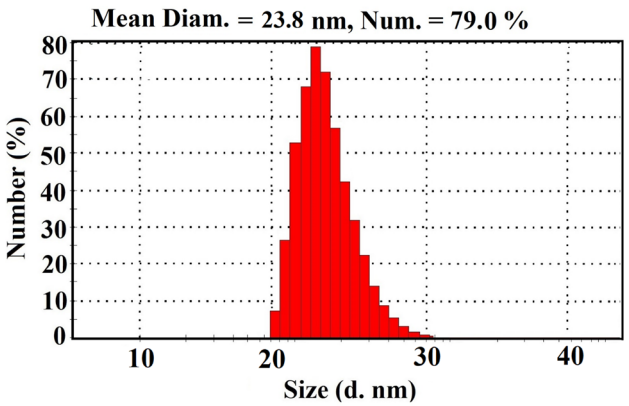

Fig. 4 DLS analysis of the green Ag NPs synthesized using ZSCE

the combination of Ag NPs with the OH group and the different functional groups introduced in ZSCE (Kumar et al. 2012). It is observed from the FTIR spectra ZSCE is used for the associated reduction and stability functions. ZSCE shows characteristic peaks which offered an essential role in Ag NPs stability (de Matos et al. 2012; Kumar et al. 2012). ZSCE may connect to Ag NPs across the electrostatic affinity between carboxylate groups (which possesses a negative charge; Arakelova et al. 2014), and consequently, they stabilized the synthesized Ag NPs from aggregation by the characteristics of ZSCE (Kumar et al. 2012).

In vitro antifungal activity of ZSCE and ZSCE-mediated biosynthesis of Ag NPs

Figure 8a shows the antifungal activity of Ag NPs and ZSCE against *F. oxysporum* using the agar well diffusion method.

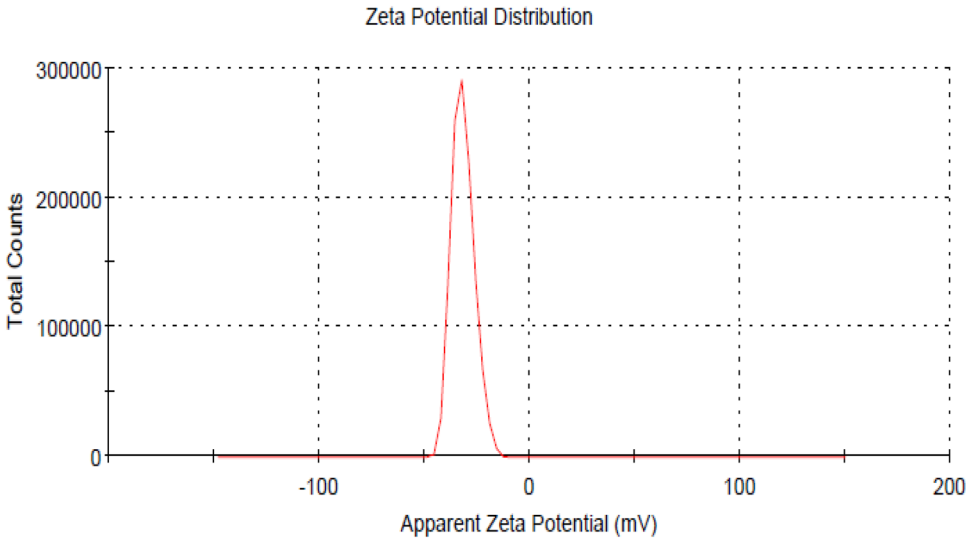

Fig. 5 Zeta potential value of Ag NPs synthesized using ZSCE

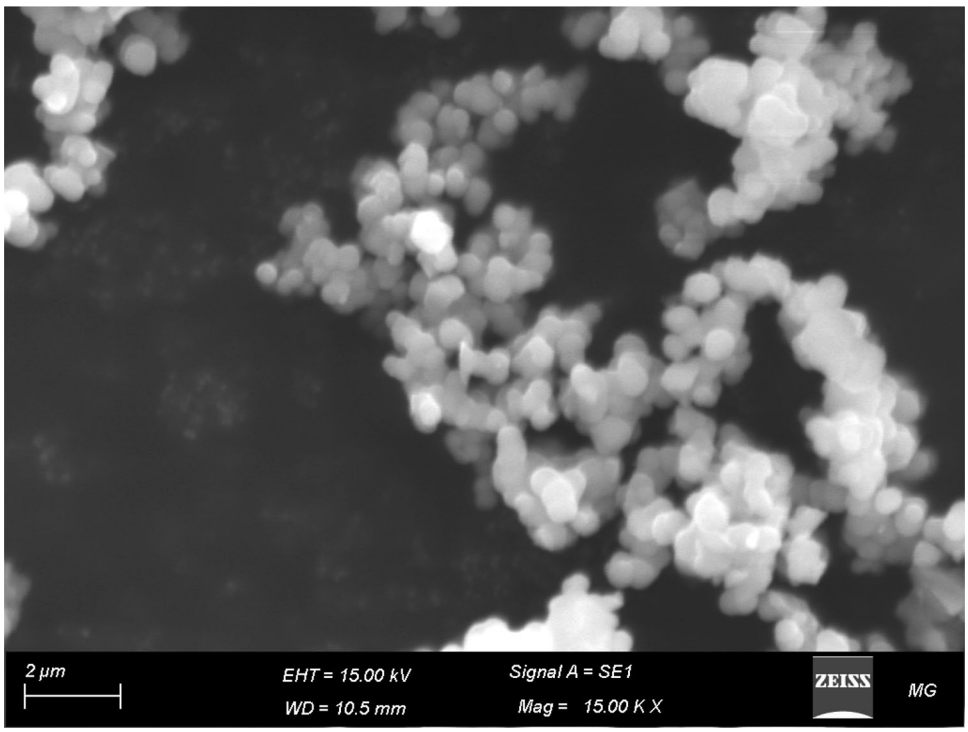

Fig. 6 SEM image of green Ag NPs synthesized using ZSCE

Results in Fig. 8b illustrated that the biosynthesized Ag NPs at concentrations 1, 0.5, 0.25 and 0.125 mM had antifungal potency against *F. oxysporum*. Moreover, 1 mM of Ag NPs had the supreme antifungal activity and offered an inhibition region of 30 mm, whereas 0.125 mM was the MIC of Ag NPs against *F. oxysporum* and presented 8 mm inhibition area. Our results are in harmony with published papers (Hashmi et al. 2019; Khalil et al. 2019; Fouda et al. 2020) which reported the anti-Fusarial effect of Ag NPs.

On the other hand, ZSCE has inhibition effect on *Fusarium* growth at 10% only. These results explained by the recent published paper (Abu-Taleb et al. 2011) which recorded that ZSCE has inhibited sporogenesis, germination, development, cellulolytic as well as pectolytic enzyme activity of *Fusarium.* In addition, other published articles (Ramaiah and Garampalli 2015; Attia et al. 2016; Alotibi et al. 2020; Daradka et al. 2021) proved the direct inhibition effect of plant extract on plant fungal pathogens including *Fusarium*.

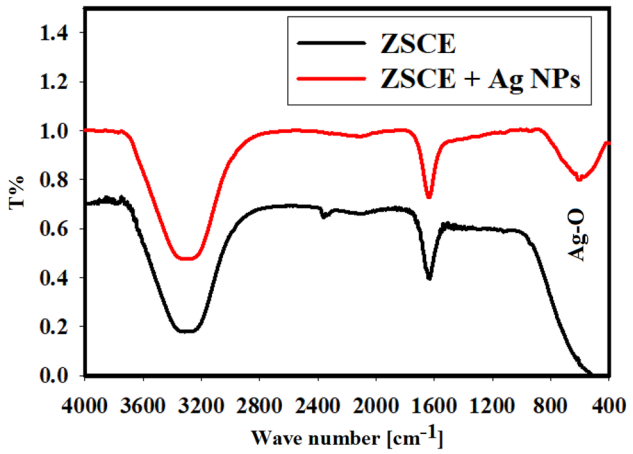

Fig. 7 FTIR spectra of *Z. spina-christi* leaves aqueous extract, and green Ag NPs synthesized using aqueous extract of *Z. spina-christi* leaves.

Evaluation and estimation of pepper systemic resistance induced by ZSCE and Ag NPs

Disease severity (DS) and protection%

The results showed in Table 3 indicate the high severity of the disease in pepper seedlings due to *F. oxysporum* infection where the severity of infection reached 83.33%. On the contrary, the results showed that the infected plants that were treated with the tested inducers, whether ZSCE or Ag NPs, recorded minimal infection rate. It is worth noting that Ag NPs gave the lowest injury severity and the highest protection rate, reaching 20.8%, 75%, respectively. In addition, treatment with ZSCE led to a decrease in the severity of the disease and an increase in the rate of recovery by 25% and 69.9%, respectively. There is no doubt that the decrease in the symptoms of infection is one of the strongest indications of the emergence of resistance against the disease. From this point of view, the use of biosynthetic Ag NPs from the plant extract is a strong antifungal, and an explanation for this was confirmed by many researches (Karbasian et al. 2008; Gorczyca et al. 2015; Madbouly et al. 2017; Al-Zaban et al. 2019; Bezerra et al. 2021).

Foliar spray of ZSCE inhibits the harmful effects of *F. oxysporum* because it contains several phenolic and antioxidant compounds that stimulate the biochemical immunity of plants such as Rutin, Naringin, Myrecetin, Quercetin, Kampferol, Hesperidin, Syringeic, Eugenol, Pyrogallol, Gallic and with Ferulic. The ability of these active substances in the plant extract to inhibit the action of pathogenic fungi and stimulate plant immunity is explained by many researchers (Matić et al. 2011; Sohal and Sharma 2011; Abd-Elsalam and Khokhlov 2015; Yang et al. 2016; YÖRÜK et al. 2018; Hassan et al. 2021b).

Growth parameters

The results obtained in Table 4 confirm that *Fusarium* wilt causes a sharp inhibition in plant height and number of leaves. Infected plants recorded the lowest stem and root length and the lowest number of leaves compared to healthy plants. These results are in agreement with the results published (El-Marzoky and Abdel-Sattar 2008; Jaber and

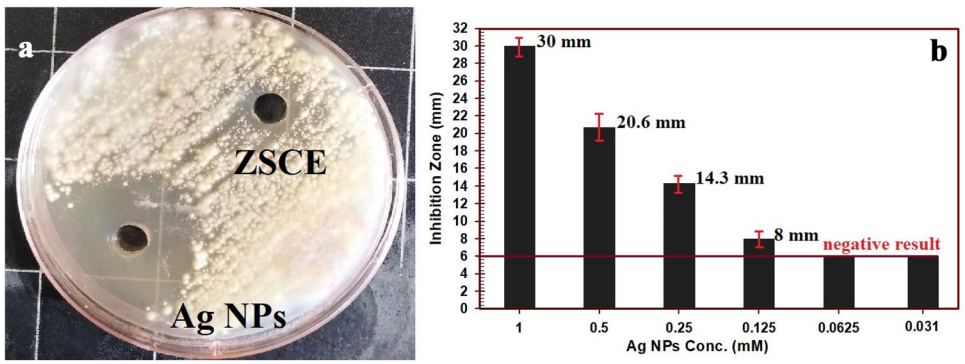

Fig. 8 In vitro antifungal activity of ZSCE, and Ag NPs as ZOI (**a**), and MIC result of the biosynthesized Ag NPs (**b**)

Table 3 Effect of ZSCE and Ag NPs on disease severity (DS) and protection %

Treatment	Disease symptoms classes					DI (disease index; %)	Protection (%)
	0	1	2	3	4		
Control healthy	6	0	0	0	0	–	–
Control Infected	0	0	1	2	3	83.3	0
Infected + ZSCE	3	1	1	1	0	25.0	69.9
Infected + Ag NPs	3	2	0	1	0	20.8	75.0

Table 4 Effect of ZSCE and the biogenic Ag NPs on plant growth parameters

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves/plant
Control healthy	75.6 ± 2^{b}	40.13 ± 1^{b}	99.72 ± 1.02^{c}
Control infected	48.3 ± 1.5^{d}	7.7 ± 0.45^{e}	65.27 ± 1.15^{d}
Healthy + ZSCE	81.15 ± 1.25^{a}	51.61 ± 4.5^{a}	179.44 ± 2.08^{a}
Healthy + Ag NPs	80.26 ± 0.7^{a}	52.26 ± 0.49^{a}	131.66 ± 1^{b}
Infected + ZSCE	54.6 ± 1^{c}	14.933 ± 1^{d}	94.72 ± 2.12^{c}
Infected + Ag NPs	75.6 ± 2.01^{b}	36.86 ± 0.43^{c}	79.722 ± 2^{cd}
LSD at 0.05	4.15	3.18	27.88

Data within the groups are analyzed using one-way analysis of variance (ANOVA) followed by [a, b, c, d, and e] Duncan'smultiple range test (DMRT)

Alananbeh 2018; Abdelaziz et al. 2021; Hassan et al. 2021c) and it can be explained that *Fusarium* causes severe disturbance in growth hormones, which leads to a clear defect in cell biology (Basco et al. 2017; Rivera-Jiménez et al. 2018).

The results in Table 4 indicated that the use of the ZSCE or the biosynthesized Ag NPs led to a significant improvement in each of the healthy or infected plants. The highest improvement in shoot and root lengths was observed in the challenged plants, which were treated with the biosynthesized Ag NPs, while number of leaves was higher in the challenged plants, which were treated with ZSCE. Our results are in agreement with many studies (Mirzaei et al. 2015; Vinković et al. 2017; Luan and Xo 2018; Ashraf et al. 2020) and these results can be explained by the plant's recovery from infection, which reduces the stress on the plant and thus improves the process of respiration, transpiration, absorption of elements from the soil and the regulation of biosynthesis pathways for growth hormones (Mirzaei and Moradi 2018; Hasanin et al. 2021).

Photosynthetic pigments

The ability of plants to carry out the process of photosynthesis is the most important aspect of health (Muhammad et al. 2021). On the contrary, the results in Fig. 9 confirmed that *Fusarium* infection resulted to a severe decrease in the photosynthetic pigments (Chlorophyll a and b), except for carotene that agree with the published results (Chávez-Arias et al. 2019; El-Abeid et al. 2020; Maqsood et al. 2020). This decrease in chlorophyll pigments can be explained by the published explanations (Choudhury and Panda 2005; Jahan et al. 2020; Singh et al. 2021), and they mentioned that the decrease in chlorophyll is a result of oxidative stress after *Fusarium* infection due to the release of free radicals, causing damage or distortion in the formation of chloroplasts, and this means the failure or inability of the plant to capture light and carry out the process of photosynthesis.

On the other hand, a significant improvement in the synthesis of photosynthetic pigments because of treatment with ZSCE or the biosynthesized Ag NPs, whether in healthy or infected plants focusing on the infected plants that were treated with the inducers. The biogenic Ag NPs were better in the synthesis of chlorophyll a pigment, followed by the treatment with the ZSCE. In addition, treatment with the ZSCE was better in the formation of chlorophyll b, followed by the synthesized Ag NP. Our results are in agreement with other published studies (Mirzaei and Moradi 2018; JIYA 2021).

Metabolic indicators

Physiological immunity results from many biological reactions, including changes in the cell wall and the synthesis of substances responsible for defense such as phytoalexins and proteins related to pathogenesis (Ramamoorthy et al. 2001; Sakaguchi and Powrie 2007; Doughari 2015). The results in Fig. 10 showed that infection with *Fusarium* caused a decrease in the total content of proteins and carbohydrates as mentioned in published articles (Farrag et al. 2017; Abdelaziz et al. 2021; Aldinary et al. 2021). The total protein and carbohydrate contents of both healthy and infected plants improved significantly due to treating with ZSCE or the biosynthesized Ag NPs. For more, it was noted that the treatment of infected plants with ZSCE was better in increasing the content of total proteins, while the treatment with Ag NPs was better in increasing the content of total carbohydrates as noted in recently published papers (Courtois et al. 2019; Hajian et al. 2022). Treatment of plants with ZSCE or the biosynthesized Ag NPs induced the photosynthesis process and caused an inhibition in the growth of *Fusarium*, which led to an increase in the total carbohydrate content as an indicator of the systemic resistance and help the affected pepper plants to tolerate the fungal infection (Tahir et al. 2018; Dikshit et al. 2021).

Based on the results obtained in Fig. 10, it was observed that the free content of proline increased in infected pepper plants, and the results are in streak with what was reported by several studies (Sziderics et al. 2007; Shishatskaya et al. 2018; Chávez-Arias et al. 2019). In addition, treating plants, whether healthy or infected, with ZSCE or the

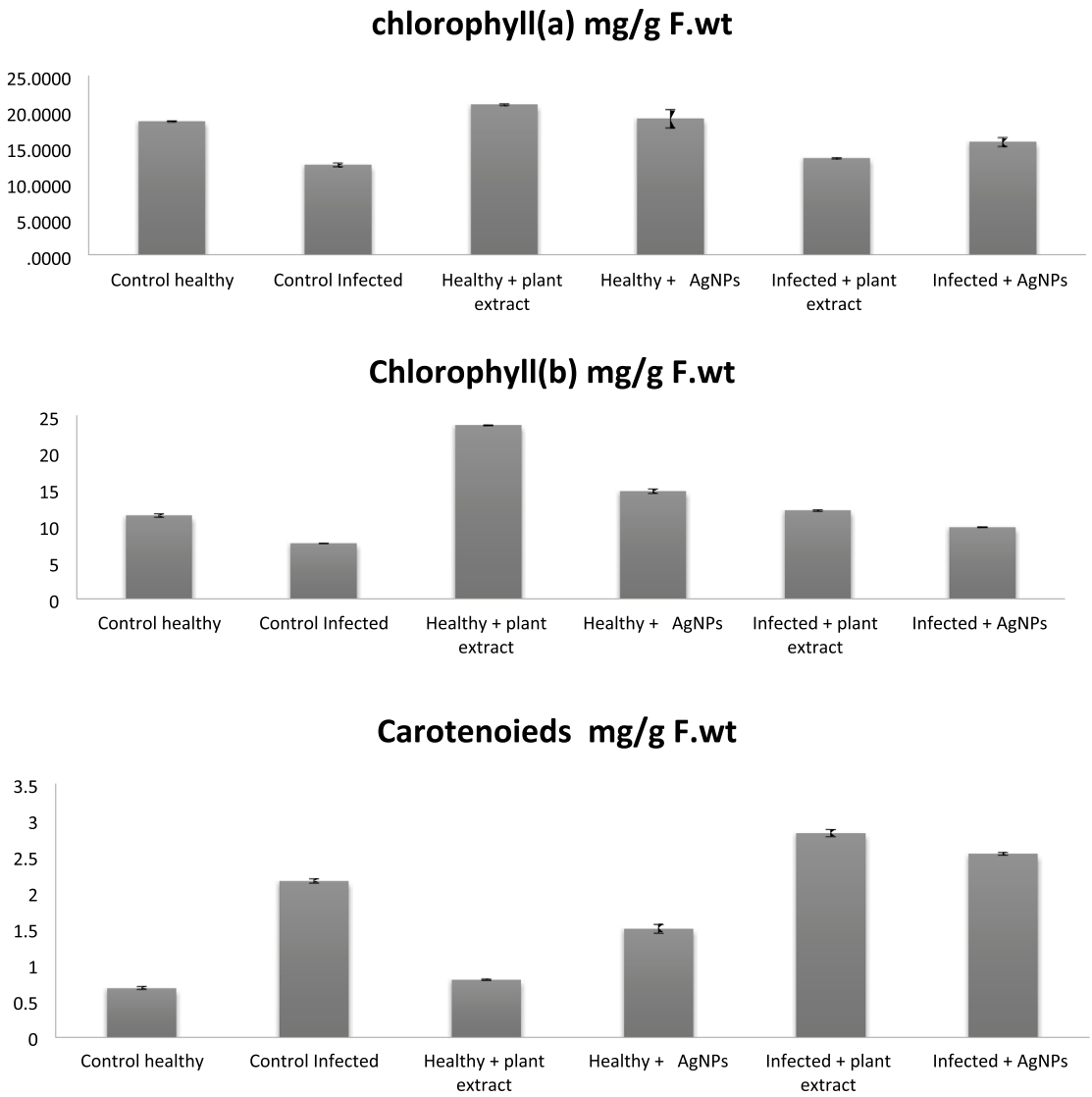

Fig. 9 Effect of ZSCE and the biosynthesized Ag NPs on photosynthetic pigments

biosynthesized Ag NPs caused an increase in the plant's content of free proline, and this is clear evidence of the activation of plant immunity (Nair and Chung 2015; Yan and Chen 2019; Dhiman et al. 2021). In general, with the biosynthesized Ag NPs, treatment was better in increasing the plant's content of proline, followed by the treatment with the ZSCE.

Phenolic compounds are characterized by high efficiency in eliminating or limiting free radicals that are formed because of pathological infections including fungi (Vance et al. 1980; Domej et al. 2014). By estimating the content of phenols in plants, the results showed infection with *Fusarium* led to a higher content of phenols. In addition, the use of with ZSCE or the biosynthesized Ag NPs, on infected or healthy plants showed a significant increase in the content of phenols. Where the plant extract was the best treatment in raising the content of phenols, followed by the biosynthesized Ag NPs, whether on healthy or infected plants. The promising results were due to the ZSCE which contains many phenolic compounds that have a clear effect in reducing the incidence of infection and stimulating the formation of substances responsible for defense and capturing free radical groups that resulted from oxidative explosions as a result of infection (Dkhil et al. 2018; Metwally et al. 2021).

Phenolic acids are involved in phytoalexin accumulation, biosynthesis of lignin and formation of structural barriers, which play a major role in resistance against the plant fungal pathogens (Matern and Kneusel 1988; Lattanzio et al. 2006). In addition, in recent studies on plants resistant to microbial infestation, it was found that there is a correlation between

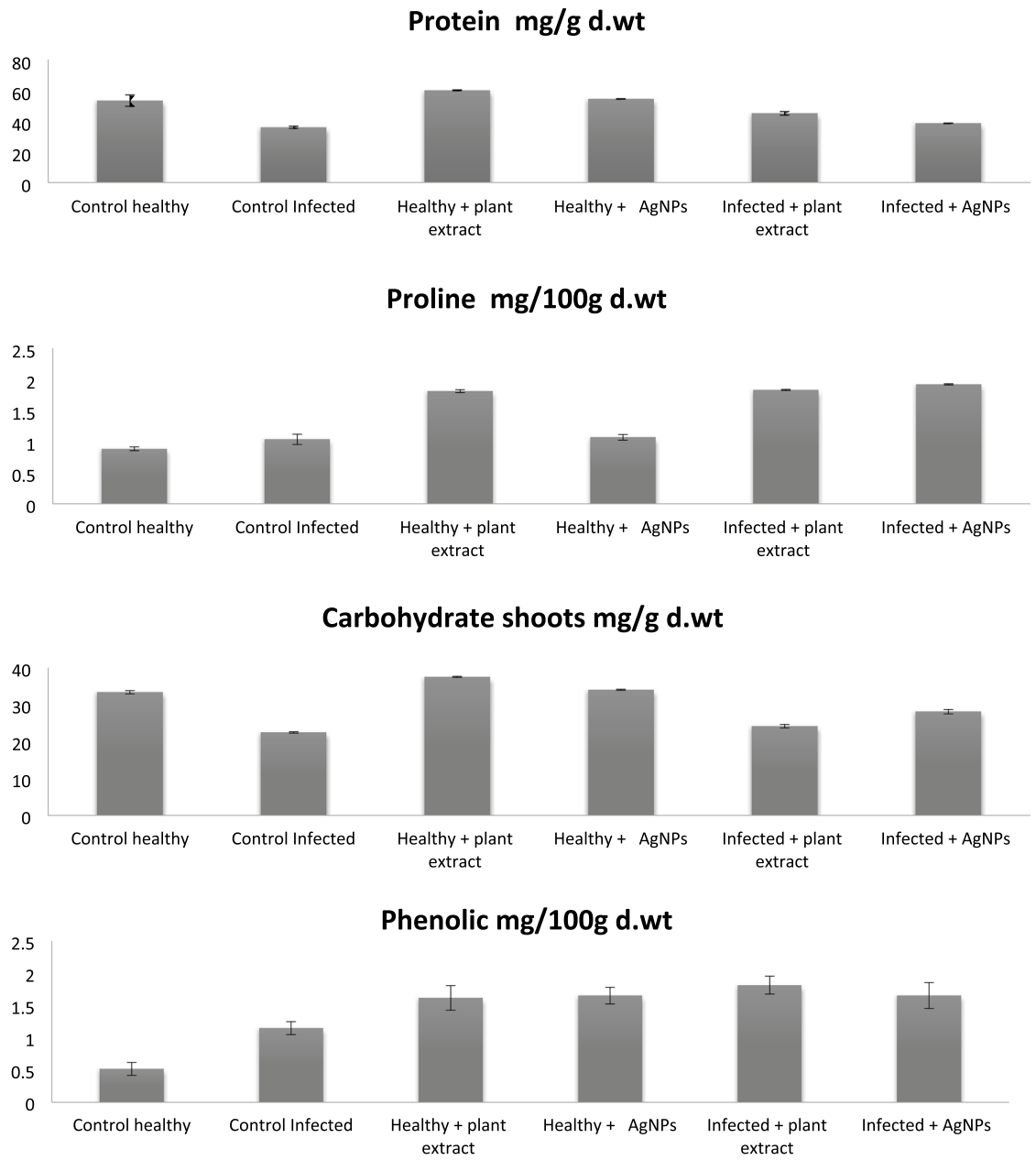

Fig. 10 Effect of ZSCE and the biosynthesized Ag NPs on metabolic indicators

the content of plant tissues of polyphenol compounds and the degree of resistance to pathogens, as the degree of resistance increases with the increase in the level of many phenols in the plant (Lattanzio et al. 2006; Minerdi et al. 2011). These increase in phenolic compounds affect the genetic material in plant cells, pushing it to increase the synthesis of some enzymes such as polyphenol oxidase, which oxidizes polyphenols and turns them into compounds known as quinone, which has the effect of inhibiting fungal spores (Makoi and Ndakidemi 2007).

Antioxidant isozymes

Isozymes enzymes play an important role in protecting the plant cell from various stresses and are an important means of controlling the metabolism process (Jamshidi et al. 2016). The results shown in Tables 5 and 6 and Fig. 11 indicate that the biosynthesized Ag NPs showed high expression in the number and density of bands, where the Ag NPs recorded the highest number and the highest density of bands, followed by the ZSCE.

Table 5 Effect of ZSCE and biogenic Ag NPs on peroxidase isozymes

Peroxidase groups	Relative mobility	C1	C2	1	2	3	4
Px1	0.2	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
Px2	0.5	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}
Px3	0.6	1^{+}	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}
Px4	0.7	1^{+}	1^{+}	1^{+}	1^{-}	1^{-}	1^{++}

C1 = Control healthy, C2 = Control Infected, 1 = healthy + ZSCE, 2 = healthy + Ag NPs, 3 = infected + ZSCE, and 4 = infected + Ag NPs

++ High density band, + moderate density band, – low-density band, 1 present band, and 0 absent band

Table 6 Effect of (ZSCE) and (Ag NPs) on polyphenol oxidase isozymes

Polyphenol oxidase groups	Relative mobility	C1	C2	1	2	3	4
PPO1	0.1	1^{+}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
PPO2	0.5	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
PPO3	0.6	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}
PPO4	0.7	1^{++}	1^{++}	1^{++}	1^{+}	1^{+}	1^{++}
PPO5	0.8	1^{-}	1^{-}	1^{-}	1^{-}	1^{-}	1^{+}

C1 = Control healthy, C2 = Control infected, 1 = healthy + ZSCE, 2 = healthy + Ag NPs, 3 = infected + ZSCE, and 4 = infected + Ag NPs

++ High density band, + moderate density band, – low-density band, 1 present band, and 0 absent band

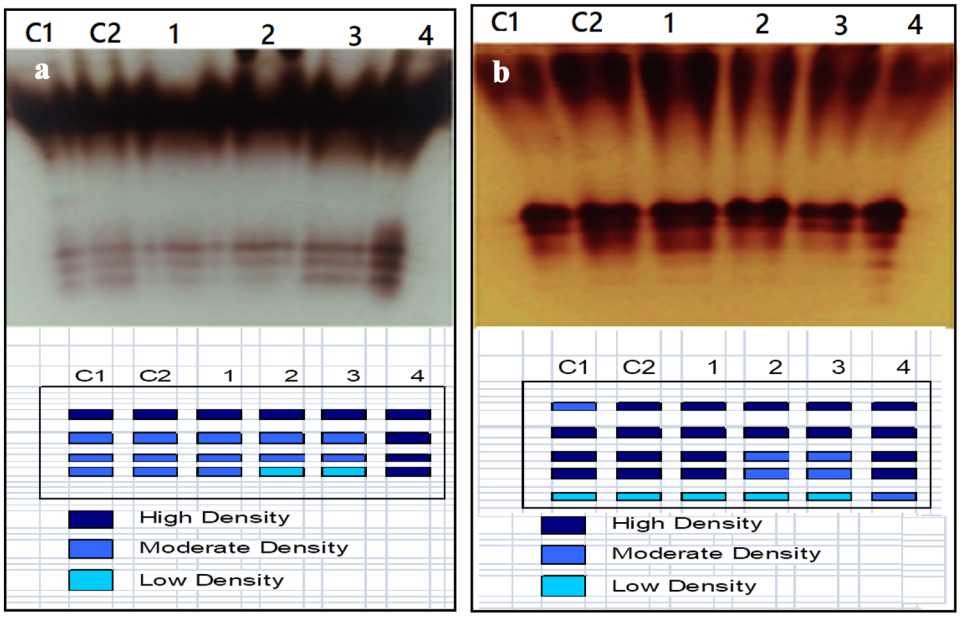

Fig. 11 Effect of ZSCE and the biosynthesized Ag NPs on antioxidant Isozymes where (**a**) for peroxidase, and (**b**) for polyphenol oxidase; (C1 = Control healthy, C2 = Control infected, 1 = healthy + ZSCE, 2 = healthy + Ag NPs, 3 = infected + ZSCE, and 4 = infected + Ag NPs)

Infected plants treated with the biogenic Ag NPs showed the strongest expression of peroxidase and polyphenol oxidase, which gave 4 high-density moieties at Rf = 0.2, 0.5, 0.6 and 0.7 for peroxide and 5 of them 4 high-density at Rf = 0.1, 0.5, 0.6 and 0.7 and one with medium density at Rf = 0.8, which agree with the published papers (Venkatachalam et al. 2017; Iqbal et al. 2019; Tuncsoy et al. 2019; Soni et al. 2021).

Conclusion

The current study showed that the green biosynthesis of Ag NPs can be obtained using the aqueous leaves' extract of *Ziziphus spina-christi.* Phenolic and flavonoid compounds obtained using the HPLC analysis are estimated as Rutin, Naringin, Myricetin, Quercetin, Kaempferol, Hesperidin, Syringeic, Eugenol, Pyrogallol, Gallic and Ferulic. They are important compounds contained in the extracts of the ZSCE. The biosynthesized Ag NPs had been validated by different techniques which confirmed the great stability of the synthesized crystalline Ag NPs with the nanoscale and identified the functional groups present in ZSCE and responsible for reduction. Ziziphus extract and the synthesized Ag NPs showed antifungal ability against *F. oxysporum* at different tested concentrations (1, 0.5, 0.25, 0.125, 0.0625 and 0.031 mM), and it is responsible for defense against pathogens. The results showed that the infected plants that were treated with the tested inducers, whether ZSCE or Ag NPs, recorded minimal infection rate. It is worth noting that Ag NPs gave the lowest injury severity and the highest protection rate, reaching 20.8% and 75%, respectively. In addition, treatment with ZSCE led to a decrease in the severity of the disease and an increase in the rate of recovery by 25% and 69.9%, respectively. The results indicated that the use of the ZSCE or the biosynthesized Ag NPs led to a significant improvement in each of the healthy or infected plants. The highest improvement in shoot and root lengths was observed in the challenged plants, which were treated with the biosynthesized Ag NPs, while number of leaves was higher in the challenged plants, which were treated with ZSCE. A significant improvement was observed in the synthesis of photosynthetic pigments because of treatment with ZSCE or the biosynthesized Ag NPs, whether in healthy or infected plants focusing on the infected plants that were treated with the inducers. Treating plants (whether healthy or infected) with ZSCE or the biosynthesized Ag NPs caused an increase in the plant's content of free proline, and this is clear evidence of the activation of plant immunity. In addition, the use of with ZSCE or the biosynthesized Ag NPs on infected or healthy plants showed a significant increase in the content of phenols and strong expression of the antioxidant enzymes of peroxidase and polyphenol oxidase enzymes. The biosynthesized Ag NPs are safe for various applications in food packaging and processing and in the control of some plant fungal pathogens (*F. oxysporum*), particularly applied for pepper plant treatment after the cultivation and before storage.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

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Informed consent Not applicable.

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