Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4):934-951

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Impact of Silver and Selenium Nanoparticles Synthesized by Gamma Irradiation and Their Physiological Response on Early Blight Disease of Potato

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ABSTRACT

Silver nanoparticles (AgNPs) were prepared by two methods: biologically using Trichoderma viride cell free supernatant and chemically with polyvinyl pyrrolidone (PVP) using gamma irradiation. The antimicrobial activity of AgNPs was evaluated in the field against Alternaria solani which cause early blight disease of potato. Disease severity, vegetative and biochemical parameters of plant were determined. AgNPs were characterized by UV-Vis spectroscopy, FT-IR spectroscopy, Dynamic Light Scattering (DLS) and Transmission electron microscope (TEM). AgNPs had a mean diameter (12.7 nm) using biological method and (23 nm) with chemical method. Application of AgNPs 150 µg/ml + K_2SO_4 (2 %) as plant foliar resulted in highly effective inhibitor of disease severity that recorded (8.39 %) compared to the untreated plants (86.17 %) after 75 days of sowing. Also, a significant increase in growth and physiological parameters was recorded. Accumulation of Ag^o in plant tissues reached minimum value. Selenium nanoparticles (SeNPs) 0.5 µg/ml, ascorbic acid 300 µg/ml and K_2SO_4 (2 %) reduced disease severity (9.81 %) significantly. SeNPs 0.5 µg/ml, AgNPs 150 µg/ml and K_2SO_4 (2 %) showed disease severity (12.63%) but all plant parameters were improved including physiological parameters and yield. It is concluded that the application of AgNPs and SeNPs are recommended as plant foliar for controlling plant pathogen and improving plant yield.

Keywords: Silver and selenium nanoparticles, potato, early blight disease, gamma irradiation.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world. In Egypt, potato has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato [1].This crop is economically important to Egypt and any disturbance in its production affects severely its local and more importantly export impact [2].

Potato plants are subjected to numerous pathogens which cause considerable loss in Egyptian quantitative and qualitative potato yield. The fungal pathogen *Alternaria solani* causes early blight disease [3].

Agricultural production is reduced worldwide every year due to plant diseases; therefore, millions of dollars have been invested in efforts to control these plant diseases.

AgNPs exhibited remarkable antimicrobial activity against both Gram-positive and Gram-negative bacterial strains regardless of their drug-resistant mechanisms and also could be considered as a potential antifungal agent. The bactericidal and fungicidal activity have proved that AgNPs kill microorganisms at such low concentrations, which do not reveal acute toxic effects on human cells, in addition to overcoming resistance and lowering cost when compared to conventional antibiotics [4]. Production of silver nanoparticles through fungi has several advantages. They include easy management in large-scale production of nanoparticles, good dispersion of nanoparticle and much higher amounts of protein expressions. One of the potential applications of silver is to manage plant disease. The antifungal activity of AgNPs is attributed to its effects on the mycelia of the pathogenic fungi [5]. Nanoparticles may improve the growth of plants by enhancing photosynthesis and nitrogen-fixation capability in leaves and roots respectively where, nanoparticles could promote the energy utilization and conversion efficiency [6].

There are three main methods for synthesis of metal nanoparticles: physical, chemical and biological methods. The radiation-induced AgNPs synthesis is a simple and clean which involves radiolysis of aqueous solution that provides an efficient method to reduce metal ions [7]. The irradiation, as a new method, had been extensively used to prepare nano-scale clusters and materials. Radiation induced reduction synthesis of AgNPs which offers some advantages over the conventional methods, because of its simplicity. It provides metal nanoparticles in fully reduced, highly pure and highly stable state [8].

Polyvinylpyrrolidone (PVP) was used as an excellent dispersant in the preparation of silver nanoparticles by chemical reduction method. PVP inhibits aggregation and stabilizes the colloidal silver even when salt is added [9].
Selenium (Se) is an essential nutrient for humans and animals to form important selenoproteins, including glutathione peroxide, thioredoxin reductase [10]. The content of Se in plants can be increased in different ways; by addition of Se to soil, soaking seeds in Se solution before sowing, hydroponic and aeroponic cultivation in a nutrient solution containing Se, and foliar application of plants with Se solution [11].

Agricultural chemical
s used to prevent microbial pathogens are poisonous to animals and other microorganisms and also are known to be harmful to humans. So, the main aim of this study is to test the effect of silver and selenium nanoparticles as antimicrobial agents to control early blight disease of potato to be applied widely and safely.

EXPERIMENTAL SECTION

2.1 Biosynthesis of silver nanoparticles by fungal supernatant

Seven fungi, *Trichoderma viride, Penicillium citrinum, Penicillium brevicompactum, Alternaria alternata, Aspergillus niger, Aspergillus oryzea* and *Fusarium oxysporum* were used to synthesize silver nanoparticles. Fungi were inoculated in sterile medium (GYP) broth containing, yeast extract 0.3%, glucose 1%, peptone 0.5%, at 25 ºC, in shaking condition (180 rpm). After 72 h of incubation, the cultures were centrifuged at 5000 rpm for 10 min and the supernatants were used in synthesis of silver nanoparticles. Aqueous solution of 1 mM $AgNO_3$ (1:1 v/v) was mixed with fungal supernatant and the mixture was put into a shaker at 32 °C (150 rpm). Control (without silver ions) was also run along with the experimental flasks [8 ,12].

The process of irradiation was carried out at the National Centre for Radiation Research and Technology (NCRRT). The facility used was ^{60}Co-Gamma chamber 4000-A-India. Irradiation was performed at a dose rate 2.9 kGy/ hr at the time of the experiment. Aqueous solution of $AgNO_3$ (5 ml) (1mM $AgNO_3$ of final concentration) was mixed with fungal supernatant (5 ml) then irradiated at different gamma radiation doses 0.0, 0.5, 1.0,1.5, 2.0, 2.5, 3.0, 5.0, 10, 15, 20, 25 and 30 kGy [8].

2.3 Chemical synthesis of silver nanoparticles

$AgNO_3$ (1 mM) and PVP solutions were used for synthesis of silver nanoparticles. 10 mL of 2% PVP solution was mixed with 90 ml of 1 mM $AgNO_3$ solution. The mixtures were exposed to gamma radiation at doses 0, 5, 10, 15, 20, 25 and 30 kGy at room temperature. After irradiation the produced AgNPs were characterized.

2.4 Effect of silver nitrate concentrations

Various concentrations, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 and 1100 ppm of silver nitrate were used as substrate. The absorbance measured by UV-Visible spectrophotometer (T60 UV/Vis spectrometer).

2.5 Characterization of silver nanoparticles

Formation of AgNPs was monitored by using UV-Visible Spectroscopy (T60 UV/Vis spectrometer). To detect silver nanoparticles the absorption range is 400 to 450 nm [7]. FTIR measurements were carried out in order to obtain information about chemical groups present in fungal supernatant as control and AgNPs sample. Average particle size and size distribution were determined by the dynamic light scattering technique (DLS) (PSS-NICOMP 380-ZLS, USA). Sample for transmission electron microscopy (TEM) shows the size and morphology of AgNPs.

2.6 Field experiment

Field experiments were carried out at Tanta town, Gharbia governorate during winter 2014 growing seasons to evaluate the efficacy of silver nanoparticles application on severity of early blight disease of potato plants, Accent variety that supplied from El- Gemmeza Agricultural Research Station (El – Gharbia Governorate- Egypt) under natural field conditions. The fungicide Kocide® (Copper (II) hydroxide, 53.8%) was used in this study as a positive control at the recommended dose (0.9 g/l that represented as 315 ppm of copper metal) and 1.8 g/l that represented as (631 ppm of copper metal). Silver nanoparticles were used at concentration of 150 ppm. Dipotassium sulphate (K_2SO_4, 99%) that obtained from El-Gomhoraia Company for Chemicals and Pharmaceuticals was used at concentrations (1 and 2 %). All treatments were applied as foliar spray three times with 10 days interval. Field experiments consist of plots 10 x 7 m each comprising of 23 rows and 10 holes /row. All plots received traditional agricultural practices such as irrigation. The average of records of the surveyed replicates (plots) for each particular treatment was calculated. Disease severity of early blight was estimated using the disease scale from 0 to 4 suggested by [13] as follows:

Zero = no leaf lesion;
1= lesions on < 25% of leaf area;
2 = lesion no 26–50% of leaf area;
3 = lesion no 51–75% of leaf area
4 = lesions no 76 up to 100% of leaf area.
Disease severity was calculated according to the equation [14]:
Disease severity = Σ (No. of infected plants × No. Scale)/Total No. of plants × highness No. scale× 100.

2.6.1 Effect of infection by *A. solani* on growth parameters and yield of potato plants

Random samples of five plants were taken from every plot at 75 days after planting. Plant height, leaves number/ plant, fresh weight, dry weight, leaf weight, number of main stems of treatments were determined. At harvest time, 90 days after planting, the average harvested yield was calculated for all applied treatments as number of tubers/ plant number of tubers/ line and weight of tubers/ plant at the end of the growing season.

2.6.2 Effect of the different treatments on chlorophyll content of potato plant leaves

Chlorophyll content in potato plants leaves after 60 days after planting was determined according to [15]. A known area (two disks, each 1 cm^2) from the fourth fresh leaf from the top was taken and extracted by 5 ml of N, N-dimethyl formamide (DMF), in dark bottles for 24 hours. The intensity of the colour was measured at λ = 647 and 664 nm wave length using spectrophotometer.

Chlorophyll content was calculated using the following equations by [16].
Chl. a = $12.46\ A_{664} - 2.49\ A_{647}$ µg/ ml.
Chl. b = $-5.6\ A_{664} + 23.26\ A_{647}$ µg/ ml.
Total chl. = $7.04\ A_{664} + 20.27\ A_{647}$ µg/ ml.
A_{664} is the absorption at λ = 664 nm.
A_{647} is the absorption at λ = 647 nm.

Then chlorophyll was calculated as assigned to the area of the leaf.

2.6.3 Specific-gravity (SG) determination

In determining the SG, healthy and marketable size grade tubers were selected randomly from the central rows of two of the six replications. Then, tubers were cleaned and weighed in both air and water by the method of [17]. Finally, the SG value was computed using the following equation:

SG = Weight of tuber in air / (Weight of tuber in air – Weight of tuber in water).

Moreover, to convert tuber specific gravity (SG) to dry matter content and starch content percentage equivalents as follow: The equation from [18] of starch (%) = 17.565 + 199.07 × (specific gravity – 1.0988) was used to convert the SG value to starch content.

The equation from [19] of dry matter (%) = -214.9206 + 218.1852 × (specific gravity) was used to convert the SG value to dry matter content.

2.7 Biochemical analysis

2.7.1 Preparation of potato sample supernatant

3 ml of 50 mM sodium phosphate buffer pH-7 (extraction buffer) in an ice cold mortar and pestle was used. The extract was centrifuged at 10000 rpm for 10 min. The supernatant was kept as such at refrigerated condition until further use and the same was employed for the measurement of all biochemical parameters studied [20].

2.7.2 Protein content assay of potato plants and tubers [21]

To an aliquot (50µl) of the supernatant diluted to 1 ml with extraction buffer. 5 ml of Coomassie brilliant blue (CBB) G-250 was added and mixed thoroughly. The absorbance was read at 595 nm in (T60 UV/Vis spectrometer) against a reagent blank. The amount of protein was calculated using standard prepared with different concentrations of bovine serum albumin (BSA) ranging from 10 µg/ml to 100 µg/ml as (mg/g dry weight of potato plants and tubers).

2.7.3 Reducing sugars content estimation of potato plants and tubers

The reducing sugars content of plant green parts and tubers extracts were determined by the dinitro salicylic acid (DNSA) method [22]. A calibration plot was drawn using a standard glucose solution in the range of 0–1.0 g L^{-1}. For the estimation of reducing sugars, 200µL of the supernatant was added to 800µL of distilled water in a test tube and hydrolyzed with 172µL of 12 N HCl at 68° C for 8 min. The hydrolyzed sample after neutralization with 20% NaOH, was used for the determination of reducing sugars. Optical density was measured spectrophotometrically (T60 UV/Vis spectrometer) at 540 nm. The reducing sugars were calculated from the standard plot of glucose as (mg/g dry weight of potato plants and tubers).

2.7.4 Catalase (CAT) assay of potato plants and tubers

CAT activity in plant green parts and tubers extracts was measured according to Chandlee and Scandalios with slight modifications [23]. The reaction mixture containing 2.5 ml of 100 mM sodium phosphate buffer (pH 7.0), 0.1 ml of 10 mM H_2O_2 and 0.2 ml of enzyme extract was used to assay CAT activity. The assay mixture without enzyme extract served as control. Optical density was measured at 230 nm up to 3 min at every 1 min time interval by taking the first reading after the addition of H_2O_2 (at 1 min).

The enzyme activity was expressed in U/ml (U = 1 mM of H_2O_2 reduction per min per mg of protein). Activity can be calculated by using the following formula [24]:

Volume activity (U/ml) = ΔA ×34.4 ×df

Where, Δ A / min is the change in absorbency per 1 min.
df: Dilution factor

2.7.5 Polyphenol oxidase (PPO) of potato plants and tubers

PPO enzyme activity was determined in plant green parts and tubers extracts with a spectrophotometer by measuring the initial rate of quinone formation as indicated by an increase in absorbance at 410 nm at 1 minute time intervals at 30°C by using catechol as substrate. The activity of PPO was determined by reaction mixture which contained of 0.1 ml freshly prepared crude enzyme extract, 3.9 ml of 100 mM phosphate buffer (pH 7.0) and 1.0 ml of 50 mM catechol. PPO activity was assayed in triplicate and the results expressed as means. One unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 0.001 $minute^{-1}$ under the conditions of the assay [25].

The following equation was used to calculate polyphenol oxidase activity [26].
Activity (U/ml) = $[(AF_{sample} – AI_{sample}) – (AF_{blank} – AI_{blank})]/ (0.001 \times t)$.

Where: AF_{sample} is the final absorption of the sample, AI_{sample} is the initial absorption of the sample, $AF_{blank}$is the final absorption of the blank, AI_{blank} is the initial absorption of the blank, t is the reaction time in minutes.

2.7.6 Determination of antioxidant activity of potato plants and tubers extracts
The assay was conducted following the procedure of [27].

1ml of potato extract (plant green parts/tubers) was added to 1 ml of 1mmol DPPH (2, 2-diphenyl-2- picrylhydrazyl) radical ethanolic solution. Absorbance of the samples was measured at λ= 517 nm. The readings were compared to the control sample consisting of ethanol 95 % instead of extract. The antioxidant activity is expressed as percent scavenging activity and calculated using the following equation:

Antioxidant activity %= [(Abs of control- Abs of sample)/ Abs of control] x 100.

2.7.7 Silver and copper metals concentrations in different plant tissues by atomic absorption spectroscopy
Plant samples were digested in concentrated nitric acid analytical grade and H_2O_2 at 5:1 ratio by using Micriwave Labstation Digestor, Milston, MLS 1200 Mega, Italy. Trace elements (Ag and Cu metals) were estimated by using Atomic Absorption (Thermo Scientific ICE 3000, serious AA Spectroscopy), England. For each elements stock solution was prepared (1000 ppm) and was diluted for serial standards for calibration curve. All solutions were of analytical grade obtained from Ultra-Pure Water Station ELGA, England with high purity and resistivity 18 Ω cm^{-1} [28].

2.8.1 Preparation of selenium nanoparticles
Selenious acid (0.04 mM) under stirring was mixed with glutathione (GSH) concentration as (0.2 mM) and 200 mg bovine albumin solution in 100 ml deionized water. The pH of the mixture was adjusted to 7.2 with 1.0 M sodium hydroxide to initiate the reaction. The reaction lasted one hour under sonication condition, during which the red elemental Se and oxidized glutathione (GSSG) formed. The red solution was dialyzed against doubly distilled water for 96 h with the water changing every 24 h to separate GSSG from Nano-Se [29, 30].

2.8.2 Effect of selenium nanoparticles on disease severity, growth and yield of potato
Selenium nanoparticles (SeNPs) at concentration 0.5 ppm and ascorbic acid at concentration 300 ppm and/or AgNPs and K_2SO_4 were applied. Disease severity and all vegetative and biochemical assays as mentioned before were carried out to potato samples.

3. Statistical analysis
All the previous results were statistically analyzed using the least significant difference test (LSD) at $P<0.05$ according to Finney [31]. The data analyzed by SPSS software ver. 15.

RESULTS AND DISCUSSION

3.1. Screening of different fungi for synthesis of Ag nanoparticles
There was a simple biological way for synthesizing the silver nanoparticles extracellulary using a supernatant of seven different fungal species. These fungi are, *Trichoderma viride, Penicillium citrinum, Penicillium brevicompactum, Alternaria alternata, Aspergillus niger, Aspergillus oryzea* and *Fusarium oxysporum.* The synthesis of nanoparticles were formed within 24 hours when silver ions were mixed with fungal supernatant and showed yellowish brown colour solution as a response of surface Plasmon resonance (SPR) with a peak at 425 nm that clearly indicates the formation of silver nanoparticles [32]. *Trichoderma viride* cultural supernatant was found to exhibit a strong potential for rapid reduction of silver ions as shown in Figure1.The strength of colour was according to the ability of fungal species to synthesize silver nanoparticles. Changing colour was due to excitation of surface Plasmon vibrations, a characteristic property of the metallic nature of the nanoparticles, which indicated formation of silver nanoparticles. The formation and stability of silver nanoparticles in colloidal solution was monitored by using UV-Vis spectral analysis.

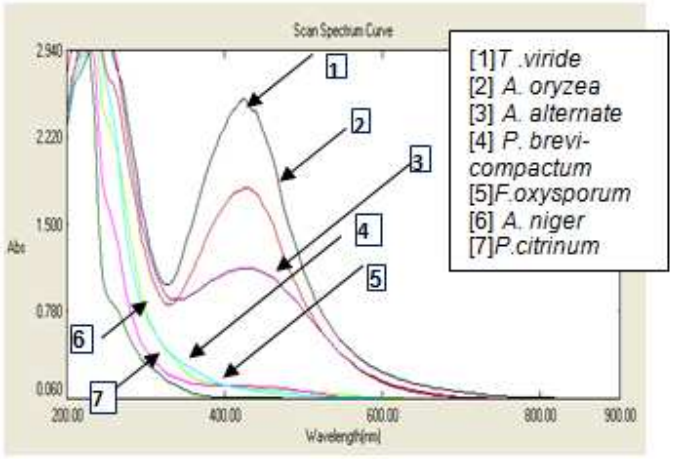


Figure1. UV-Vis spectra of AgNPs synthesized by fungal supernatants

The narrow peak indicates that nanoparticles were well dispersed in the solution without aggregation. The peak absorbance of 280 nm arises due to electronic excitations in tyrosine and tryptophan residues of the protein founded in the supernatant that are act as capping agents of AgNPs [33].

3.2 Synthesis of Ag nanoparticles using gamma irradiation

A method using gamma radiation provides more convenient and a cleaner approach. $AgNO_3$ separated to Ag^+ and NO^{-3} ions in the aqueous solution as shown in Equation 2. Gamma radiolysis of aqueous solution yields several products including hydrated electrons and hydrogen atoms ($H^\bullet$) having the powerful reducing ability that reduce Ag^+ into Ag^0 [34].

$$H_2O \longrightarrow e^-_{aq}, H_3O+, H_2, H^\bullet, OH^\bullet, H_2O_2 \quad (1)$$
$$AgNO_3 \longrightarrow Ag^+ + NO_3^- \quad (2)$$
$$Ag^+ + e^-_{aq} \longrightarrow Ag^0 \quad (3)$$
$$Ag^+ + H^\bullet \longrightarrow Ag^0 + H^+ \quad (4)$$

The growth of silver nanoparticles by reduction of Ag^+ to Ag is stepwise. These neutral Ag^0 atoms at first dimerize when they encounter or associate with the excess Ag^+ ions trapped in the individual loops of cell free supernatant. The charged dimer clusters Ag^{2+} may further react with excess silver cations by a -cascade of coalescence processes to form trimer, tetramer and higher order silver ion clusters (Ag^+_{n+1}) and also the doubly charged $3Ag^{2+}$, $4Ag^{2+}$, etc. The aggregation of these clusters into higher metallic clusters and nanoparticles occurs as the nucleation in the solution increases. The competition between the reduction of free silver ions and absorbed ones is controlled by the rate of reducing radical formation [35]. Silver atoms formed by the irradiation tended to coalesce into oligomers (Equation 5), which progressively grew into large clusters (Equation 6). The aqueous electrons reacted with the Ag^+ clusters to form the relatively stabilized Ag clusters (Equation 7) as illustrated in [8].

$$Ag^0 + Ag^+ \longrightarrow Ag^+_2 \quad (5)$$
$$nAg^+ + Ag^+_2 \longrightarrow (Ag^+)_n \quad (6)$$
$$(Ag^+)_n + n\, e^-_{aq} \longrightarrow (Ag^0)_n \quad (7)$$

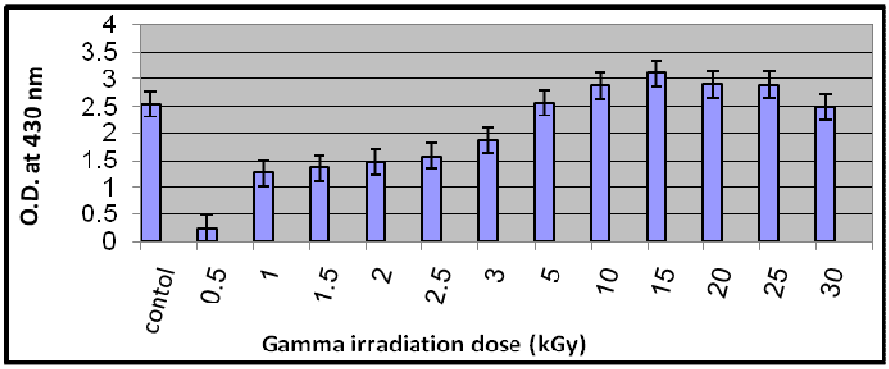


Figure 2. Optical density of AgNPs after gamma irradiation at different doses

Figure 2 shows that, after gamma irradiation of mixture of *T. viridie* supernatant and $AgNO_3$ from 0.5 up to 30 kGy compared with unirradiated control. For all doses, the characteristic surface Plasmon resonance peak for silver nanoparticles appear, that is indication of AgNPs formation while, the maximum AgNPs production was at 15kGy. On the other hand, over than 15 kGy the production of AgNPs was decreased.

3.3 Effect of different silver nitrate concentrations on silver nanoparticles synthesis

Different concentrations of silver nitrate in the reaction mixture were used in order to evaluate the maximum concentration of the substrate for nanoparticles production after gamma irradiation at 15 kGy as shown in table 1. By gradual increase in concentration of $AgNO_3$ up to 1000 ppm, the nanoparticles production was increased. However, by further increasing of $AgNO_3$ concentration the production of AgNPs decreased.

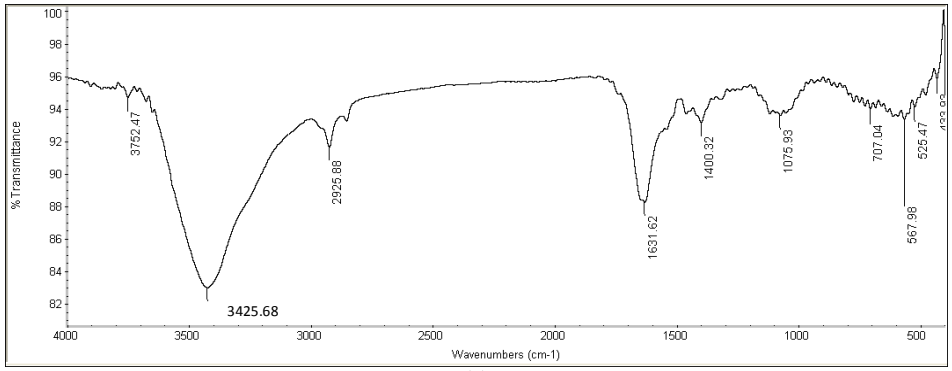
Table (1). Effect of silver nitrate concentration on silver nanoparticles synthesis by gamma irradiated *T. viride* (15 kGy)

$AgNO_3$ conc. (ppm)	Optical density at 430 nm
50	0.876
100	1.912
200	3.542
300	4.154
400	4.726
500	5.234
600	5.362
700	5.381
800	5.537
900	5.595
1000	5.601
1100	4.492

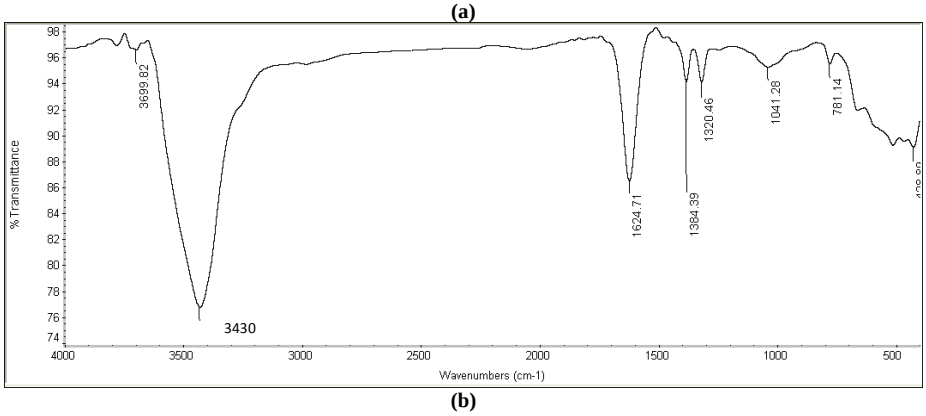
3.4 Characterization of silver nanoparticles synthesized by gamma irradiated *T. viride* supernatant

3.4.1 Fourier Transmission Infra-Red (FT-IR)

Fourier Transform Infrared spectroscopy (FT-IR) measurements are carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and protein molecules that act as capping agent. Figure 3. shows the FT-IR spectrum of 15 kGy gamma irradiated *T. viride* supernatant (control) and silver nanoparticles. It was observed that the N–H stretching band at 3425.6 cm^{-1} of blank was shifted to 3430 cm^{-1} in AgNPs. This suggests that the formation of AgNPs was promoted by N-H bond [36]**.** The frequency of the C=O stretching vibration in *T. viride* supernatant shifted from 1631.62 cm^{-1} to 1624.71 cm^{-1} in AgNPs. The decreasing frequency of the C–O stretching vibration implies that *T. viride* supernatant molecules use the oxygen atom in carbonyl to coordinate with the metal ion in the AgNPs solution [7]. In AgNPs FT-IR spectrum indicate formation of a new peak at 781.14 cm^{-1} which may be assigned to one of the vibration modes of Ag–O [37]. In the case of silver nanoparticles, the disappearance of a peaks (2925.88 cm^{-1}) and appearance of a peak (1320 cm^{-1}) may be attributed to the contribution towards the stabilization of the AgNPs by OH bond deformation by silver metal surface [35].

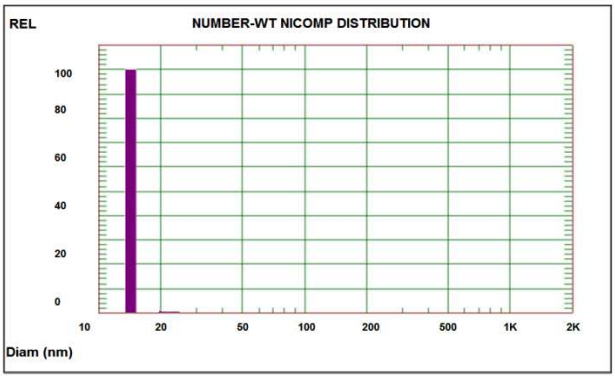


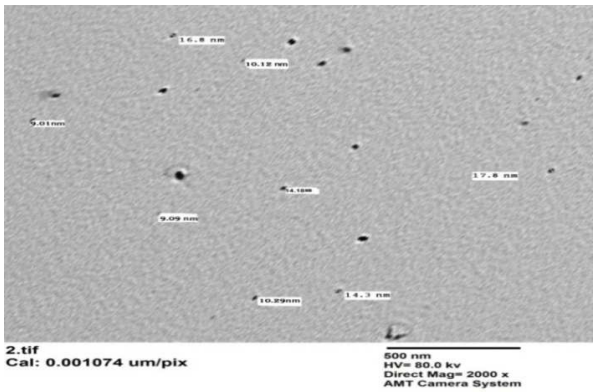
(a)



(b)

Figure 3. (a):FT-IR spectrum of *T. viride* supernatant.(b):FT-IR spectrum of AgNPs *T. viride* supernatant





a) b)

Figure 4. a) DLS (mean diameter = 14.5 nm, Num = 100 %)
b) TEM graphs (average diameter of 12.7 nm) of AgNPs synthesized by *T. viride* cell free supernatant

3.4.2 Dynamic Light Scattering (DLS) and Transmission electron microscope (TEM) graphs of AgNPs

Average particle size was determined by DLS. Figure 4 represents graph of AgNPs solution exhibit a very narrow size distribution with small particles size. The distribution of the hydrodynamic diameter of the nanoparticles show peak (approximately 100 % of the particle diameter) had its maximum diameter at 14.5 nm. TEM image of AgNPs with spherical shape and the average diameter of 12.7 nm. The nanoparticles were monodispersed without aggregation indicating stabilization of the nanoparticles by a protein capping agent [7].

3.5 Chemical synthesis of silver nanoparticles using gamma irradiation

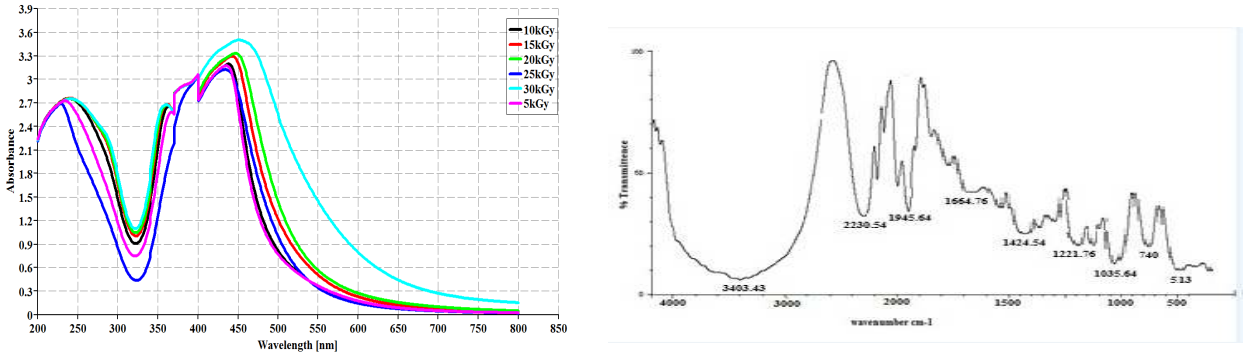
After mixing of PVP solution and $AgNO_3$(1mM) were exposed to gamma irradiation at doses 0, 5, 10, 15, 20, 25 and 30 kGy deep brown colour appeared indicating the formation of polyvinylpyrrolidone coated silver nanoparticles (PVP-AgNPs).

3.6 Characterization of silver nanoparticles synthesized chemically

3.6.1. Effect of different doses of gamma irradiation on synthesis of PVP-AgNPs (UV-vis) and FT-IR spectroscopy

In case of PVP-AgNPs, as the irradiation dose increases, the surface Plasmon resonance (SPR) band red shifted which mean an increase in particles size. At gamma irradiation 30 kGy, a characteristic SPR band for PVP-AgNPs was obtained at 450 nm (Figure 5a).

FT-IR spectrum of PVP-AgNPs was used in the range of 500- 4000 cm^{-1}. As shown in (Figure 5b) FT-IR results revealed that bands on this spectrum are centered at 1424.54 cm^{-1} which results from vibration of the tertiary nitrogen, and at 3403.43 cm^{-1} assigned to environmental (-OH). Also, the peak at 1190 cm^{-1} represents the functional (-C-N) present in PVP [38], shifts to 1221.75 cm^{-1} after embedding of silver nanoparticles. The peak shifting corresponding to (-C-N) bond towards higher wavenumber may be attributed to chemical coordination of AgNPs with (-C-N) bond .On the other hand, the peaks at the range of 1630 up to 1660 cm^{-1} in pure PVP due to (-C=O) bonds [39], shift to 1664.76 cm^{-1} after the formation of AgNPs within PVP polymer. Such a change may occur due to the bond weakening as a result the partial bond formation with the surface of silver atoms which eventually passive the surface of AgNPs. That result confirms bonding between AgNPs with polyvinylpyrrolidone structure. Thus polyvinylpyrrolidone can be used as effective stabilizer because the nitrogen atom of PVP interacts with silver and forms a protective shell [38].

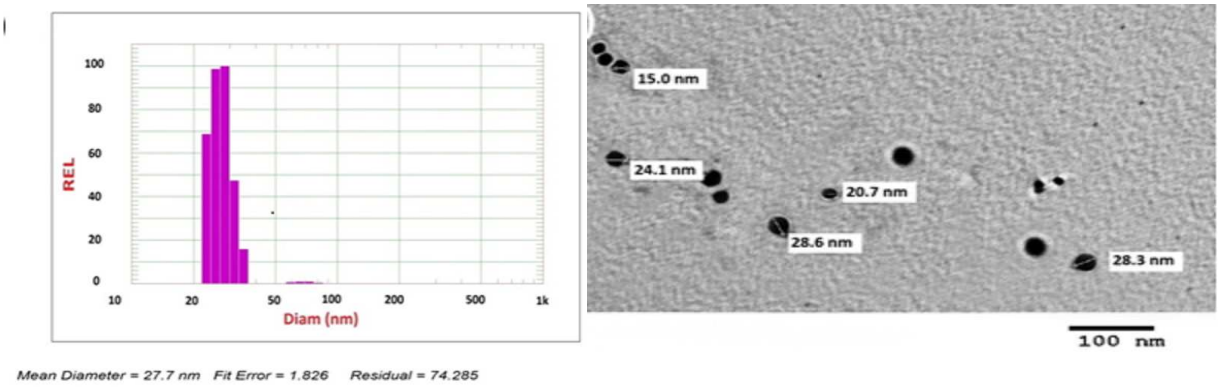


(a) **(b)**

Figure 5. (a) Effect of different doses of gamma irradiation on synthesis of PVP-AgNPs at 30 kGy
(b) FT-IR spectrum of gamma irradiated PVP-AgNPs at 30 kGy

3.6.2 Dynamic light scattering (DLS) and TEM analysis

DLS indicates for particles size with mean diameter of 27.7 nm for PVP-AgNPs at 30 kGy of gamma irradiation dose.TEM micrograph of PVP-AgNPs showed the particles appear to be spherical and remain fairly dispersed with diameter of 23 nm as presented in Figure 6.



a) **b)**

Figure 6. a) Particle size distribution of PVP-AgNPs by DLS, b) TEM image of PVP-AgNPs

It was clear that, particle size AgNPs synthesized biologically by gamma irradiated of *T. viride* supernatant is smaller than the particle size of PVP-AgNPs. So, we used the smaller nanoparticles to be applied in the field.

3.7 Field experiment

3.7.1 Effect of treatments on early blight disease severity and vegetative growth of potato plants

Eighteen combinations of treatments recorded, disease severity ranging from (8.39 %) to (28.33 %) as compared with control (86.17 %) after 75 days of sowing as shown in table (2). In addition, a combination between AgNPs 150 ppm and K_2SO_4 (2%) exhibited the lowest disease severity compared with other combinations to give (8.39 %) as shown in table (2).

The application of silver nanoparticles 150 ppm significantly limited the development of early blight disease on leaves of potato compared with control and other treatments which do not containing silver nanoparticles (like fungicides and K_2SO_4 individually or in combination with each other).

It is worthy to mention that, 100 ppm concentration of silver nanoparticles was used as foliar application also, but it gave less effects in disease severity, vegetative and biochemical parameters of plant as compared with 150 ppm concentration of AgNPs. So, application of AgNPs at 150 ppm was more favourable and was used in all field experiments.

Similar results were observed by Jung *et al* [40] in the field when green onion plants were treated with different concentrations of nano-silver (10, 25, 50, and 100 ppm). The effective inhibition to control *Sclerotium cepivorum* was observed from the dry weight of the onion. Same trend was found by El-Batal *et al* [41] who found that, the foliar application of gum acacia-AgNPs and $AgNO_3$ at 0.0, 5, 10, 20 and 60 ppm significantly increased plant height, root length, number of leaves/plant, the leaves' area, total fresh and dry weights/plant and yield of two varieties (Bronco and Nebraska) of the common bean (*Phaseolus vulgaris* L.).

Data in table (2) revealed that, the highest value of plant height, leaves number/ plant, fresh weight and dry weight of the potato plants were obtained with the treatment of nano silver 150 ppm and K_2SO_4 2% to give (57.3 cm , 17 leaves , 84.82 g and 9.21 g, respectively). Therefore, combination of AgNPs with K_2SO_4 was best result. So, we can avoid using very harm fungicide treatment. In contrast, the number of stems did not affect by silver nanoparticles treatment. So, there is no significant difference between treatments in number of stems/plant. Basiri *et al* [42] recognized that, adding silver nanoparticles increased water uptake in carnation (*Dianthus caryophyllus*) more than the control. Uptake rate depends on the size and the surface properties of nanoparticles.

Nanoparticles could promote the energy utilization and conversion efficiency [43]. Also, application of potassium increases plant height, crop vigour and impart resistance against diseases.

Table 2.Effect of different treatments on potato plant growth in the field

Treatments	PlH (cm)	LN/p	DS%	Eff %	NS	FW (g)	DW (g)
Control (untreated)	27^{a}	8^{a}	86.17^{a}	0.00^{a}	2^{a}	20.93^{a}	2.24^{a}
Koc. 631 ppm	39^{c}	10^{c}	22.27^{d}	74.15^{e}	4^{a}	45^{d}	4.84^{bc}
Koc. 315 ppm	37.5^{b}	9^{b}	28.19^{d}	69.51^{c}	4^{a}	42.37^{c}	$4.^{56b}$
AgN 150 ppm	44^{e}	12^{f}	13.94^{b}	83.85^{fg}	4^{a}	74.15^{l}	8.10^{e}
K_2SO_4(2 %)	43.6^{de}	13^{g}	24.97^{d}	71.02^{d}	4^{b}	55.09^{g}	5.73^{c}
K_2SO_4 (1 %)	42^{d}	12^{f}	27.04^{d}	68.62^{bc}	3^{c}	59.77^{l}	6.54^{d}
Koc. 315ppm+AgN150ppm	47.3^{gh}	14^{h}	11.60^{b}	86.54^{hi}	5^{c}	75.65^{l}	7.89^{e}
Koc. 631 ppm+AgN 150 ppm	48.7^{gh}	13^{g}	14.9^{b}	82.70^{f}	5^{a}	50.83^{f}	5.43^{c}
Ko315ppm+AgN 150ppm+ K(1%)	51^{j}	12^{f}	13.12^{e}	84.77^{gh}	4^{b}	84.61^{o}	9.14^{g}
Koc. 631ppm+AgN 150ppm+K(1%)	47^{g}	12^{f}	10.91^{b}	87.34^{i}	3^{c}	47^{e}	4.94^{b}
Koc.315ppm+AgN150ppm+K(2%)	49.6^{i}	13^{g}	15.15^{b}	82.41^{f}	4^{c}	57.01^{h}	5.83^{c}
Koc.631ppm+AgN150ppm+K(2 %)	53.3^{k}	11^{e}	12.01^{b}	86.06^{hi}	4^{a}	68.14^{k}	6.78^{d}
Koc.315 ppm+K(1%)	48.3^{hi}	12^{f}	23.07^{d}	73.23^{e}	3^{b}	81.90^{n}	8.78^{fg}
Koc.631 ppm+K(1%)	45.3^{f}	10^{c}	28.33^{d}	67.12^{b}	5^{a}	78.57^{m}	8.24^{e}
Koc.315 ppm+K(2 %)	54.3^{l}	11^{d}	21.84^{c}	74.65^{e}	3^{a}	60.67^{j}	6.73^{d}
Koc.631 ppm+K(2 %)	43.7^{de}	11^{e}	24.67^{d}	71.37^{d}	4^{b}	41.25^{b}	4.45^{b}
AgN150 ppm+K(1 %)	48.8^{i}	13^{g}	9.11^{b}	89.42^{j}	3^{c}	84.25^{o}	9.04^{g}
AgN150 ppm+K (2 %)	57.3^{m}	17^{i}	8.39^{b}	90.26^{j}	5^{b}	84.82^{o}	9.21^{g}
LSD	0.75	0.85	1.07	1.60	0.6	1.06	0.89

** Koc.: Kocide, AgN: silver nanoparticles, K: K_2SO_4, PlH: Plant height, LN/p: leaves number/plant, DS%: disease severity*
FW: Fresh wt./ plant (gm), DW: Dry weight/ plant (gm), NS: number of main stems, Eff %: Efficiency, LSD: Least significant difference. Mean values were followed by the same letters column are not significantly different (Duncan's multiple range test)

3.7.2 Effect of different treatments on chlorophyll content, potato tubers yield and quality of tubers in the field

In the same accordance, Kocide® 315 ppm + AgNPs 150 ppm + K_2SO_4 (1 %) gave the best chlorophyll a, b and total chlorophyll (the values of 0.1002, 0.0238 and 0.1243 mg/ cm^2, respectively) followed by AgNPs 150 ppm + K_2SO_4 (2%) that recorded (0.0895, 0.0195 and 0.1094 mg/ cm^2, respectively) to be the best treatments in between control and other treatment, table (3). Enhanced photosynthetic pigment contents were recorded in leaves of *Pelargonium zonale* treated with silver nanoparticles when compared to the control [44].

Data in table (3) showed that, all treatments raise potato yield, but AgNPs 150 ppm + K_2SO_4 2% followed by AgNPs 150 ppm + K_2SO_4 1% recorded the highest number of tubers/plant, number of tubers/ line and weight of tubers/ plant. Similar results were observed by Tahmasbi *et al* [45] who showed that, Nitroxin fertilizer + 50 mg/L nanosilver gave best effects compared to control. Also, Trehan *et al* [46] recorded that, potassium increases the size of tubers. So, potassium developed the yield by increasing the number and yield of large sized tubers.

Specific gravity was influenced by different treatments. The highest SG, starch content (%) and dry matter (%) values were obtained from AgNPs 150 ppm + K_2SO_4 2 % (1.113 g/cm^3, 28.66 % and 20.40 %, respectively) followed by AgNPs 150 ppm + K_2SO_4 1% which recorded (1.102 g/cm^3, 25.52 % and 18.20 %, respectively) as shown in table (3). But, we noted that the combination of AgNPs and K_2SO_4 gave the highest improving effect that means AgNPs improve the enhancing effect of K_2SO_4 in a significant way. So, we can indicate that AgNPs and K_2SO_4 had a positive effect in increasing potato quality. So that, high specific gravity values were resulted from stimulation of photosynthetic efficiency of growing crop and starch deposition in the tuber [47].

3.7.3 Effect of treatments on reducing sugar content, protein content, enzymes and absorbed Cu and Ag of potato plants and tubers in field

As shown in table (4) silver nanoparticles 150 ppm in combination with K_2SO_4 (2%) significantly decreased reducing sugar contents in tubers which recorded as (23.29 mg/g dry weight) comparing with untreated control and other combinations. The processing industry requires potatoes with well-defined tuber characteristics of suitable consumer preference and processing qualities. Low content of reducing sugars is preferred as they result in light colours of desirable quality [48].

Data in table (4) showed that, in potato green parts, AgNPs 150 ppm + K_2SO_4 1% gave the highest protein content (31.83 mg/g dry weight) followed by AgNPs 150 ppm + K_2SO_4 2 % (28.23 mg/g DW). While, in potato tuber, AgNPs 150 ppm + K_2SO_4 2 % recorded the highest protein content (38.18 mg/g dry wt.). From the results it was clear that, application of AgNPs 150 ppm + K_2SO_4 1 % and 2 % improve the protein content of potato. Although potato is not considered a main source of proteins for human nutrition, its protein is of high quality and its biological value compares with that of whole eggs [49]. Silver nanoparticles with their antimicrobial and hormone-like

characteristics increased total protein content [50]. Also, Salama [51] revealed that, application of silver nanoparticles at 60 ppm caused an increase in protein content of *Phaseolus vulgaris* and *Zea mays*.

Table 3.Effect of different treatments on chlorophyll content, potato tubers yield and quality of tubers in the field

Treatments	Ch.a	Ch.b	Tot.ch	NTP	NTL	WTP	SG	DM	St
Control (Untreated)	0.0448^{a}	0.0090^{a}	0.0548^{a}	4^{a}	96^{a}	115.4^{a}	1.031^{a}	10.03^{a}	4.07^{a}
Koc. 631 ppm	0.0647^{b}	0.0163^{bc}	0.0810^{b}	4^{a}	100^{b}	172.9^{b}	1.056^{b}	15.48^{b}	9.04^{b}
Koc. 315 ppm	0.0721^{a}	0.0166^{bc}	0.0887^{b}	4^{a}	96^{a}	210.65^{d}	1.058^{b}	15.92^{b}	9.44^{b}
AgN 150 ppm	0.0852^{a}	0.0183^{c}	0.1035^{ef}	6^{c}	144^{f}	270.8^{g}	1.079^{ef}	20.50^{g}	13.62^{g}
K(2 %)	0.0883^{a}	0.0192^{c}	0.1077^{c}	5^{b}	120^{d}	296.15^{h}	1.078^{ef}	20.28^{g}	13.42^{g}
K (1 %)	0.0602^{a}	0.0236^{c}	0.0841^{d}	5^{b}	120^{d}	257.6^{f}	1.062^{c}	16.79^{c}	10.24^{c}
Koc.315ppm+AgN150ppm	0.0693^{a}	0.023^{c}	0.0940^{e}	7^{d}	168^{g}	353.85^{k}	1.093^{h}	23.56^{j}	16.41^{j}
Koc.631ppm+AgN150ppm	0.0730^{a}	0.0185^{ab}	0.0915^{c}	4^{a}	115^{c}	196.3^{c}	1.067^{cd}	17.88^{d}	11.23^{d}
Koc.315ppm+AgN150ppm+ K(1%)	0.1002^{a}	0.0238^{c}	0.1243^{g}	6^{c}	144^{e}	310.65^{i}	1.096^{h}	24.21^{k}	17.01^{k}
Koc.631ppm+AgN150ppm+K (1 %)	0.0523^{a}	0.0163^{c}	0.0687^{bc}	7^{d}	168^{g}	320.25^{j}	1.077^{ef}	20.06^{g}	13.23^{g}
Koc.315ppm+AgN150ppm+K (2%)	0.0814^{a}	0.0203^{c}	0.1019^{c}	6^{c}	144^{e}	268.05^{g}	1.087^{g}	22.25^{i}	15.22^{i}
Koc.631ppm+AgN150ppm+K (2 %)	0.0793^{a}	0.0217^{c}	0.1010^{d}	6^{c}	146^{f}	250.5^{f}	1.095^{h}	24.01^{k}	16.81^{jk}
Koc.315 ppm+ K (1%)	0.0791^{a}	0.0190^{c}	0.0981^{f}	8^{e}	192^{h}	355.7^{k}	1.075^{e}	19.63^{f}	12.83^{h}
Koc.631 ppm+ K (1%)	0.0660^{a}	0.0197^{bc}	0.0857^{ef}	5^{b}	120^{d}	208.35^{d}	1.064^{c}	17.23^{c}	10.64^{e}
Koc.315 ppm+ K (2%)	0.0696^{a}	0.0192^{c}	0.0889^{d}	10^{f}	240^{i}	365.45^{l}	1.083^{fg}	21.37^{h}	14.42^{l}
Koc.631 ppm+ K (2%)	0.0742^{a}	0.0195^{c}	0.0937^{b}	8^{e}	192^{j}	244.9^{e}	1.071^{de}	18.76^{e}	12.03^{m}
AgN150 ppm+ K (1 %)	0.0796^{a}	0.0197^{c}	0.0993^{g}	10^{f}	240^{f}	369.45^{l}	1.102^{i}	25.52^{l}	18.20^{f}
AgN150 ppm+ K (2 %)	0.0895^{a}	0.0195^{c}	0.1094^{g}	11^{g}	264^{h}	376.7^{m}	1.113^{j}	28.66^{m}	20.40^{c}
LSD	0.25	0.081	0.01	0.95	5.5	3.6	0.014	0.77	0.53

**Ch a: Chlorophyll a (µg/ cm^2), Ch b: Chlorophyll b (µg/ cm^2), Tot.ch: total chlorophyll (µg/ cm^2), NTP: number of tubers/plant, NTL: number of tubers/line, WTP: weight of tubers/plant (g), SG: Specific gravity (g/cm^3), DM: Dry matter content (%), St: Starch content (%), LSD: Least significant difference. Mean values were followed by the same letters column are not significantly different (Duncan's multiple range test)*

Results in table (4) showed that, in case of both potato green parts and tubers the most value of catalase activity was reported by AgNPs 150 ppm + K_2SO_4 (2 %) (had 28.78 and 46.92 U/ml, respectively). As the same results mentioned before stile the combination of AgNPs with K_2SO_4 had a significant role in enhancement of biochemical parameters of potato. Catalase plays a central role in maintaining balance of cellular hydrogen peroxide in plants. The significance of catalases in the antioxidant defence system of plants has been proven by various studies [52]. According to Priyadarshini *et al* [53] nanosilver particles decreased H_2O_2 production and increased the efficiency of redox reactions. Similar result was reported by Krishnaraj *et al* [20] that high activity of catalase was recorded from leaf samples of plants subjected to nanosilver treatment, In contrast, Pozveh *et al* [54] found that, catalase activity in shoot of canola (*Brassica napus* L.) treated by silver nanoparticles remained without significant changes in comparison to the control plants.

Data in table (4) showed that, combination of AgNPs 150 ppm + K_2SO_4 (2%) recorded the lowest polyphenol oxidase activity (9.67 U/ml of potato plant extract and 17.34 U/ml of tuber extract) that cause enzymatic browning which improved the phytochemical parameters of potato to be more acceptable in consumer using and marketing and cooking processes. Enzymatic browning of polyphenol oxidase of raw agro-materials is a major problem in the food industry and one of the main causes of quality loss during postharvest handling and processing [55]. It results in unpleasant appearance and the concomitant development of off flavours catalyzed oxidation of mono- and diphenols to o-quinones. The o-quinones are highly reactive compounds and can polymerize spontaneously to form brown pigment (melanin), or react with amino acids and proteins [56].

Natural antioxidants that are present in plants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. As shown in table (4) the antioxidant activity of AgNPs 150 ppm + K_2SO_4 (2 %) that reported (68.48 in tuber) was the highest antioxidant activity among the untreated control or all other tested treatments, but with potato plant antioxidant evaluation recorded that Kocide® 631 ppm + AgNPs 150 ppm + K_2SO_4 2 % gave the highest antioxidant (67.56 %) followed by AgNPs 150 ppm+ K_2SO_4 1 % (65.06 %). Potatoes are regarded as a significant antioxidant source in human nutrition. Vinod *et al* [57] concluded that, antioxidants may be useful in preventing the deleterious consequence of oxidation processes and so, the importance of natural antioxidants is increasing day by day. Also potatoes have received substantial interest as a valuable source of antioxidants.

Naturally, the accumulations of both metals (Ag and Cu metals) in plants are higher in plant green parts than tubers. The data in table (4) revealed that, the rate of accumulation of Cu in treatments using fungicides significantly increased compared by control and other treatments not contain fungicides. On the other hand, the accumulation of

Ag in treatments containing AgNPs rarely increased over control and indicated safety of silver nanoparticles application as fungicides. Generally, the rate of accumulation of Cu in different potato parts (plant and tubers) is more than the rate of Ag accumulation. The sprayed nanoparticles may get absorbed through the stomata of leaves and translocated in plant. Nanoparticles have high reactivity because of more specific surface area, more density of reactive areas, or increased reactivity of these areas on the particle surfaces. These features in nano-scale simplify their absorption in plants [58].

In the accordance of animal toxicity to AgNPs, Amin *et al* [59] reported that, LD_{50} of AgNPs in normal and irradiated (4Gy) mice is 268.781(ppm) and 425.990 (ppm), respectively.

3.8 Effect of selenium nanoparticles (SeNPs) on plant growth

Hartikainen *et al* [60] applied Se in low concentrations to control plant diseases where Se was used in ryegrass (*Lolium perenne*) at concentrations 0.1 and 1.0 ppm Se. So, in the present investigation selenium at 0.5 ppm was used as a trial to control early blight disease of potato using the previous best combination (150 ppm AgNPs and K_2SO_4 2%) in addition to 300 ppm ascorbic acid.

In this respect, results in table (5) revealed that, the early blight disease is sufficiently controlled with all treatments compared with highest infected control (86.17 %). But by comparing results, treatment no.3 was the most effective one in controlling the disease (DS of 9.81 %) followed by treatment no. 2 (DS of 12.63 %) and finally treatment no. 4 (DS of 15.10 %). As the same results, Sameer [61] found that, Se slightly reduced the incidence of early blight on tomato plants.

Concerning the vegetative growth treatment no. 4 was the best effective treatment on stem no./plant, leaves no./plant, plant height, fresh weight and dry weight (5, 16, 47.5 cm, 105.29 g and 13.92 g, respectively). As mentioned before AgNPs and K_2SO_4 have a significant promoting growth effect. So, addition of SeNPs and ascorbic acid to that combination gave additional promoting effect. Selenium can exert beneficial effects at low concentrations; it has been shown to promote the growth of plants subjected to stress [62]. Selenium addition resulted in a significant enhancement of dry matter production of vegetative parts as well as pod and seed dry weight of canola (*Brassica napus* L.) plants when applied in 20 μg Se $plant^{-1}$[63].

Results in table (5) and figure (7) also, reported that treatment no. 4 (SeNPs 0.5 ppm, ascorbic acid 300 ppm, AgNPs 150 ppm and K_2SO_4 2%) effected in high degree in increasing potato yield and potato quality that made it a recommended treatment. The combinations of these factors with each other enhanced the plant growth as mentioned before and as a result the same effect exert on the yield and quality. Tubers no./ plant, tubers wt./plant, specific gravity, dry matter and starch content results were as follow; (12, 520.07 g, 1.321 g/cm^3, 30.21% and 23.15 %, respectively).

The additional promoting effect of Se may be due to its action as an antioxidant, inhibiting lipid peroxidation as reported by Hartikainen *et al* [60] in ryegrass (*Lolium perenne*) when applied at concentrations 0.1 ppm and 1.0 ppm Se. Results reported by Khalifa and Sameer [64] showed that, antioxidants slightly reduced the incidence of green mold on orange fruits. Se also increased yield in pumpkins (*Cucurbita pepo*) at a concentration1.5 ppm [65].

Chlorophyll content was increased by all applied treatments over the untreated control. But treatment no. 4 was the best treatment. Chlorophyll a, chlorophyll b and total chlorophyll were as (0.1002, 0.035321 and 0.13532 mg/cm^2, respectively). At similar research, the respiratory potential of *Eruca sativa*, measured by electron transport system (ETS) activity, significantly increased in selenium treated plant seeds. Possible explanations are as follows: (1) some of respiratory enzymes might need Se for their activity; (2) Se may intensify plant metabolism [66].

Dong *et al* [67] reported that, selenium (10–50 ppm Se) significantly increased chlorogenic acid, chlorophyll and carotenoids by 200–400% of leaves of *Lycium chinense* L. Moreover, Moldovana *et al* [68] observed amazing results increasing chlorophyll content in onion (*Allium cepa*) plants by supplementation of zinc and selenium in doses 50 and 100 ppm, respectively.

Concerning our results table (6) showed that, the sugar content reduced by all treatments compared with untreated control that recorded (45.9 mg/g DW of potato tubers). While, treatment no. 4 was more effective in decreasing reducing sugar content (25.46 mg/g DW of potato tubers). High sugar content reduced potato quality and affecting

on the colour of potato. In the forming work, we observed that AgNPs and K_2SO_4 2% reduced reducing sugar content. Also, application of Se reduced the sugar content. These results agree with the results of Kumar and Knowles [69] who recorded that, soluble sugars decreased by Se treatment of potato.

In this study, we reported that all treatments showed higher protein content compared with untreated control. The most effective treatment was no. 4 (45.31 mg/g DW of potato tubers). It may be attributed that most of the added Se was allocated in tubers into the organic fraction (soluble proteins, residue and amino acids) in addition to the effect of AgNPs and K_2SO_4 2% in increasing protein content as mentioned before. Se enrichment improved the nutritive quality of potato by increasing the amount of organic Se in tubers [70]. Similar results observed by Hajiboland and Keivanfar [63] who recorded higher photosynthesis rate and protein content in the leaves of Se treated plants of canola (*Brassica napus* L.) when applied in 20 ppm Se compared with control.

Also, as shown in table (6) treatment no. 4 gave the highest catalase activity (43.25 U/ml of tuber extract) as compared with catalase activity of control (16.92 U/ml). In addition, the untreated control had the highest polyphenol oxidase activity (46.67 U/ml of tuber extract). But, all treatments decrease PPO activity. Treatment no.3 decreases significantly the PPO activity to be the inferior treatment (19.02 U/ml of tuber extract). Darkening is an enzymatic reaction caused by the oxidation of phenolic compounds catalyzed by PPO following cell injury [71]. Therefore, raw darkening of fresh-cut potatoes has been prevented by using antibrowning agents (inhibitors) as ascorbic acid [72].

In this study, Se application lead to decrease of raw darkening in the tubers when applied in 0.5 ppm. This beneficial effect of Se can be ascribed to its antioxidative function, delaying the oxidation reaction in the enzymatic discolouration process. It is well documented that at low concentrations Se acts as an antioxidant [73]. So, it is suggested that a low Se concentration can directly affect the activity of PPO in tubers. This assumption is supported by results of Nowak *et al* [74] demonstrating that, the selenite at 0.45 mmol Se kg^{-1} caused decrease in the activity of PPO.

As mentioned before, AgNPs + K_2SO_4 2% gave high antioxidant activity. Here we noted that, the highest antioxidant activity was reported by treatment no.3 (72.34 %) compared with untreated control (47.73%). Cultivation of plants enriched with Se could be an effective way of producing Se-rich foodstuffs which can be beneficial to health.

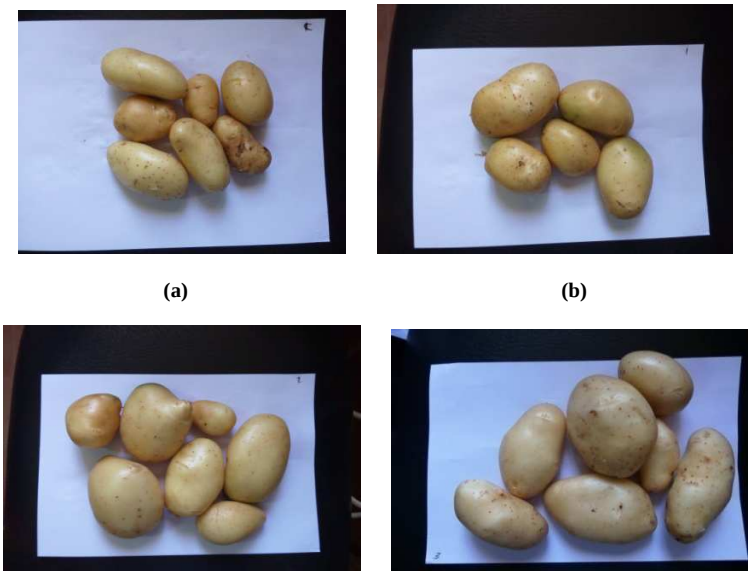
Recently it has been shown that Se is able to regulate the water status of plants under conditions of drought. As done by previous search where, selenate via foliar spray (0, 2, or 20 mg Se $plant^{-1}$) resulted in Se-biofortified tomato fruits, with Se content levels which are low enough and do not pose a health risk. The Se-biofortified fruits showed enhanced levels of the antioxidant [75]. Se is important in the metabolism of cyanobacteria and some plants, being involved in their antioxidative processes [76].

Ascorbic acid is one of antioxidants present in plants. Ascorbic acid is abundant in leaf tissue and is present in millimolar concentrations in the chloroplast stroma. Ascorbic acid serves many functions in plants. For example, it is a major redox buffer and serves as a required cofactor for several enzymes and as a major antioxidant [77]. Ascorbic acid also regulates cell division and growth [78].

Table 4. Effect of treatments on reducing sugar content, protein content and polyphenol oxidase of potato plants and tubers in the field

Treatments	RST	RSP	PCT	PCP	AAT	AAP	PPOT	PPOP	CATT	CATP	CuT	CuP	AgT	AgP
Control(untreated)	45.9^{a}	25.7^{a}	14.5^{a}	6.97^{a}	47.73^{a}	43.94^{a}	46.67^{a}	27.67^{a}	16.92^{a}	13.80^{a}	5.02^{a}	19.11^{a}	0.406^{a}	1.487a
Koc. 631 ppm	38.54^{j}	18.31^{g}	12.62^{b}	10.23^{b}	58.81^{e}	49.01^{e}	28.34^{i}	16.30^{g}	29.59^{f}	15.41^{b}	8.03^{i}	96.11^{p}	0.2938^{c}	1.29^{a}
Koc. 315 ppm	41.05^{l}	16.17^{f}	21.19^{d}	12.31^{c}	52.71^{c}	46.78^{c}	46.67^{a}	22.15^{l}	24.56^{e}	23.59^{h}	6.10^{d}	62.97^{j}	0.454^{d}	1.013^{c}
AgN 150 ppm	28.12^{d}	12.66^{c}	32.7^{j}	15.91^{d}	60.68^{f}	53.44^{g}	24.3^{f}	13.46^{d}	31.26^{g}	25.5^{j}	5.86^{d}	16.57^{c}	0.5714^{e}	2.434^{i}
K(2 %)	28.39^{d}	24.11^{j}	29.30^{h}	14.11^{d}	65.49^{i}	58.6 ^{i}	27.15^{h}	15.3^{f}	30.93^{g}	20.04^{f}	6.79^{fg}	17.84^{d}	0.762^{g}	1.50^{e}
K (1 %)	34.31^{h}	28.11^{l}	37.89^{m}	11.8^{c}	56.35^{d}	43.65^{a}	36.34^{l}	19.7j	22.30^{d}	18.5^{d}	6.13^{de}	18.46^{d}	0.500^{d}	2.064^{g}
Koc.315ppm+AgN150ppm	29.90^{e}	20.55^{h}	31.46^{i}	11.59^{bc}	70.21^{k}	45.44^{b}	27.85^{h}	14.7^{e}	44.53^{m}	25.78^{j}	6.15^{de}	62.07^{h}	1.396^{j}	3.850^{j}
Koc. 631ppm+AgN150 ppm	38.45^{j}	13.38^{d}	17.49^{c}	19.74^{f}	64.77^{g}	52.90^{g}	28.24^{i}	14.3^{e}	45.75^{n}	26.27^{k}	7.02^{gh}	75.93^{l}	0.5818^{e}	4.698^{l}
Koc.315ppm+AgN150ppm+ K(1%)	30.27^{e}	14.23^{e}	29.22^{h}	25.58^{i}	75.18^{n}	59.11^{j}	23.51^{e}	13.18^{d}	35.64^{i}	23.37^{h}	6.82^{fg}	61.99^{h}	1.039^{i}	3.744^{i}
Koc.631ppm+AgN 150ppm+K(1 %)	29.67^{e}	11.43^{b}	36.39^{l}	15.90^{d}	64.38^{g}	66.13^{m}	25.85^{g}	16.3^{h}	42.49^{l}	27.22^{k}	5.61^{c}	60.71^{g}	0.764^{g}	7.828^{n}
Koc.315ppm+AgN 150ppm+K(2%)	26.5^{c}	11.61^{b}	28.88^{g}	19.30^{f}	73.45^{l}	54.70^{h}	33.67^{j}	13.05^{d}	32.59^{h}	15.30^{b}	7.01g^{h}	65.^{37j}	1.681^{k}	4.468^{k}
Koc.631ppm+AgN 150ppm+K(2 %)	33.30^{g}	21.01^{i}	25.69^{f}	22.01^{h}	72.99^{l}	67.56^{n}	18.34^{c}	23.45^{m}	36.41^{j}	17.34^{c}	8.60^{i}	79.92^{m}	0.767^{g}	7.365^{m}
Koc.315ppm+K(1%)	30.87^{f}	16.56^{f}	16.77^{c}	20.19^{fg}	58.74^{e}	51.92^{f}	43.3^{o}	24.21^{n}	19.26^{c}	25.51^{j}	7.71^{h}	44.63^{f}	0.414^{a}	1.941^{f}
Koc.631ppm+K(1%)	36.58^{i}	21.74^{i}	30.34^{i}	17.19^{e}	50.01^{b}	44.08^{a}	37.34^{m}	17^{i}	24.56^{e}	22.45^{g}	5.76^{c}	83.80^{n}	0.863^{h}	1.174^{d}
Koc.315ppm+K(2 %)	43.49^{m}	26.54^{k}	24.81^{f}	21.43^{gh}	66.52^{j}	47.67^{d}	40.26^{n}	20.6^{k}	35.16^{i}	18.66^{d}	7.52^{h}	70.17^{k}	0.2164^{b}	0.900^{b}
Koc.631ppm+K(2 %)	39.91^{k}	18.06^{g}	22.53^{e}	17.55^{e}	65.12^{h}	45.23^{b}	35.34^{k}	27.7^{a}	18.78^{d}	19.24^{e}	6.73^{ef}	90.21^{o}	0.409^{a}	1.022^{d}
AgN150ppm+K(1 %)	27.79^{d}	14.51^{e}	33.53^{k}	31.83^{k}	73.99^{m}	65.06^{i}	21.05^{d}	11.6^{c}	39.60^{k}	24.56^{i}	3.78^{b}	15.18^{b}	0.714^{f}	2.299^{gh}
AgN150ppm+K(2 %)	23.29^{b}	11.9^{b}	38.18^{n}	28.23^{j}	75.86^{n}	62.48^{k}	17.34^{b}	9.67^{b}	46.92^{o}	28.78^{l}	5.06^{a}	20.06^{e}	0.883^{h}	2.350^{h}
LSD	1.47	0.74	1.35	1.38	0.75	0.81	0.67	0.80	0.78	0.69	0.49	0.70	0.049	0.12

**RST: Reducing sugar in tubers (mg/dry wt.), RSP: Reducing sugar in plant (mg/g dry wt.).PCT: Protein content in tubers (mg/g dry wt), PCP: Protein content in plant (mg/g dry wt). AAT: Antioxidant activity in tubers (%), AAP: Antioxidant activity in tubers (%), PPOT: Polyphenol oxidase activity in tuber extract (U/ml), PPOP: Polyphenol oxidase activity in plant extract (U/ml). CATT: Catalase activity in tubers extract (U/ml), CATP: Catalase activity in plant extract (U/ml). CuT: Absorbed Cu in tubers (Mg/ml), CuP: Absorbed Cu in plant (Mg/ml). AgT: Absorbed Ag in tubers (Mg/ml), AgP: Absorbed Ag in plant (Mg/ml), LSD: Least significant difference, Mean values were followed by the same letters column are not significantly different (Duncan's multiple range test)*



(a) **(b)**

(c) **(d)**

Figure 7. (a): Untreated control, (b): SeNPs 0.5 ppm+ ascorbic acid 300 ppm +AgNPs150 ppm, (c): SeNPs 0.5 ppm+ ascorbic acid 300 ppm + K_2SO_4 2%,
(d): SeNPs0.5ppm+ ascorbic acid 300 ppm+ AgNPs 150 ppm + K_2SO_4 2%

Table 5. Effect of different combined treatments on potato plant growth and yield to relation to early blight disease

Treat no.	Treatments	PIH	LN/P	DS%	Effe%	NS	FW	DW	NTP	WTP	SG	DM	ST
1	Control (untreated)	27^{a}	8^{a}	86.17^{a}	0.00^{a}	2^{a}	33.85^{a}	4.73^{a}	6^{a}	323^{a}	1.031^{a}	10.03^{a}	4.07^{a}
2	SeNPs0.5ppm+ Ascor.300ppm+AgNPs150ppm	38.2^{b}	12^{b}	12.63^{c}	85.34^{c}	3^{b}	85.59^{b}	10.86^{b}	4^{b}	469.2^{b}	1.095^{b}	28.95^{c}	18.14^{b}
3	SeNPs0.5ppm+ Ascor.300ppm+K 2%	43.6^{c}	14^{c}	9.81^{b}	88.61^{d}	4^{c}	90.21^{c}	12.71^{c}	6^{a}	465.56^{b}	1.092^{b}	27.38^{b}	16.72^{c}
4	SeNPs0.5ppm+Ascor.300ppm +AgNPs 150ppm+K 2%	47.5^{d}	16^{d}	15.10^{d}	82.47^{b}	5^{d}	105.2^{d}	13.9^{d}	12^{c}	520.07^{c}	1.321^{c}	30.21^{d}	23.15^{d}
	LSD	4.25	1.85	2.73	2.93	0.95	4.66	1.11	1.85	54.9	0.60	1.38	1.44

Table 6. Effect of different combined treatments on potato plant biochemical parameters to relation to early blight disease

Treat no.	Treatments	Ch.a	Ch.b	Tot.ch.	RST	PCT	CATT	PPOT	AAT
1	Control (Untreated)	0.0448^{a}	0.0090^{a}	0.0548^{a}	45.9^{a}	14.5^{a}	16.92^{a}	46.67^{a}	52.87^{a}
2	SeNPs0.5ppm+Ascor.300ppm+AgNPs150ppm	0.0871^{b}	0.02949^{b}	0.11633^{b}	32.16^{c}	39.64^{b}	31.65^{b}	22.33^{c}	57.65^{b}
3	SeNPs0.5ppm+Ascor.300ppm+K_2SO_4 2%	0.0901^{c}	0.02966^{b}	0.11989^{c}	35.38^{d}	41.25^{c}	35.87^{c}	19.02^{b}	72.34^{d}
4	SeNPs0.5ppm+ascor.300ppm+AgNPs150ppm+ K_2SO_4 2%	0.1002^{d}	0.0353^{c}	0.1353^{d}	25.46^{b}	45.31^{d}	43.25^{d}	31.34^{d}	65.54^{c}
	LSD	0.0039	0.006	0.0038	3.11	4.20	1.69	3.20	4.73

PIH: Plant height, LN/p: leaves number/plant, DS%: disease severity, Eff %: Efficiency, NS: number of main stems, FW: Fresh wt./plant (gm),DW: Dry weight/ plant (gm), Ch a:Chlorophyll a (mg/ cm^2), Ch b:Chlorophyll b (mg/ cm^2), Tot.ch: total chlorophyll, RST: Reducing sugar in tubers (mg/dry wt.), PCT: Protein content in tubers (mg/g dry wt),CATT: Catalase activity in tuber extract (U/ml), PPOT: Polyphenol oxidase activity in tuber extract (U/ml), AAT: Antioxidant activity in tubers (%), LSD: Least significant difference. Mean values were followed by the same letters column are not significantly different (Duncan's multiple range test)

CONCLUSION

In conclusion we can say that, using silver and selenium nanoparticels limited environmental pollution and excessive use of chemical compounds in the field. Silver and selenium nanoparticles reduced disease severity in potato and improved vegetative and chemical parameters of the plant. It is expected that the application of silver and selenium nanoparticles at low concentrations will be economic, eco-friendly, and decrease farm management costs.

Acknowledgements
The authors would like to thank the Nanotechnology Research Unit (P.I. Prof. Dr. Ahmed El-Batal), Drug Radiation Research Department, National Center for Radiation Research and Technology (NCRRT), Egypt, for financing and supporting this study under the project'' Nutraceuticals and Functional Foods Production by using Nano/Biotechnological and Irradiation Processes.

Conflict of interests
The authors declare that there is no conflict of interest regarding the publication of this manuscript

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