British Biotechnology Journal
12(3): 1-11, 2016, Article no.BBJ.24155
ISSN: 2231–2927, NLM ID: 101616695

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of *in vitro* Antifungal Activity of Silver and Selenium Nanoparticles against *Alternaria solani* Caused Early Blight Disease on Potato

Abdel-Wahab A. Ismail[1], Nagwa M. Sidkey[2], Rawhia A. Arafa[2], Rasha M. Fathy[3] and Ahmed I. El-Batal[3*]

[1]Plant Diseases Institute, Agriculture Research Centre, Giza, Egypt.
[2]Department of Botany and Microbiology, Faculty of Science, Al Azhar University, Cairo, Egypt.
[3]Department of Drug Radiation Research, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AIEB, AWAI and NMS wrote the protocol. Authors AIEB, AWAI and RMF designed and analyzed the present study. Authors AIEB, NMS and RAA wrote the first draft of the manuscript and shared in analysis of the study. Author RMF managed the literature searches, performed the practical studies and statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/24155
Editor(s):
(1) Chan Yean Yean, Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Malaysia.
Reviewers:
(1) Gyula Oros, Plant Protection Institute HAS, Budapest, Hungary.
(2) Ishola Vincent, University of Abomey- Calavi, Benin.
Complete Peer review History: http://sciencedomain.org/review-history/13604

Original Research Article

Received 6th January 2016
Accepted 4th February 2016
Published 9th March 2016

ABSTRACT

Aim: This study investigated the effect of silver and selenium nanoparticles on *Alternaria solani*, the pathogenic fungus causing early blight disease of potato.
Place and Duration of Study: Drug Radiation Research Department, National Centre for Radiation Research & Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, 2013.
Methodology: The fungus was isolated from infected potato leaves that showed brown circular spots as early blight disease symptoms. Silver nanoparticles (AgNPs) were prepared biologically using gamma irradiated *Trichoderma viride* cell free supernatant. Selenium nanoparticles (SeNPs)

**Corresponding author: E-mail: aelbatal2000@gmail.com;*

were prepared by glutathione method. The synthesized AgNPs were characterized by UV-Vis spectroscopy, Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM). Kocide® fungicide was used as reference.
Results: The fungus isolated of leaf spot was identified both microscopically and genetically as *Alternaria solani* causing early blight disease of potato. AgNPs were spherical in shape with average size of 12.7 nm. Selenium nanoparticles were prepared by glutathione as reducing agents. Under laboratory conditions, 25 µg/ml concentration of silver nanoparticles completely inhibited *A. solani* as compared to Kocide®, fungicide that gave maximum inhibition at 600 µg/ml. The selenium nanoparticles completely inhibited the fungal growth at 800 µg/ml.
Conclusion: AgNPs completely inhibited the growth of *A. solani* at low concentrations. Silver nanoparticles might be suitable alternative to chemical fungicides. While, SeNPs can be used as antioxidant for enhancing plant immunity.

Keywords: Potato; Alternaria solani; early blight; silver and selenium nanoparticles; antifungal.

1. INTRODUCTION

Potato is the fourth main food crop in the world. The economical outlets for this crop are great; however, numerous either soil- or airborne diseases can cause huge losses in the production. Early blight caused by *Alternaria solani* is one of the most serious biotic threat to potato production worldwide causing severe yield losses in Egypt [1].

This pathogen has the ability to grow over a wide range of temperatures [2] and is reported to infect tomato and potato plants under both dry and wet conditions [3], and its propagules survive between crops as mycelia or conidia in soil, plant debris and seed [4].

Values in the literature for measured yield losses due to early blight vary within large limits from 5-78% [5,6].

Diagnosis of a disease in its very early stage can play important role in successful control. Nanotechnologists look for a nano solution for protecting the cultivated plants from bacteria, fungus and viral agents. New molecular and cellular biology tools are expected to provide apropriate pest management practicies in agriculture [7].

There are three main methods of synthesizing metal nanoparticles: Chemical, physical and biological methods. The radiation-induced AgNPs synthesis is a simple, clean which involves radiolysis of aqueous solution that provides an efficient method to reduce metal ions [8].

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 [9].

Antifungal activity of ionic or nanoparticle silver has a great potential for use in controlling spore-producing fungal plant pathogens. Silver may be less toxic to humans and animals than synthetic fungicides. Multiple modes of action targeting a broad range of biological pathways of microbes provide an important benefit for avoiding the development of resistance, which has been increasingly important in terms of current issues for the chemical management of many plant fungal diseases [10].

Selenium is an essential nutrient for humans and animals to form important selenoproteins, including glutathione peroxide, thioredoxin reductase [11]. Selenium has not been classified as an essential element for plants, although its role has been considered to be beneficial in plants capable of accumulating large amounts of the element [12].

2. MATERIALS AND METHODS

2.1 Isolation and Identification of the Pathogenic Fungus, *A. solani*

Standard isolation technique was followed to obtain *A. solani* isolate. For this purpose potato plants showing typical early blight symptoms were collected. The infected leaves were cut into small bits measuring about 2 mm and surface sterilized in 0.1 per cent sodium hypochlorite solution for 2 min. Such bits were transferred into

Petri-dishes containing 15 ml Potato Dextrose Agar (PDA) and incubated at 28±1°C for 7 days [13]. Pure culture of the fungus was obtained by hyphal tip isolation method [14]. *A. solani* was maintained in the refrigerator on PDA medium and subcultured periodically at an interval of 30 days during the course of this study. The pathogen was identified on the basis of colony characters, viz., colour, growth, pigmentation etc. and morphological characteristics of its mycelium and conidia produced on host and in culture under electron microscope. The fungus was further identified genetically. The partial sequences of 18S rRNA obtained from *Alternaria* isolate was aligned with the available 18S rRNA sequences in Gene Bank data base.

2.2 Biosynthesis of Silver Nanoparticles

Trichoderma viride fungus (provided by Drug Microbiology lab, NCRRT) was used to synthesize silver nanoparticles. The fungus was inoculated in sterilized medium, glucose yeast peptone (GYP) broth containing yeast extract 0.3%, glucose 1%, peptone 0.5%, at 25°C, in shaking condition (180 rpm). After 72 h of incubation, the culture was centrifuged at 5000 rpm for 10 min and the supernatant was used in synthesis of silver nanoparticles [8]. Aqueous solution of $AgNO_3$ (5 ml) (1 mM $AgNO_3$ of final concentration) was mixed with fungal supernatant (5 ml) then irradiated at different gamma radiation doses, 0.5, 1.0,1.5, 2.0, 2.5, 3.0, 5.0, 10, 15, 20, 25 and 30 kGy. AgNPs were characterized by UV-Visible Spectroscopy (T60 UV/Vis spectrometer), Dynamic Light Scattering (DLS) and Transmission electron microscope (TEM).

2.3 Preparation of Selenium Nano-particles

Selenious acid (0.04 mM) under stirring was mixed with (0.2 mM) glutathione (GSH) as product from *Saccharomyces cerevisiae* (provided by Drug Microbiology lab, NCRRT) 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 [15,16].

2.4 *In vitro* Comparison Fungicidal Activity between Kocide®, Silver and Selenium Nanoparticles

Inhibitory effect of Kocide®, silver and selenium nanoparticles were tested *in vitro*. A laboratory study was performed to examine the sensitivity of the pathogenic fungus *A. solani* in relation to Kocide®. Nine concentrations of Kocide® were used as follows: 50, 100, 150, 200, 250, 400, 500, 600 and 800 ppm. Six concentrations of silver nanoparticles were used as follows: 5, 10, 15, 20, 25 and 50 ppm respectively. Concentrations of selenium nanoparticles were used as follow: 50, 100, 200, 300, 400, 500, 600, 700 and 800 ppm, respectively. The required concentrations were obtained by adding the appropriate amount of stock solution used, to 100 ml portions of autoclaved potato dextrose agar medium (PDA) which was cooled to about 45°C (not solidified), three Petri dishes were used as replicates for each concentration and three were left without any treatment as control. After solidification of the medium, each dish was inoculated centrally with a mycelial disk (5 mm in diameter) taken from the cultures of the fungus (7 days – old). Plates were incubated at 28°C and colony diameters were measured till untreated control had just covered the plate. Linear growth was measured daily in mm. The average of two perpendicular diameters were recorded. The percentage of inhibition (I %) was calculated according to [17] as follows:

$$I\ \% = (A - B) / A \times 100$$

Where:

I % = Inhibition percentage.
A = Mean diameter of growth in the control.
B = Mean diameter of growth in treatment.

IC_{50} of the each treatment was determined (IC_{50}, the concentration of each treatment that is required to cause fungitoxic inhibition of the tested fungus by 50 % in comparison with the control).

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of the Pathogen

According to Mayee and Datar [18], the early blight disease of potato was characterized by the appearance of brown to dark brown colour necrotic spots. Appearance of concentric rings

inside the spots produced target board effect. Singh et al. [19] reported that, the spots were oval to angular in shape measuring up to 0.3-0.4 cm in diameter and usually with a chlorotic zone around the spot.

The fungus was identified microscopically and confirmed by its morphological characterization and gave the name of *Alternaria solani*. The sequences of the isolate studied were compared with those of NCBI databases using BLAST network. The partial sequences of 18S rRNA obtained from *Alternaria* isolate was aligned with the available 18S rRNA sequences in Gene Bank data base, and the fungus was found to have 99-100% similarity with *A. solani* strains (Table 1).

Partial sequencing of 18S and 28S rRNA and complete sequence of internal transcribed sequence 1 (ITS1), internal transcribed sequence 2 (ITS2) and 5.8S rRNA gene has been submitted in Sigma Aldrich Company (Fig. 1). Multiple sequences alignments and phylogenetic tree illustrated graphically in Fig. 2.

3.2 Effect of Kocide Fungicide on the Growth of *A. solani*

The control of plant diseases is one of the most important topics. Scientists all over the world are trying to solve the complicating problems of plant diseases. The control of plant diseases has been possible by the use of chemicals or fungicides.

Data in Fig. 3 showed that, the growth of *A. solani* was completely inhibited at 600 ppm of Kocide, while at 50 up to 250 ppm has no effect against the same fungus and starting from 400 ppm; the inhibition was increased gradually up to 600 ppm which gives complete inhibition (100%).

Table 1. Sequence producing significant alignments for *A. solani* isolate

Accession No.	Description	Identity %
KC78609.1	*Alternaria solani* strain UP T 10	100%
KJ634071.1	*Alternaria solani* strain SL 1	100%
KF998549.1	*Alternaria solani* strain 043021	100%
AY154716.1	*Alternaria solani* strain IA300	100%
AY154715.1	*Alternaria solani* strain IA295	100%
KF308830.1	*Alternaria solani* isolate IM HW A6	100%
KF998550.1	*Alternaria solani* strain A7AXTL14e 2	100%
AF314576.1	*Alternaria solani* strain AS3	99%
JQ625583.1	*Alternaria solani* f sp lycopersici isolate UP 7	99%
JQ625580.1	*Alternaria solani* f sp lycopersici isolate HR 12	99%
JF331442.1	*Alternaria* porri isolate CNU093037	99%
JQ625586.1	*Alternaria solani* f sp lycopersici isolate RJT 1	99%
JX418359.1	*Alternaria* potato strain A38	99%

GGGCTTGGTGGATGCTAGACCTTGGGGCTGGAAGAGAGCGCGACTTGTGCTG
GAAAAAAAAGGCTTGGTGGATGCTAGACCTTGGGGCTGGAAGAGAGCGCGACTTGTGCTG
CGCTCCGAAACCAGTAGGCCGGCTGCCAATGACTTTAAGGCGAGTCTCCCGCAAGGGGAG
CGCTCCGAAACCAGTAGGCCGGCTGCCAATGACTTTAAGGCGAGTCTCCCGCAAGGGGAG
ACAAAAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCAT
ACAAAAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCAT
TGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGA
TGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGA
TCCGTTGTTGAAAGTTGTAAATTATTACATTGTTACTGACGCTGATTGCCATTGCAAAAA
TCCGTTGTTGAAAGTTGTAAATTATTACATTGTTACTGACGCTGATTGCCATTGCAAAAA
GGTTTATGGGTTGGTCCTTGTGGTGGGCGAGCCCACCAAGGAAACAAGAAGTACGCAAAA
GGTTTATGGGTTGGTCCTTGTGGTGGGCGAGCCCACCAAGGAAACAAGAAGTACGCAAAA
GACACGGGTGGAATAATTCAGCAAGGCTGGCCACCCCGGGAGGTGCCAGCCCGCCTTCAT
GACACGGGTGGAATAATTCAGCAAGGCTGGCCACCCCGGGAGGTGCCAGCCCGCCTTCAT
ATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGA
ATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGA

Fig. 1. *A. solani* isolate sequence based on partial 18S rRNA sequences

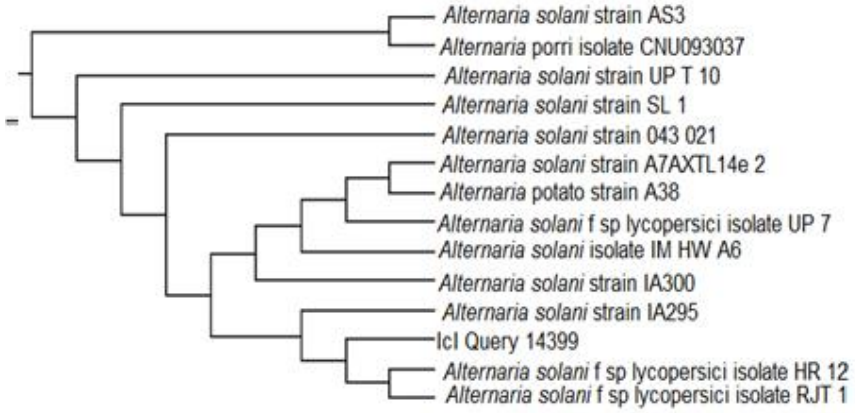

Fig. 2. Phylogenetic tree showing the position of isolate *A. solani* derived from 18S rRNA gene sequences

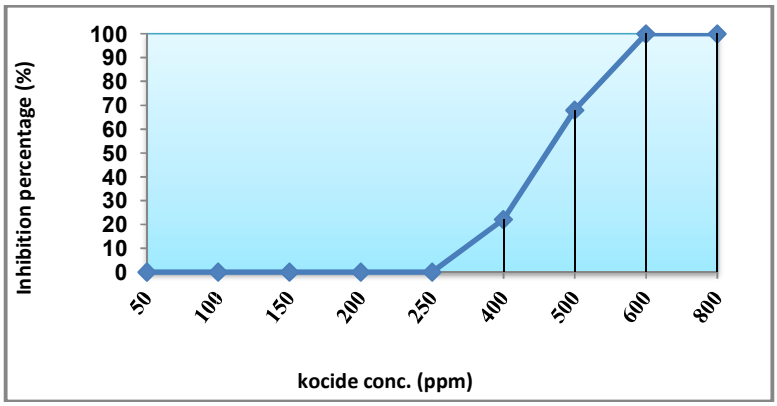

Fig. 3. Effect of Kocide on the growth of *A. solani in vitro* after 7 days

Fungicides can be divided into two groups based on mode of action in fungal cells: a. Site-specific inhibitors: Site-specific inhibitors target individual sites within the fungal cell. b. Multi-site inhibitors that target many different sites in each fungal cell. Where, many fungicides in the market having the same mode of action where they are sterol and demethylation inhibitors or interfering with respiration and interruption of energy compound as ATP production in sensitive fungi [20]. Also, as reported by Meena et al. [21] fungicides mancozeb and carbendazim caused 100% reduction in mycelial growth of *Alternaria brassicae* over control *in vitro*.

3.3 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 ($H^•$) having the powerful reducing ability that reduce Ag^+ into Ag^0 [22].

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

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 [23]. 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 [24].

$$Ag^0 + Ag^+ \longrightarrow Ag^+_2 \quad (5)$$

$$nAg^+ + Ag^+_2 \longrightarrow (Ag^+)_n \quad (6)$$

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

Fig. 4 showed that, after gamma irradiation of mixture of *T. viride* 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 15 kGy. On the other hand, over than 15 kGy the production of AgNPs was decreased.

3.4 Characterization of Silver Nanoparticles Synthesized by Gamma Irradiated *T. viride* Supernatant

Average particle size was determined by DLS. Fig. (5a) represented graph of AgNPs solution exhibit a very narrow size distribution with small particles size. The volume distribution of the hydrodynamic size of the nanoparticles show peak (approximately 100% of the particle volume) had its average size at 14.5 nm. TEM image of AgNPs solution with the average size of nanoparticles to be 12.7 nm Fig. (5b). The nanoparticles were monodispersed without aggregation indicating stabilization of the nanoparticles by a protein capping agent.

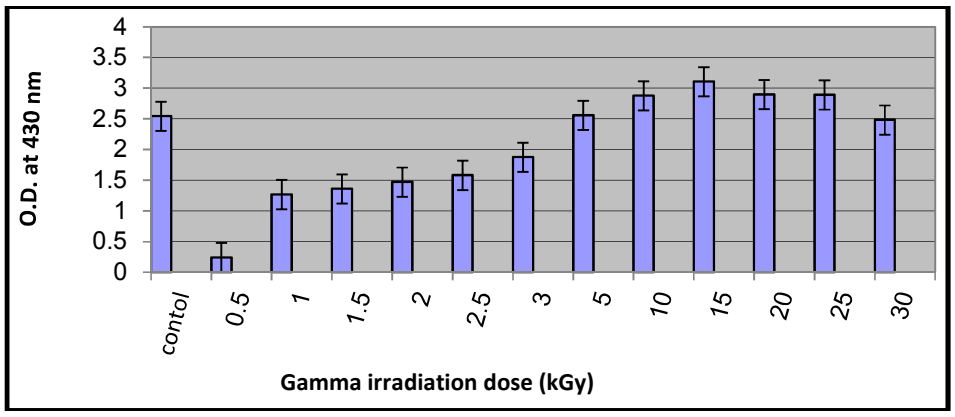

Fig. 4. Optical density of AgNPs after gamma irradiation at different doses

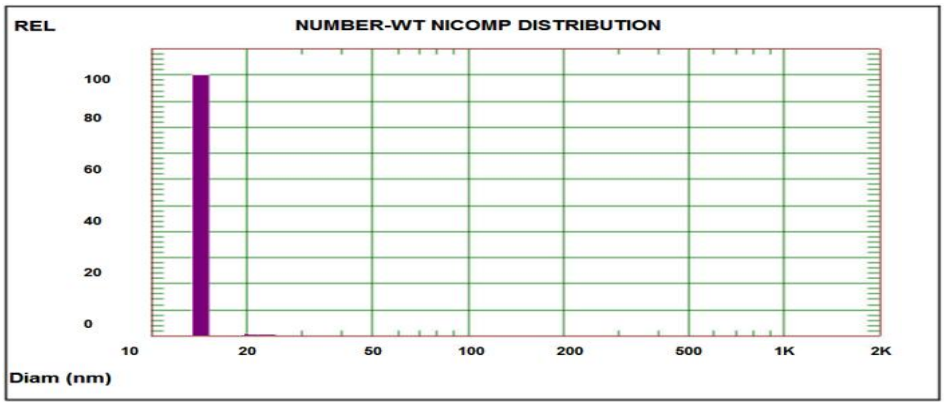

Fig. 5(a). DLS (mean diameter = 14.5 nm, Num = 100 %)

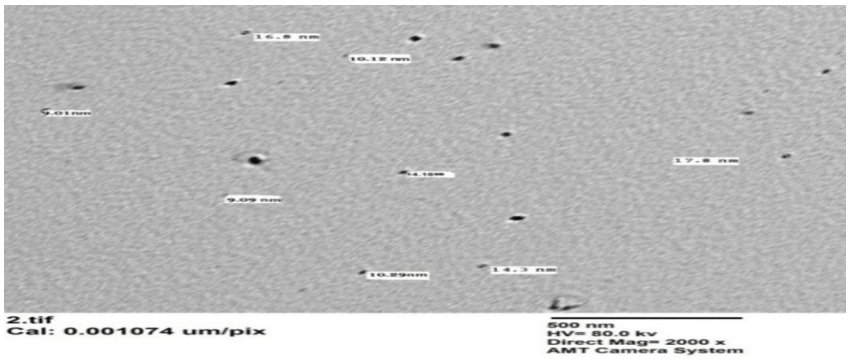

Fig. 5(b). TEM graphs (average diameter of 12.7 nm) of AgNPs synthesized by *T. viride* cell free supernatant

3.5 Effect of Silver Nanoparticles on the Growth of *A. solani*

Applications of nanoparticles based fungicides have been increasing gradually in recent years because of many advantages over conventional chemical fungicides. This study presents fungicidal efficiency of silver nanoparticles against the phytopathogen, *A. solani*.

Data in Fig. 6 showed that, the growth of *A. solani* was completely inhibited at 25 ppm AgNPs, while at 5 ppm the lowest effect against the same fungus was obtained. AgNPs were highly effective against the pathogenic fungus *A. solani in vitro*.

In previous studies it was observed that, silver nanoparticles disrupt transport systems including ion efflux. The dysfunction of ion efflux can cause rapid accumulation of silver ions, interrupting cellular processes at their lower concentrations such as metabolism and respiration by reacting with molecules. Also, silver ions are known to produce reactive oxygen species via their reaction with oxygen, which is detrimental to cells, causing damage to proteins, lipids, and nucleic acids [25,26].

Different parameters, such as particle size and particle concentration, which affect the efficiency of the fungicidal effect, showed that small sized particles (12.7 nm) are very effective in preventing the fungal growth. It is well known that silver nanoparticles are highly toxic to microorganisms. Silver nanoparticles have been known to have inhibitory and antimicrobial effects and thus we extend its application as an antimicrobial agent. Several studies propose that silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell. It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the organism [27]. In addition, Naghsh et al. [28] found that growth and colony number of *Aspergillus niger* decreased significantly in 12.5 ppm concentration of nano-silver.

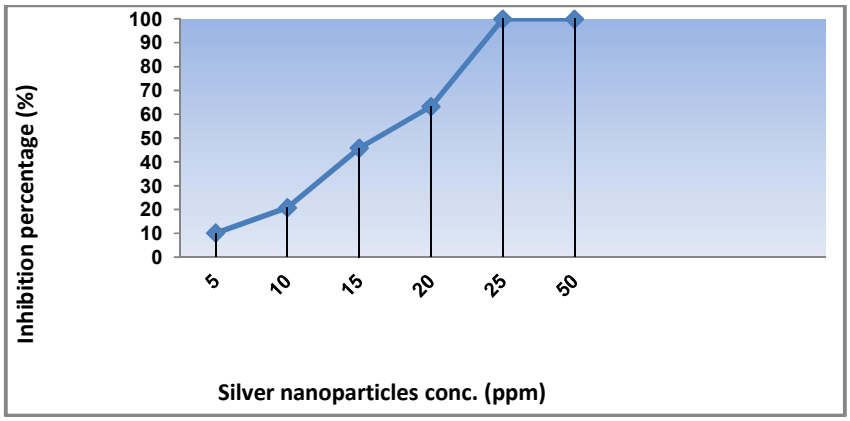

Fig. 6. Effect of AgNPs on the growth of *A. solani in vitro* after 7 days

Silver stops the metabolism of pathogenic microorganisms. The electrical load of an emitted silver anion kill the pathogenic microbes by controlling a procreation function [29,30]. It was found that there are no bacteria which can survive after touching silver for 6 minutes [31]. These results suggested that, nanosilver is a potential compound in the treatment of fungal infectious diseases. Many studies have shown the antimicrobial effect of nanosilver [32]. The same results was recorded with Gajbhiye et al. [33] who observed that, the transmission electron microscopy analysis revealed the interaction between nano-Ag and the membrane structure of *Candida albicans* cells during nano-Ag exposure results in changes in the membranes of *C. albicans*, which can be observed as the "pits" on the membrane surfaces. The formation of pores subsequently leads to cell death. In this mechanism, the nano-silver particles diffuse gradually silver ions. These ions substitute the -SH bonds to -SAg ones in microorganism's membrane in a substitution reaction, resulting in death of microorganism [34]. Rathod et al. [35] studied synthesizing highly stabilized monodispersed silver nanoparticles by *Rhizopus stolonifer* and the antifungal efficacy of silver nanoparticles (AgNPs) against *Candida* sp. Also, Lamsal et al. [36] applied silver nanoparticles at various concentrations. Silver nanoparticles caused a detrimental effect on mycelial growth of *Colletotrichum* species *in vitro* and in the field. Molecular mechanism provided by silver nano-particles can be attributed to production of free radicals. These free radicals were induced oxidative stress and program cell death in microorganisms. These free radicals result in apoptosis, Programmed death of live cells, by attacking the intracellular organelles of fungi [37].

3.6 Effect of Selenium on Pathogenic Fungus, *A. solani in vitro*

As shown in Fig. 7 selenium nanoparticles exert gradual inhibiting effect on the growth of the pathogenic fungus, *A. solani*. The lowest concentration 50 ppm gave 8.33% of inhibition while, at 800 ppm of selenium nanoparticles completely inhibit the fungus growth (100% inhibition). *A. solani* tolerate high concentrations of SeNPs.

Many literatures attributed the antibacterial effects of different selenium compounds to the formation of free radicals [38]. Moreover, selenium has also been found to trigger the generation of ROS, with both elements capable of reacting with intracellular thiols and forming intermediates that cause oxidative stress as a consequence of the formation of superoxide radicals [39,40]. Nanoparticles can contribute to functional damages of cell membrane or wall by disrupting the integrity of these important envelopes [41].

At similar research, Khalifa and Sameer [42] indicated that, selenium started to inhibit the fungal growth of *Penicillium digitatum,* causing green mold disease of orange fruit growth at 100 ppm with 5.56% inhibition. However, at 500 ppm, selenium gave fungal growth inhibition rate of 85.22%. Also, Sameer [43] found that, *Alternaria solani* tolerated high concentrations of selenium according to its IC_{50} value (263.03 ppm). In this respect Zohri et al. [44] reported that, the fungal biomass of *Aspergillus parasiticus* was slightly decreased at higher concentrations of selenium.

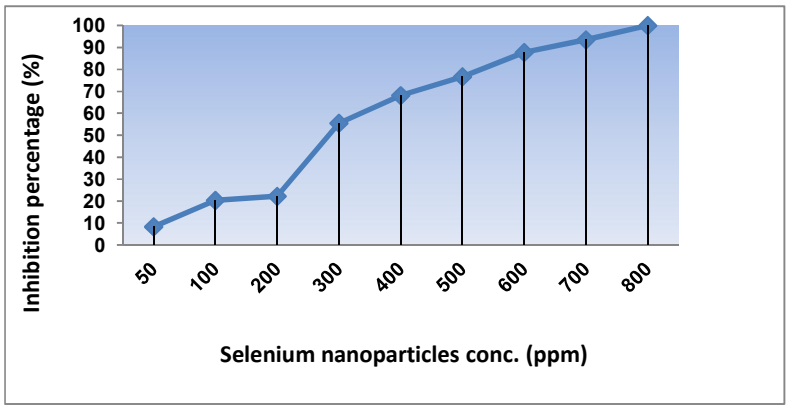

Fig. 7. Effect of SeNPs on the growth of *A. solani in vitro* after 7 days

4. CONCLUSION

In the present work it was demonstrated that, silver nanoparticles has significant antifungal activity against potato pathogenic fungus, *A. solani*. So, AgNPs can be effectively used as antimicrobial agents to control early blight disease of potato. SeNPs have a moderate effect on *A. solani*. So, AgNPs may be applied widely and safely instead of using the commercially available synthetic fungicides, which show higher toxicity to humans.

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 Research and Radiation Technology (NCRRT), Egypt, for financing and supporting this study under the project'' Nutraceuticals and Functional Foods Production by using Nano/Biotechnological and Irradiation Processes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fiers M, Edel-Hermann V, Chatot C, Hingrat YL, Alabouvette C. Steinberg potato soil-borne diseases a review. Agro. Sustain. Develop. 2012;32:93-132.
2. Pound GS. Effect of air temperature on incidence and development of the early blight disease of tomato. Phytopathol. 1951;41:35-127.
3. Waggoner PE, Horsfall JG. Epidem: A Simulator of Plant Disease written for a Computer. Bulletin No. 698. New Haven, Connecticut: Connecticut Agri. Exper. Stat; 1969.
4. Sherf AF, MacNab AA. Vegetable diseases and their control. John Wiley and Sons, New York; 1986.
5. Waals JE, Korsten L, Slippers B. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. Plant Dis. 2004;88:959-964.
6. Pasche JS and Piche LM, Gudmestad NC. Effect of the F129L mutation in *Alternaria solani* on fungicides affecting mitochondrial respiration. Plant Dis. 2005;89:269-278.
7. Sharma K, Sharma R, Shit S, Gupta S. Nanotechnological application on diagnosis of a plant disease. Inter. Conf. Adv. Biologic. Medic. Sci. (ICABMS'2012). 2012;15-16:149-150.
8. El-Batal AI, Haroun BM, Farrag AA, Baraka A, El-Sayyad GS. Synthesis of silver nanoparticles and incorporation with certain antibiotic using gamma irradiation. Brit. J. Pharma. Res. 2014;4:1341-1363.
9. El-Batal AI, Amin MA, Mona MK, Shehata, Merehan MA Hallo. Synthesis of silver nanoparticles by *Bacillus stearothermophilus* using gamma radiation and their antimicrobial activity. World Appl. Sci. J. 2013;22:1-16.
10. Jo YK, Kim BH, Jung G. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis. 2009;93: 1037-1043.
11. Zhu YG, Pilon-Smits EAH, Zhao FJ, Williams PN, Meharg AA. Se in higher plants: Understanding mechanisms for biofortification and phytoremediation. Tren Plan Sci. 2009;14:436-442.
12. Shanker AK. Countering UV-B stress in plants: Does selenium have a role? Plant and Soil. 2006;282:21-26.
13. Ganie SA, Ghani MY, Nissar Q, Jabeen N, Anjum Q, Ahanger FA, Ayaz A. Status and symptomatology of early blight (*Alternaria solani*) of potato (*Solanum tuberosum* L.) in Kashmir valley. Afri. J. Agri. Res. 2013;8:5104-5115.
14. Pathak VN. Essentials of plant pathology. Prakesh Publishers Jaipur, India. 1972; 448.
15. Huang B, Zhang J, Hou J, Chen C. Free radical scavenging efficiency of Nano-Se *in vitro.* Free Rad. Biol. Med. 2003;35:805-813.
16. El- Batal AI, Thabet NM, Osman A, Abdel Ghaffar AB, Azab KS. Amelioration of oxidative damage induced in gamma irradiated rats by nano selenium and lovastatin mixture. World Appl. Sci. J. 2012;19:962-971.
17. Topps JH, Wain RL. Investigation of fungicides. III. The fungitoxicity of 3- ans 5-alkyl salicylanilide and P- chloronilines. Ann. Appl. Biol. 1957;45:506-511.
18. Mayee CD, Datar VV. Phytopathometry Technical Bulletin-1. Marathwad Agricultural University, Parabhani. 1986;25.
19. Singh RS. Diseases of vegetable crops. Pub. Co. Pvt. Ltd., New Delhi, Bombay, Culcutta; 1987.

20. Burpee L. Integrated disease management, an introduction to fungicides. Integrated disease management, an introduction to fungicides. Courses Support; 2006.
21. Meena PD, Meena RL, Chattopadhyay C, Kumar A. Identification of critical stage for disease development and biocontrol of *Alternaria* blight of in dianmustard (*Brassica juncea*). Phytopathol. 2004;152: 204-209.
22. Yoksan R, Chirachanchai S. Silver nanoparticles dispersing in chitosan solutions: Preparation by gamma-ray irradiation and their antimicrobial activity. Mater. Chem. Phys. 2009;115:296.
23. Rao YN, Banerjee D, Datta A, Das SK, Guin R, Saha A. Gamma irradiation route to synthesis of highly re-dispersible natural polymer capped silver nanoparticles. Rad. Phys. Chem. 2010;79:1240-1246.
24. El-Batal, AI, El-Baz AF, Abo Mosalam FM, Tayel AA. Gamma irradiation induced silver nanoparticles synthesis by *Monascus purpureus*. J. Chem. Pharma. Res. 2013;5:1-15.
25. El-Batal AI, El-Sayed MH, Refaat BM, Askar AA. Marine *Streptomyces cyaneus* strain alex-sk121 mediated eco-friendly synthesis of silver nanoparticles using gamma radiation. Briti. J. Pharma. Res. 2014;4:2525-2547.
26. Hwang ET, Lee JH, Chae YJ, Kim YS, Kim BC, Sang BI, Gu MB. Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. Small. 2008;4:746-750.
27. Srivastava AA, Kulkarni AP, Harpale PM, Zunjarrao RS. Plant mediated synthesis of silver nanoparticles using a bryophyte: *Fissidens minutus* and its anti-microbial activity. Inte. J. Eng. Sci. Technol. 2011;3:8342-8347.
28. Naghsh N, Ghyasiyan M, Soleimani S, Torkan S. Comparison between alcoholic eucalyptus and nano-silver as a new nanocomposition in growth inhibition of *Aspergillus niger.* Ind. J. Sci. Technol. 2012;5:2445-2447.
29. Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Escherichia coli*. Can. J. Microbiol. 1974;20:9- 883.
30. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. 2000;52:8-662.
31. Kim JH. Nano silver chemotherapeutic agents and its applications. The Nat. Instit. Health Care Excel. 2004;22:60-656.
32. Klasen HJ. A historical review of the use of silver in the treatment of burns. Renewed interest for silver. Burns. 2000;26:131-138.
33. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed Nanotech. Biol. Med. 2009;5:382-386.
34. Somayyeh M, Delavar HA, Motallebi A. Toxicity study of nano-silver on osteoblast cancer cell line. Inter. Nano. Lett. 2011;1:11-16.
35. Rathod V, Banu A, Ranganath E. Biosynthesis of highly stabilized silver nanoparticles by *Rhizopus stolonifer* and their anti-fungal efficacy. Inter. J. Molecu. Clin. Microbiol. 2011;1:65-70.
36. Lamsal K, Kim SW, Jung JH, Kim Ys, Kim KS, Lee YS. Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. Mycobiol. 2011;39:94-199.
37. Christoforidis GA, Frank JA, Lindau M, Lockman DH, Geho CD, Jones EF, Petricoin LA, Liotta SP, Manninger Y, Qiang AMS, Stupp SI. Nanoparticles: Potential biomarker harvesters. Curr. Opinion Chem. Biol. 2006;10:56-61.
38. Tran PL, Hammond AA, Mosley T, Cortez J, Gray T, Colmer-Hamood JA, et al. Organoselenium coating on cellulose inhibits the formation of biofilms by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Appl. Environ. Microbiol. 2009;75:3586-3592.
39. Zonaro E, Lampis S, Turner RJ, Qazi SJ, Vallini G. Biogenic selenium and tellurium nanoparticles synthesized by environ-mental microbial isolates efficaciously inhibit bacterial planktonic cultures and biofilms. Frontiers in Microbiol. 2015;6:1-11.
40. Zannoni D, Borsetti F, Harrison JJ, Turner RJ. Thebacterial response to the chalcogen metalloids Se and Te. Adv. Microbiol. Physiol. 2008;53:1-72.
41. Pi J, Yang F, Jin H, Huang X, Liu R, Yang P, et al. Selenium nanoparticles induced membranebio- mechanical property changes in MCF-7 cells by disturbing

membrane molecules and F-actin. Bioorgan Med. Chem. Lett. 2013;23:6296-6303.
42. Khalifa HMS, Sameer WM. Control of the green mold of orange using fungicides alone and in combination with antioxidants. Middle East J. Appl Sci. 2014;4:200-206.
43. Sameer WM. Synergistic action of profenofos insecticide and selenium to some fungicides against tomato early blight (*Alternaria solani*). Afri. J. Mycol. Biotechol. 2013;18:37-53.
44. Zohri AA, Sabah MS, Mostafa ME. Effect of selenite and tellurite on the morphological growth and toxin production of *Aspergillus parasiticus* var. globosus IMI 120920. Mycopathol. 1997; 139:51-57.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13604