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Biological Synthesis of Silver Nanoparticles Exposed to Gamma Irradiation for Control of Early Blight Disease in Tomatoes

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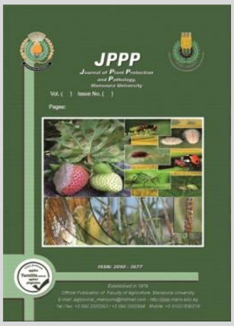

ABSTRACT

Early blight disease causes a significant loss in tomato production. This study focuses on the effective suppression of *Alternaria solani*, which causes huge losses in tomato yield. The study was conducted at a nuclear research center in Egypt. Infected plants were treated with irradiated and non-irradiated silver nanoparticles.The fungiced Mancozeb was used as a positive control. Three sprays were carried out at 10-day intervals. Two biological agents, *Alternaria alternata* and *Fusarium oxysporum,* were used to produce silver nanoparticles. AgNPs were exposed to several doses of gamma radiation (0, 1.5, 3, 6, 12 and 24 kGy) in order to enhance and maximize the effect of AgNPs on *Alternaria solani*. U.V., DLS, FITR, and TEM were used to characterize AgNPs, and AgNPs + gamma irradiation. Gamma irradiation decreased the size of AgNPs. All treatments, particularly AgNPs supported by gamma irradiation, reduced disease severity when compared to the untreated control. The highest shoot fresh weight was recorded in *A. alternata* AgNPs +3 kGy; 174.38 g as the mean of two seasons. The highest shoot dry weight was obtained by *A. alternata* AgNPs +24 kGy with a mean of 151 g. All treatments elevated peroxidase and catalase as well as total chlorophyll as compared with the untreated control and healthy plant. $AgNO_3$ decreased the efficacy of peroxidase (5). *Alternaria alternata* exhibited the lowest efficacy of catalase (9.09) after healthy plants and control. *F. oxysporum* + 24 kGy and *A. alternata* AgNPs + 24 kGy achieved the highest reduction of the mycelial growth *in vitro*

Keyword: Tomato, Early Blight, *Alternaria solani*, Silver nanoparticles, Gamma irradiation

INTRODUCTION

Tomatoes are considered one of the most vital and extensively grown vegetable crops worldwide (Chanthini *et al.,* 2018). Early blight disease, , caused by the pathogenic fungus *Alternaria solani* is a most destructive and causes sesvere lossess in tomato yield and quality, worldwide. The disease symptoms can be seen on the stems, leaves, and fruits of tomatoes (Tomazoni *et al*., 2018; Shoaib *et al*., 2019) *Alternaria solani* produce celluloses enzyme that break down the host cell wall and pectin methyl galacturonase that promotes host colonisation (Shahbazi *et al*., 2011). According to reports, silver nanoparticles (AgNPs) act as cellular system protectors while preventing microbial growth (Liu *et al*.,2010). The effects of the particles on plant and soil microflora during the tripartite interaction of plant pathogens and nanoparticles are unknown, even though numerous studies have demonstrated that silver nanoparticles have antifungal and antibacterial properties against plant diseases in field and greenhouse trials (Mishra *et al.,* 2014; Ocsoy *et al*., 2013). The nanomaterials are characterized using a variety of analytical techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and Fourier transform infrared spectroscopy (FTIR), (Zhang *et al.,* 2009). Synthesis of silver nanoparticles using fungus has attracted a lot of interest. The fungus is a separate group of organisms that produces various proteins and enzymes that can decrease and stabilize metal nanoparticles (Gajbhiye *et al.,* 2009; Li *et al.,* 2012). Gamma irradiation of silver solution resulted in the production of silver nanoparticles from1 to 4 nm (Hornebecq *et al.,* 2003). AgNPs exposure may lead to the accumulation of reactive oxygen species (ROS) in tomato seedlings, which in turn triggers the expression of genes involved in chlorophyll (Song *et al*., 2013). In addition to the root pathway, AgNPs can also be taken up through plant leaves (Geisler-Lee *et al.,* 2014). Biosynthesis and ultimately results in an increase in total chlorophyll content (Singh *et al*. 2019). Furthermore, AgNPs have been shown to interact with various biomolecules such as proteins and enzymes, which can interfere with the normal functioning of these molecules and ultimately lead to cell death (Gurunathan *et al*., 2016). This study aimed to examine the possible effects of AgNPs exposed to gamma irradiation to reduce early blight disease on tomato plants.

MATERIALS AND METHODS

Isolation of pathogen and disease evaluation

In the 2018 and 2019 growing seasons, various tests were performed to assess the effect of silver nanoparticles produced by biological agents to control tomato early blight disease. The nanoparticles were subjected to different dosages of gamma irradiation, 1.5 3.0, 6.12, and 24 Kilogray.Laboratory experiments were conducted at the Plant Research Center of the Atomic Energy Authority in Egypt (EAEA). The early blight-affected tomato plant leaves were gathered from several fields , El-Minia, Al-Sharqiyah, and Qalyubia. small sections of the diseased tissues were then taken off and the surface sterilized using a sodium hypochlorite solution. The tissues were then repeatedly cleaned with distilled-sterillized water. The edges of the sanitized pieces' fragments were air-dried before being placed on PDA medium in 9 cm Petri plates. and then incubated 27± at 12 h of light and 12 h of darkness according to Naik *et al.* (2010). Pure cultures of the growing fungs were kept on PDA slants at 5 and 10 degrees Celsius. (Singh, 1982., Barnett and Hunter's ,1987). Super strain B tomato plants were sown in 30-centimetre diameter plastic pots.. Three seedlings were

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transplanted into each pot contain sterilized soil. Each of the three replicate plants was inoculated with 30 mL of spore suspension ($5X10^6$/ ml) using an atomizer. Plants were sprinkled with the same quantity of distilled water as a control. After inoculation, the plants were covered with polyethylene sacks for 48 hours to increase the relative humidity and encourage spore germination. The plants were then maintained in greenhouse conditions after the bags were taken off.. After 45 days, disease severity were estimated. scale with six categories- 0 for not infected, 1 for sporadic places of infection less than 10% of the leaf area, 2 for greater than 10% >20%, 3 for 20% >30%, 4 for 30% >40%, and 5 for 40% of the leaf area was used to gauge the severity of the disease. The severity of the illness was calculated using the newly developed formula.

Diseases severity (%) = (nV / NV) x 100

Where:

n = the degree of infection as measured by the scale in the category with the highest rating.

V = the total number of samples evaluated for every category v = the number of samples evaluated in the category with the highest rating NV = the total number of samples (Townsend and Heuberger, 1943).

Biological synthesis of silver nanoparticles

Two species of fungi (*Alternaria alternata* and *Fusairum oxysporum*) were obtained from, Microbiological Resources Centres MIRCEN Ain Shams University for synthesis of AgNps. A reaction mixture without $AgNO_3$ was employed as a control (Devi and Joshi, 2012).

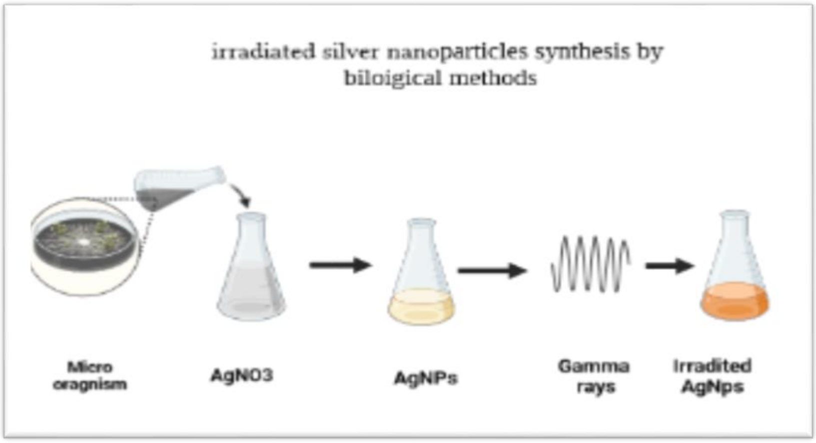

Fig. 1. Irradiate silver nanoparticles synthesis by biological agent

Irradiation Technique

The irradiation process took place at the Nuclear Research Institute. The silver nanoparticles were irradiated with gamma irradiation in a cyclotron project using a Cobalt-60 source (gamma cell) at specified doses of 0, 1.5, 3, 6, and 24 kGy.

Characterizations

At the Nawah Centre, characterizations using UV and DLS were carried out. (TEM) and (FTIR) procedures were carried out at Egypt's National Research Center.

U.V Vis. Spectrophotometer

AgNPs UV-Vis Spectra were recorded by UV Spectrophotometer as a function of frequency (JENWAY 6305). The UV-Vis spectrum of a spectrophotometer is 300 to 800 nm.

Dynamic Light Scattering (DLS)

The size distribution and average particle size were calculated using the Dynamic Light Scattering (DLS) Zetasizer (Nano-ZS Malvern). The irradiation process took place at the Nuclear Research Institute. The silver nanoparticles were irradiated with gamma radiation in a cyclotron project using a Cobalt-60 source (gamma cell) at specified doses of 0, 1.5, 3, 6, and 24 kGy.

Transmission Electron Microscopy (TEM)

Size and form measurements of the produced nanoparticles were made using TEM JEOL, JEM-2100 Tokyo, Japan.

Fourier Transform Infrared (FT-IR)

FT-IR measurements were carried out to identify the chemical groups that are present around AgNPs to ensure their stability and to reach a conclusion about the modification of functional groups brought on by the reduction process measurement tools were used to (FT-IR VERTEX 80 Germany).

Spectrophotometric techniques were used to measure the absorption at 430 nm, which represents the peroxidase activity as described by Allam and Hollis., (1972).

Activity of catalase (CAT)

According to, the oxidation of H_2O_2 at 240 nm catalase was determined (Nogueirol et al., 2015).

Antifungal test

Silver nanoparticles was evaluated *in vitro* for their **efficacy** against the a virulent *A. solani* isolate; each flask contained 500 mL of each treatment and 45 mL of warmed PDA **medium** and was carefully **shaken.** The mixture was then poured **into sterilized Petri dishes** (9 cm Ø) **at** a constant volume of 10 **mL** and allowed to **solidify.** The control was distilled water. 7-day-old **fungal** cultures were placed in the **center** of Petri dishes with **mycelial** discs (5 mm Ø) **extending** from the edge. **Plates** were kept in an **incubator at 27** ± 1**°C.** When **the** mycelium completely **covers** the surface **of** the control **treatment medium,** the **test is terminated. Two diagonal lines were drawn at** the back of each Petri **dish** to **estimate** the amount of fungal growth (Sallam 2017).

Filed experiment

This experiment was conducted at the Experimental Farm of Research Plants, Nuclear Research Centre, Egyptian Atomic Energy Authority, during two successive seasons (2018 and 2019) from August to November using seeds for the tomato cultivar Super Strain B in order to assess the severity of the early blight disease, shoot fresh weight and shoot dry weight by (3.5×3) m^2 field plots were planted with three rows and four plants per row using a fully randomized block design. Three plots were used as replications for each treatment as well as the untreated control treatment. Tomato seedlings from the Super Strain B variety were transplanted at 28 days old and were provided with all recommended farming practices, such as irrigation and fertilization. Seedlings were later sprayed three times, each ten days apart. After the previously stated five days of spraying, the disease's severity was evaluated.

The study consisted of 18 treatments that were each replicated three times in a field experiment. *F.oxysporum* AgNPs, *F.oxysporum* AgNPs +1.5 kGy, *F.oxysporum* AgNPs +3 kGy, *F.oxysporum* AgNPs +6 kGy, *F.oxysporum* AgNPs +12 kGy, *F.oxysporum* AgNPs +24 kGy, *A.alternata* AgNPs, *A.alternata* AgNPs +1.5 kGy, *A. alternata* AgNPs +3 kGy, *A.alternata* AgNPs +6 kGy, *A.alternata* AgNPs +12kGy, *A. alternata* AgNPs +24kGy Control (infected), healthy plant, $AgNO_3$+, *F.oxysporum*, *A. alternata*, and Mancozeb 200 g/100 L.

Statistical Analysis

The results were statistically examined using SPSS 14.0. (ANOVA). The means for all comparisons and measurements were fixed at $P \leq 0.05$ in accordance with Duncan's multiple range tests. (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Results

Characterization of silver nanoparticles

Characterization of irradiated silver nanoparticles prepared by *Fusarium oxysporum* and *Alternaria alternata*, conducted by using a UV spectrophotometer, DLS, FTIR, and TEM

U.V Spectrophotometry.

Fig. 2 shown UV for preliminary confirmation of *A.alternata* AgNPs**.** Results show that a peak was seen between 410 and 450 nm after the color change of the *A. alternata* AgNPs irradiated by 0,1.5,3,6,12 and 24 kGy the color of filtrate changed to brown-dark after the addition of $AgNO_3$. Although color changed after irradiated 3- days incubated. Particularly, dose at 24 kGy the colloidal silver still stable.

Dynamic Light Scattering (DLS)

Figures. 4 a, b, c, and d show the average particle size determined by DLS for *A. alternata* AgNPs at doses of 1.5 and 24 kGy, as well as *F. oxysporum* AgNPs *at* 1.5 and 24 kGy. However, it was of average size for the mentioned treatments (89, 26, 90, and 28 nm, respectively). On the other hand, a dose of 24 kGy decreases the size of the nanoparticle for both biological agents as compared with 1.5 kGy.

Fourier Transform Infrared Spectroscopy (FT-IR)

The reduction of the Ag+ ions and the protein molecules that serve as capping agents are investigated using Fourier transform infrared spectroscopy (FT-IR) measurements. The FT-IR spectrum of silver nanoparticles is displayed in Fig 3.

Transmission Electron Microscope (TEM)

A. *alternata* AgNPs *F. oxysporum* AgNPs measured by TEM analysis of the solution containing these particles revealed particles in the nano range less than 100 nm (Fig 5a-b)

Characterization irradiated AgNPs and non-irradiated using DLS- FTIR- TEM

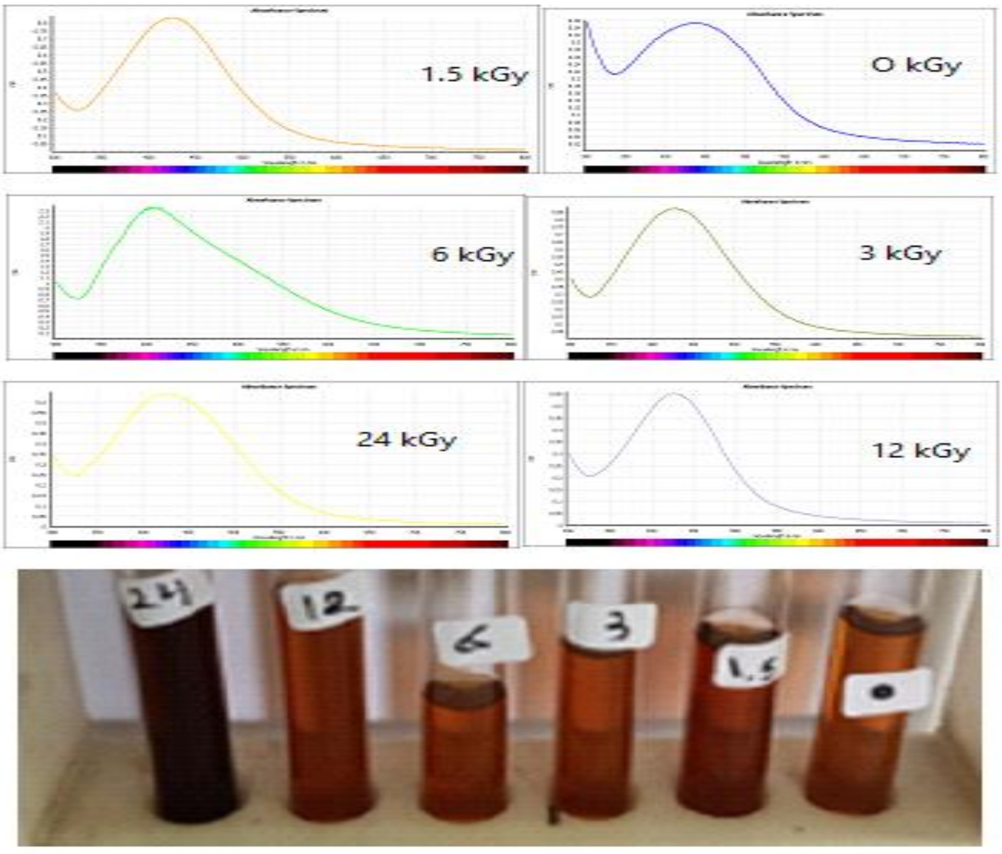

Fig. 2. (U.V) *A. alternata* AgNPs after irradiated with several doses of gamma irradiation .Three days incubated

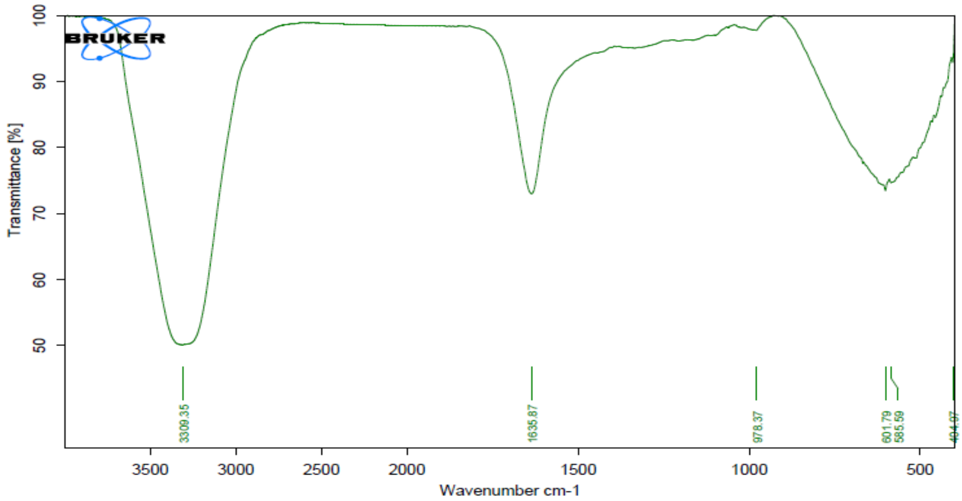

Fig. 3. (FTIR) *Fusarium oxysporum* AgNPs non-irradiated

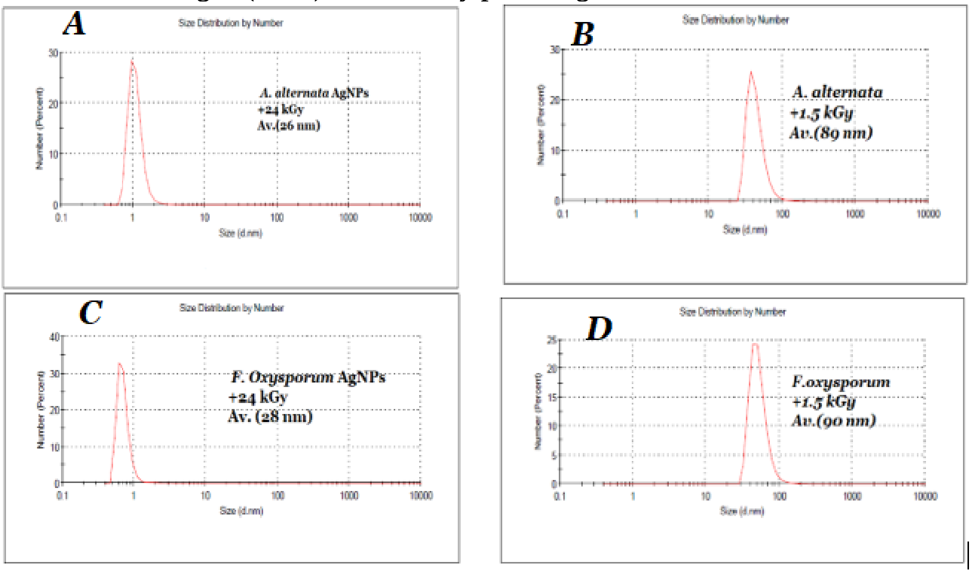

Fig 4. (DLS) irradiation AgNPs produced form biological agents with two different doses of gamma *(A): Alternaria alternata* +24 kGy *(B): A. alternata* +1.5 kGy (c): *F. oxysporum*+24 kGy (D): *F. oxysporum*+1.5 kGy.

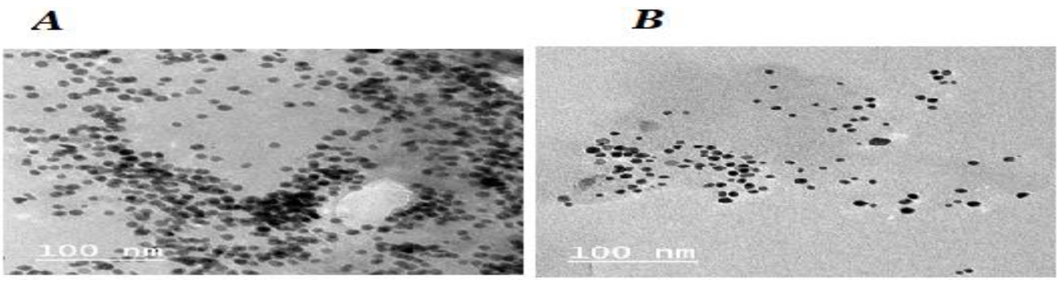

Fig.5 Transmission electron microscope (TEM) A): *A. alternata* AgNPs B): F. *oxysporum*AgNPs

Effect of *F. oxysporum* AgNPs and *A. alternata* AgNPs at certain doses of gamma irradiation on linear growth of *Alternaria solani in vitro.*

Data in Table 1 show that *F. oxysporum* + 24 kGy and *A.alternata* AgNPs + 24kGy caused the highest reduction of the mycelial growth of *A. solani* (100% both of them), followed by *F. oxysporum* + 6 kGy, *A.alternata* AgNPs + 3 kGy, and Mancozeb (96.29%, 93.82% and 91.35% respectively,). However, $AgNO_3$ inhibited the pathogen's mycelium growth the least (11.11%).

Efficacy of irradiated and non-irradiated silver nanoparticles as foliar applications on the disease severity of tomato early blight.

The data in Table 2 show the impact of all silver nanoparticles, irradiated and non-irradiated, synthesized by biological methods using *Fusarium oxsporum* and *Alternria alternata* on disease severity against early blight disease. All treatments reduced disease severity in both seasons, through a mean of three sprays for each season. In the first season, *F. oxysporum* AgNps + 24 kGy resulted in the lowest disease severity (4.23), followed by, as compared to the control (untreated) (44.11), *F. oxysporum* AgNps + 12 kGy and *F. oxysporum* AgNps + 6 kGy (5.46 and 6.61, respectively). The second season showed the same trend: *F. oxysporum* AgNPs +24 kGy achieved the lowest disease severity (4.13), followed by *F. oxysporum* AgNps +12 kGy and *F. oxysporum* AgNps +6 kGy (6.43 and 7.10, respectively). However, AgNPs and AgNps+ gamma irradiation significantly reduced disease severity while *F.* oxysporum, *A. alternata*, and AgNO3 achieved the highest disease severity, but they were more effective as compared to the control.

Effect of irradiated and non-irradiated silver nanoparticles as foliar applications on shoot fresh and dry weight.

Table 3 shows the effect of two techniques for the biological synthesis of silver nanoparticles using *F. oxysporum* and *A. alternata* on shoot fresh weight and shoot dry weight of infected tomato plants during both seasons of 2018 and 2019. All treatments increased both growth parameters. The highest shoot fresh weight was observed with *A. alternta* +3 kGy; 174.38 g, followed by *F. oxsyporum* AgNPs +6 kGy and *A. alternata* +6 kGy (173.93 and 173.53 g, respectively) in the mean of the two seasons. In comparison to the untreated control (106.75 g). On the other hand, the highest shoot dry weight was obtained by *A. alternata* + 24 kGy with a mean of 151 g, followed by *F. oxsyporum* AgNPs + 24 kGy and Mancozeb (147 and 143.17 g, respectively.

Table 1. Effect of *F. oxysporum* AgNPs, *A. alternata* AgNPs at certain doses of gamma Irradiation on linear growth of *Alternaria solani in vitro*

Treatment	Mycelia Linear Growth (L.G) (mm)	Reduction %
Mancozeb 200g/100 L	7	91.35
F. oxysporum AgNPs	28	65.43
F. oxysporum AgNPs +1.5 kGy	23	71.60
F. oxysporum AgNPs+ 3 kGy	10	87.65
F. oxysporum AgNPs+ 6 kGy	3	96.29
F. oxysporum AgNPs +12 kGy	9	88.88
F. oxysporum AgNPs +24 kGy	0	100
*A.altrnata*AgNPs	21	74.07
A.alternata AgNPs + 1.5 kGy	19	76.54
A.alternata AgNPs + 3 kGy	5	93.82
A.alternata AgNPs + 6kGy	0	100
A.alternata AgNPs + 12kGy	12	85.18
A.alternata AgNPs + 24kGy	0	100
$AgNO_3$	72	11.11
control	81	0

Table 2. The effect of irradiated and non-irradiated silver nanoparticles produced by biological agents as foliar applications on Early blight disease severity

Treatment	Disease Severity							
	First Season				Second Season			
	1stSpray After 25 days	2ndSpray After 35 days	3rd Spray After 45 days	Mean	1st Spray After 25 days	2nd Spray After 35 days	3rdSpray After 45 days	Mean
$AgNO_3$	22.33 ^{j}	24.33 ^{f}	26.33 ^{j}	24.33 ^{h}	23.66 ^{g}	26.33 ^{g}	27.00 ^{j}	25.66 ^{h}
F. oxysporum	25.33 ^{jk}	25.10 ^{f}	27.41 ^{j}	25.95 ^{h}	25.60 ^{h}	27.33 ^{g}	29.93 ^{k}	27.62 ^{h}
F. oxysporum AgNPs	13.33 ^{hi}	14.02 ^{e}	15.93 ^{i}	14.43 ^{g}	14.60 ^{f}	17.02 ^{f}	18.23 ^{i}	16.62 ^{g}
F. oxysporum AgNPs +1.5 kGy	12.33 ^{hi}	12.50 ^{de}	13.30 ^{gh}	12.71 ^{fg}	13.40 ^{f}	14.00 ^{de}	16.22 ^{h}	14.50 ^{fg}
F. oxysporum AgNPs +3 kGy	7.33 ^{cd}	7.99 ^{bc}	8.20 ^{cd}	7.84 ^{bc}	8.30 ^{d}	11.33 ^{cd}	11.6 ^{fg}	10.41 ^{de}
F. oxysporum AgNPs +6 kGy	5.83 ^{bc}	6.30 ^{ab}	7.70 ^{bc}	6.61 ^{bc}	6.80 ^{bc}	6.70 ^{a}	7.80 ^{bc}	7.10 ^{bc}
F. oxysporum AgNPs +12 kGy	4.21 ^{b}	5.95 ^{ab}	6.23 ^{ab}	5.46 ^{ab}	5.3 ^{b}	6.90 ^{a}	7.10 ^{b}	6.43 ^{ab}
F. oxysporum AgNPs +24 kGy	3.06 ^{a}	4.60 ^{a}	5.02 ^{a}	4.23 ^{a}	3.00 ^{a}	4.10 ^{a}	5.30 ^{a}	4.13 ^{a}
A. alternata	30.91 ^{l}	32.03 ^{g}	33.25 ^{k}	32.06 ^{i}	28.6 ^{i}	30.60 ^{h}	33.23 ^{l}	30.81 ^{i}
A. alternata AgNPs	12.08 ^{hi}	14.05 ^{e}	14.20 ^{h}	13.44 ^{g}	13.06 ^{f}	14.85 ^{e}	15.30 ^{h}	14.40 ^{fg}
A. alternata AgNPs +1.5 kGy	10.90 ^{gh}	12.60 ^{de}	13.6 ^{fg}	12.37 ^{fg}	11.30 ^{e}	12.10 ^{d}	13.03 ^{g}	12.14 ^{ef}
A. alternata AgNPs +3 kGy	9.90 ^{fg}	10.60 ^{cd}	12.36 ^{f}	10.95 ^{ef}	8.37 ^{d}	9.63 ^{bc}	10.63 ^{ef}	9.54 ^{cd}
A. alternata AgNPs +6 kGy	8.53 ^{ef}	8.86 ^{bc}	12.30 ^{f}	9.90 ^{de}	8.10 ^{d}	9.04 ^{b}	10.20 ^{de}	9.11 ^{bc}
A. alternata AgNPs +12 kGy	7.84 ^{de}	8.33 ^{bc}	10.63 ^{ef}	8.93 ^{cd}	7.30 ^{cd}	8.50 ^{ab}	9.04 ^{cd}	8.28 ^{bc}
A. alternata AgNPs +24 kGy	6.60 ^{bc}	7.65 ^{bc}	9.46 ^{de}	7.90 ^{cd}	6.22 ^{bc}	7.60 ^{ab}	8.36 ^{bc}	7.39 ^{bc}
Mancozeb 200g/100L	8.10 ^{de}	9.27 ^{c}	9.73 ^{de}	9.03 ^{cd}	8.36 ^{d}	9.33 ^{bc}	10.43 ^{ef}	9.37 ^{cd}
Control un treated	42.00 ^{m}	44.00 ^{i}	46.33 ^{l}	44.11 ^{j}	43.00 ^{j}	46.00 ^{i}	47.00 ^{m}	45.33 ^{j}

Values assigned to similar letters are not significantly different (*$P \leq 0.05$*) According Duncan's multiple range tests. Values are the means of three replicates. Disease severity was detected 5 days after each spray.

Table 3. The effect of irradiated and non-irradiated silver nanoparticles produced by biological agents as foliar applications on shoot fresh weight and shoot dry weight

Treatment	Gamma Irradiation (KGy)	Shoot Fresh Weight (g)		Mean	Shoot Dry Weight (g)		Mean
		1stseason	2nd Season		1st season	2nd season	
	0	136.00 ^{c}	118.30 ^{c}	127.15	91.50 ^{b}	96.46 ^{b}	93.98
	1.5	160.70 ^{fg}	164.97 ^{fg}	162.84	113.80 ^{d}	120.13 ^{d}	116.97
Fusarium oxysporum	3	163.83 ^{g}	174.53 ^{h}	169.18	126.56 ^{e}	130.86 ^{e}	128.71
AgNPs	6	171.23 ^{h}	176.63 ^{h}	173.93	136.10 ^{gh}	143.80 ^{f}	139.95
	12	152.00 ^{e}	156.90 ^{e}	154.45	127.73 ^{e}	131.70 ^{e}	129.72
	24	174.03 ^{h}	167.23 ^{g}	170.63	142.33 ^{g}	151.66 ^{g}	147.00
	0	133.17 ^{c}	141.97 ^{bc}	137.57	88.37 ^{b}	92.13 ^{b}	90.34
	1.5	141.00 ^{d}	143.90 ^{d}	142.45	104.40 ^{c}	113.10 ^{c}	108.75
Alternaria alternata	3	171.53 ^{h}	177.23 ^{h}	174.38	133.10 ^{f}	119.00 ^{d}	126.05
AgNPs	6	171.53 ^{h}	175.53 ^{h}	173.53	134.30 ^{fg}	135.10 ^{e}	134.70
	12	158.53 ^{f}	162.46 f	160.49	113.60 ^{d}	117.80 ^{d}	115.70
	24	170.20 ^{h}	174.53 ^{h}	172.37	148.30 ^{i}	153.70 ^{g}	151.00
AgNo3	0	141.05 ^{e}	144.33 ^{d}	142.96	123.33 ^{e}	130.50 ^{e}	126.92
Fusarium oxysporum	0	125.00 ^{ab}	114.00 ^{b}	119.50	91.50 ^{b}	92.13 ^{b}	94.82
Alternaria alternata	0	128.00 ^{b}	116.00 ^{b}	122.00	88.37 ^{b}	96.46 ^{b}	92.42
Mancozeb 200g/100L	0	170.33 ^{i}	175.20 ^{h}	172.77	138.03 ^{gh}	148.30 ^{g}	143.17
Control (infected)	0	112.20 ^{a}	101.30 ^{a}	106.75	60.20 ^{a}	64.83 ^{a}	60.52

Values in the same column assigned to similar letters are not significantly different (*$P \leq 0.05$*) According Duncan's multiple range tests. Values are the means of three replicates.

Efficacy of irradiated and non-irradiated silver nanoparticles as foliar applications on the severity and yield of early blight in tomato

The results in Table 4 show that all treatments specifically containing AgNPs achieved increased tomato yield compared to the control. In the first seasons, the highest yield was achieved by *F. oxysporum* AgNPs + 24 kGy recorded 49308 kg fed-1, followed by *A. alternaria* AgNPs + 12kGy and *A. alternaria* AgNPs + 6kGy which recorded 47900 and 46210 kg fed-1 respectively compared with 24102 kg fed-1 in the control. As for the second season, AgNPs +24 kGy followed by Mancozeb recorded the highest that 45209 and 44517 respectively compared with 23524 kg fed-1 in the control. On the average of two seasons, the highest yield was achieved by *F. oxysporum* AgNPs + 24 kGy followed by *A. alternaria* AgNPs + 6kGy. The least effective treatment in increasing yield was *A. alternaria.*

Table 4. The effect of irradiated, non-irradiated silver nanoparticles as foliar applications produced by biological agents on yield.

Treatment	Yield kg fed^{-1}		Mean	
	First season	Second Season	Yield kg fed^{-1}	Yield kg ha^{-1}
$AgNO_3$	33206 ^{e}	32114 ^{e}	32660.00	80670.20
F. oxysporum	27653 ^{c}	26540 ^{c}	27096.50	66928.40
*F. oxysporum*AgNPs	36609 ^{h}	37883^{h}	37246.00	66838.20
*F. oxysporum*AgNPs +1.5 kGy	38207 ^{g}	36231 ^{f}	37219.00	91930.90
*F. oxysporum*AgNPs +3 kGy	31704 ^{d}	32114 ^{e}	31909.00	78815.20
*F. oxysporum*AgNPs + 6 kGy	35413 ^{f}	37887 ^{h}	36650.00	90525.60
F. oxysporum AgNPs + 12 kGy	39708 ^{h}	39186 ^{i}	39447.00	97434.10
F. oxysporum AgNPs + 24 kGy	49308 ^{n}	48605 ^{p}	48956.50	120922.60
A. alternata	26319 ^{b}	26303 ^{b}	26311.00	64988.20
A. alternata AgNPs	41510 ^{i}	43404 ^{k}	42457.00	104868.80
A. alternata AgNPs + 1.5 kGy	36611 ^{h}	36300 ^{g}	36455.50	90045.10
A. alternata AgNPs + 3 kGy	43610 ^{j}	43303 l	43456.50	107337.60
A. alternata AgNPs + 6kGy	46210 ^{l}	47904 ^{o}	47057.00	116230.80
A. alternata AgNPs + 12kGy	47900 ^{m}	45216 ^{n}	46558.00	114998.30
A. alternata AgNPs + 24kGy	41306 ^{i}	43179 ^{j}	42242.50	104338.90
Control infected	24102 ^{a}	23524 ^{a}	23813.00	58818.10
Mancozeb 200g/100 L	44397 ^{k}	44517 ^{m}	44457.00	109808.80

Values assigned to similar letters are not significantly different ($P \leq 0.05$) According Duncan's multiple range tests. Values are the means of three replicates.

Effect of irradiated and non-irradiated silver nanoparticles and some foliar applications on total chlorophyll

Data in Table 5 represent the efficacy of different treatments in terms of the total chlorophyll content (in mg g-1 FW). The control (untreated) had the lowest total chlorophyll (2.1 mg g-1 FW), while the treatment with *A. alternata* AgNPs at 1.5 kGy had the highest total chlorophyll (25.4 mg g-1 FW) with efficacy 1109.52. AgNO3 resulted in the lowest total chlorophyll after control treatment (5.3 mg g-1 FW); efficacy was 152.38; and *F. oxysporum* AgNPs + 24 kGy treatments also showed an increase in total chlorophyll (25.1 mg g -1FW**).** Overall, the treatments with *A. alternata*AgNPs + 1.5 kGy and *F. oxysporum* AgNPs + 24 kGy were the most effective in improving the total chlorophyll content.

Effect of irradiated and non-irradiated silver nanoparticles as foliar applications on oxidative enzymes activity

The data illustrated in Table 6 shows the efficacy of AgNPs and AgNPs + gamma irradiation as foliar applications on peroxidase and catalase efficacy. However, the healthy plant had the lowest activities of peroxidase and catalase (11 and 8, respectively) with the lowest efficacy (-45 and -27.27, respectively). On the other hand, the rest treatments elevated both peroxidase and catalase activity. The highest peroxidase activity was achieved by Mancozeb and *F. oxysporum* AgNPs (36) in each treatment. On the other hand, the catalase activity showed the same trend: Mancozeb, *F.oxysporum,* and AgNps A*.alternata* significantly elevated the catalase (30) in each treatment as compared with the control (11). Meanwhile, AgNO3 decreased the efficacy of peroxide (5). *A. alternata* exhibited the lowest efficacy of catalase (9.09) after healthy plants and control.

Table 5. The effect of irradiated and non-irradiated silver nanoparticles and some foliar applications produced by biological agents on total chlorophyll.

Treatement	Total Chlorophyll mg g^{-1} FW	Efficacy
Healthy plant	26 ^{o}	1138.10
Control infected	2.1 ^{a}	0
Mancozeb 200g/100 L	15.2 ^{e}	623.81
*F. oxysporum*AgNPs	23.1 ^{l}	1000.00
*F. oxysporum*AgNPs +1.5 kGy	21.6 ^{j}	928.57
*F. oxysporum*AgNPs +3 kGy	20.2 ^{i}	861.90
*F. oxysporum*AgNPs + 6 kGy	18.8 ^{g}	795.24
F. oxysporum AgNPs + 12 kGy	24.4 ^{m}	1061.90
F. oxysporum AgNPs + 24 kGy	25.1 ^{n}	1095.24
A. alternata AgNPs	22.3 ^{k}	2020.00
A. alternata AgNPs + 1.5 kGy	25.4 ^{n}	1109.52
A. alternata AgNPs + 3 kGy	24.6 ^{m}	1071.43
A. alternata AgNPs + 6kGy	20.1 ^{i}	857.14
A. alternata AgNPs + 12kGy	19.2 ^{h}	814.29
A. alternata AgNPs + 24kGy	17.0 ^{f}	709.52
$AgNO_3$	5.3 ^{c}	152.38
F. oxysporum	4.6 ^{b}	119.05
A. alternata	6.9 ^{d}	228.57

Table 6. The effect of irradiated and non-irradiated silver nanoparticles produced by biological agents as foliar applications on peroxidase and catalase enzyme.

Treatement	Peroxidse	Catalase	Effcacy %	
			Peroxidse	Catalase
Healthy plant	11 ^{a}	8 ^{a}	-45	-27.27
Control infected	20 ^{b}	11^{b}	0	0.00
Mancozeb 200g/100 L	36 ^{k}	30 ^{i}	80	172.73
*F. oxysporum*AgNPs	36 ^{k}	30 ^{i}	80	172.73
*F.oxysporum*AgNPs+1.5kGy	31 ^{i}	21 ^{cd}	55	90.91
*F.oxysporum*AgNPs+3kGy	28 ^{h}	28 ^{h}	40	154.55
*F.oxysporum*AgNPs+6kGy	27 ^{gh}	26 ^{fg}	35	136.36
F.oxysporum AgNPs+12kGy	26 ^{fg}	25 ^{f}	30	127.27
F.oxysporum AgNPs+24kGy	23 ^{cd}	22 ^{de}	15	100.00
A. alternata AgNPs	30 ^{i}	27 ^{gh}	50	145.45
A. alternata AgNPs + 1.5kGy	33 ^{j}	21 ^{cd}	65	1000.00
A. alternaria AgNPs + 3 kGy	26 ^{fg}	23 ^{e}	30	109.09
A. alternata AgNPs + 6kGy	25 ^{ef}	22 ^{de}	25	100.00
A. alternata AgNPs + 12kGy	23 ^{cd}	21 ^{cd}	15	90.91
A. alternata AgNPs + 24kGy	33 ^{j}	30 ^{i}	65	172.73
$AgNO_3$	21 ^{c}	20 ^{c}	5	81.82
F. oxysporum	26 ^{fg}	21 ^{cd}	30	47.62
A. alternata	24 ^{de}	12 ^{b}	20	9.09

***Values assigned to similar letters are not significantly different (*$P \leq 0.05$*) According Duncan's multiple range tests. Values are the means of three replicates.**

***Peroxidase activity was expressed as the increase in absorbance at 430 nm/gram fresh weight/15 minutes.**

*** Catalase activity was expressed as the increase in absorbance at 240nm/g fresh weigh/ minutes**

Discussion

The present work demonstrated that silver nanoparticles could be used as alternatives chemical fungicide to control early blight disease of tomato plant caused by *Alternaria solani*. Several studies reported that AgNPs have been demonstrated to possess a variety of antifungal abilities, including those against contagious fungal plant diseases. (Rai *et al.,* 2014). Included in these are *Rhizoctonia solani*, *Curvularia lunata*, *Alternaria solani*, *Colletotrichum sp*., and *Fusarium sp*. (Bera *et al*., 2014 and Balakumaran *et al.* ,2015, The data of DLS indicated that gamma radiation can decrease the size of silver nanoparticles. These outcomes are consistent with Flores *et al.,* 2020 who indicated that gamma irradiation-induced synthesis of NPs may offer special benefits, such as the capacity to adjust size, shape, and scalability with few steps; Using fewer chemical reagents or nontoxic solvents, fewer toxic or non-toxic precursors, and generating fewer reaction byproducts and hazardous waste results in a more environmentally friendly process. In the present work biological synthesis was conducted, the colloidal silver still stability after three days on incubation also the gamma radiation has not negative effect for stability Similarly, Durán *et al.,* (2011) they reported that the bioproducts of the metabolism of organisms, including bacteria, fungus, and plants, which act as reducing and stabilizing agents, can be used in the biogenic synthesis of nanoparticles.. Biomolecules from the organism employed in the synthesis are used to cap these nanoparticles, which can increase stability and possibly exhibit biological activity (Ballotin *et al*., 2016). Greater biocompatibility is provided via biological synthesis, which is relatively easy to do, clean, sustainable, and affordable (Gholami-Shabani *et al*., 2014). U.V characterization of all AgNps treatments shows the beak range between (400-450 nm). Similarly, with Devi and Joshi (2015) synthesized silver nanoparticles using the endophytic fungi *Aspergillus tamarii*, and *Aspergillus niger*, UV-Vis absorption analysis revealed peaks at 419, 430, and 430 nm, respectively. A peak at 280 nm was attributed to the presence in the filtrate of amino acid residues such as tryptophan and tyrosine, which were secreted by the fungi. All silver nano particles that syntheses by biological methods increased shoot fresh weight and shoot dry weight compared with control. Results indicated that the diseases severity had decreased with all treatments of silver nanoparticles due to the properties of AgNPs and their small size that make silver nanoparticles can adhere to the cell walls and membrane of *Alternaria solani*. Similarly, Kumari *et al.,* (2017) they reported that the nanoparticles were able to reduce the pathogenic population of *A. solani* of tomato both *in vitro* and *in vivo*. Nanoparticles can enter the interior of cells by adhering to their cell walls and membranes in bacteria. They affect the signal transduction pathways, harm cellular structures, and produce reactive oxygen species (Kim *et al.,* 2001 and Dakael *et al.,* 2016). The obtained show the nanoparticles enhanced total chlorophyll and oxidative enzymes. These results agree with Torres *et al.,* (2006) who indicated that significant increase in oxidative enzymes was also observed when plants were pre-treated with SNP. Control infected untreated has been clearly symptoms. However, after applying SNP, the symptoms were clearly lessened, and the plant appeared to be as healthy (Ocsoy *et al*., 2013). Another proposed mechanism is that AgNPs produce reactive oxygen species (ROS), which can cause oxidative stress in the cells of the fungi. This oxidative stress can damage cellular components such as DNA, proteins, and lipids, leading to cell death (Yah *et al.,* 2015).

CONCLUSION

The current study showed the it is possible to biologically synthesis of silver nanoparticles using *Alternaria alternata* and *Fusarium oxysporum*, as well as the irradiation of the silver nanoparticles at various gamma doses. Gamma irradiation decreases the size of AgNPs. Plant growth including shoot fresh weight and shoot dry weight was increased by irradiated or non-irradiated silver nanoparticles. Also, total chlorophyll, peroxidase, and catalase enzymes avtivity in treated plants have been elevated. Furthermore, AgNPs and irradiated AgNPs reduced Early blight disease severity.

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جزيئات الفضة النانوية المشععة بأشعة جاما والتي تم تخليقها بواسطة العوامل الحيوية لمكافحة مرض اللفحة المبكرة فى الطماطم

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الملخص

أجريت هذه الدراسة فى هيئة الطاقة الذرية مركز البحوث النووية وهدفت الدراسة إلى مكافحة مرض اللفحة المبكرة الذى يسبب خسائر فادحة فى محصول الطماطم. أستخدمت جزيئات الفضة النانوية (AgNPs)والتى تم توليفها بطريقة التخليق الحيوي باستخدام فطري (*Alternaria alternata*) *وفطر* *Fusarium oxysporum*. تم تشعيع جزيئات الفضة النانوية لتحسين خواصها الفيزيائية والكيمائية بعدة جرعات من أشعة جاما هى (0 و 1.5 و 3 و 6 و 12 و24) كيلو جراي. تم استخدام المبيد الفطرى Mancozeb للمقارنة. تم تقييم تأثير المعاملات المختلفة على شدة المرض في الحقل ضد المسبب المرضى لمرض اللفحة المبكرة فى الطماطم *Alternaria solani* وكذلك على نمو النبات وبعض القياسات الحيوية. تم استخدامU.V و FT-IR و DLS و TEM لتوصيف جزيئات الفضة النانوية خلال موسمين لنمو الطماطم وتم حساب النتائج كمتوسط. تحقق اعلى وزن طازج للنبات مع معاملات (*Alternaria alternata* AgNPs) +3 كيلو جراى (174.38 جرام). وكان أعلى وزن جاف قد تحقق مع المعاملة (*A. alternata* AgNPs) +24 كيلو جراى (151 جرام). جميع المعاملات أدت إلى زيادة نشاط إنزيم البيروكسيديز وإنزيم الكتاليز وكذلك زيادة المحتوى الكلى من الكلوروفيل مقارنة بالنباتات السليمة والنبات المعدى غير المعامل وتم رش النباتات بجميع المعاملات بثلاث رشات متتالية عند عمر 25 و35 و45 يوم وقياس شدة المرض بعد كل رشة من هذه الرشات .حققت جميع معاملات النانو فضة المشععة والغير مشععة خفض شدة الإصابة بصورة معنوية مقارنة بالنباتات المصابة (الكنترول) بينما معاملة محلول نترات الفضة ومعلق الجراثيم *Alternaria Alternata*و *Fusariurm oxysorum* تحقق معهم اختزال طفيف فى شدة الإصابة مقارنة بمعاملة الكنترول (النباتات المعدية الغير معاملة)،كما أدت جميع المعاملات زيادة فى نسبة المحصول مقارنة بالكنترول . معظم الجرعات الإشعاعية العالية أدت إلى تحسين فاعلية جزيئات الفضة وزيادة تثبيطها لنمو مسيليوم فطر *Alternaria solani* تحت ظروف المعمل حيث أدت الجرعات الإشعاعية 24 كيلو جراى لكل من *F. oxysporum*AgNPs) و*A. alternata* AgNPs)إلى نسبة خفض وصلت إلى 100%.