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Irradiated Silver Nanoparticles Synthesized by Plant Extracts and Their Effect on Early Blight Disease of Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

Nanoparticles of silver (AgNPs) were prepared by the green synthesis method using neem (*Azadirachta indica*) and lemon leaf extract. AgNPs were irradiated with several doses of gamma irradiation: 0, 1.5, 3, 6, 12, and 24 kGy. AgNPs were tested in the field against *Alternaria solani*, a tomato disease that causes early blight. There was a notable rise in various growth and physiological measurements. Every aspect of the plant's performance, including physiological traits and crop yield, showed an improvement. FT-IR, TEM, UV-Vis, and DLS are used to characterize AgNPs. The results were computed as an average of two seasons. Application of neem AgNPs under 6 kGy and lemon AgNPs under 12 kGy were used as foliar spraying. A highly effective inhibitor of disease severity was obtained after mancozeb as compared with control (untreated). The improvement of all aspects of the plant was observed as well as yield, compared with the control (untreated). The results also showed that there were significant differences in enzymes activity compared with the infected control (untreated). The highest value of peroxidase activity occurred with neem AgNPs under 3 kGy (39). The highest value of catalase was obtained by combining lemon AgNPs with 6 kGy (31). The highest value of polyphenol oxidase was achieved by neem AgNPs under 1.5 kGy (35). All treatments were evaluated for total chlorophyll content in comparison to the control treatment, with the healthy plant achieving the highest value (26), followed by the neem AgNPs under 6 kGy, which recorded 23.1.

Key words: Early Blight, Silver Nanoparticles, *Alternaria solani*, Gamma Irradiation, Green Synthesis, Characterization, Oxidative Enzymes, Chlorophyll, Neem.

INTRODUCTION

One of the most important and widely cultivated vegetable crops in the world is the tomato (Ahmed *et al.*, 2017; Chanthini *et al.*, 2018). The tomato is vulnerable to a number of bacterial, viral, nematode, and fungal diseases (Verma *et al.,* 2018). Early blight, Fusarium wilt, damping-off, late blight, tomato mosaic virus, Verticillium wilt, and bacterial wilt are the most common tomato diseases. Tomato stems, leaves, and fruits show indications of the disease. Disease development is triggered by a crowded plantation, excessive rainfall, and a protracted period of leaf wetness (Gondal *et al.,* 2012). If this disease is not controlled, yields may suffer (Malik *et al.,* 2014). Silver nanoparticles (AgNPs) have been reported as protective agents for cellular systems while inhibiting microbial growth (Liu *et al.,* 2010). Although several studies have shown

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that silver nanoparticles have antifungal and antibacterial properties against plant diseases in the field and greenhouse. The effects of particles on plants and soil microflora during the tripartite interaction of plant pathogens and nanoparticles are unknown (Mishra *et al.,* 2014; Ocsoy *et al.,* 2013). Many processes can produce silver nanoparticles, such as green synthesis, chemical, and physical reduction processes (Narayanan *et al.,* 2005), or possibly a combination of ultrasonic and gamma irradiation (Ahmad *et al.,* 2009). Numerous analytical methods, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), Infrared spectroscopy using a Fourier transform infrared (FTIR), X-ray diffractometry (XRD), and X-ray photoelectron spectroscopy (XPS) were used to characterize the nanomaterials (Zhang *et al.,* 2009). The primary characterization of produced nanoparticles can be accomplished with the help of UV-vis spectroscopy, which is also used to track the synthesis and stability of AgNPs (Sastry *et al.,* 2018). Dynamic light scattering can measure the size distribution of tiny particles in suspension or solution at scales from submicron to one nanometer (Lin *et al*., 2014). The effective, well-liked, and significant of TEM technique is employed to assess particle or grain size, size distribution, and morphology (Joshi and Bhattacharyya, 2008). According to studies, terpenoids make up most of the reducing phytochemical in neem (*Azadirachta indica*). According to Fourier transmission infrared (FTIR) research, these reducing substances also functioned as capping and stabilizing agents in addition to reducing substances. The main benefit of employing neem leaves is that they are a widely available medicinal plant, and by capping the biosynthesized silver nanoparticle with neem leaf extract, the antibacterial activity of the silver nanoparticle may have been improved. Nimbin and quercetin were found to be the extract's two main chemical components (Shankar *et al.,* 2004; Tripathy *et al.,* 2009). Radiation processing is considered a greener approach since it has numerous environmental benefits over other procedures, such as being quick and economical, producing pure chemicals with catalyst needles, reducing and oxidizing agents, and so on (Ghobashy *et al.,* 2018). Furthermore, the procedure for gamma rays might take place in either a liquid or solid state (Ghobashy *et al.,* 2020) An effective way to decrease metal ions is provided by radiolysis of an aqueous solution, which is used in the gamma radiation-induced silver nanoparticle synthesis (El-Batal *et al.,* 2016). The aim of this study is to look into the effectiveness of silver nanoparticles as a replacement for chemical fungicides in the control of tomato early blight disease.

Materials and Methods

The present experiments were executed to study the effects of silver nanoparticles treated by gamma irradiation on the early blight disease of tomatoes; these experiments were done during the 2018 and 2019 growing seasons. Lab and farm experiments were conducted in the lab and farm of the Plant Research Center Department and the Nuclear Research Center in the Egyptian Atomic Energy Authority, Egypt. The characterization of silver

nanoparticles by Transmission Electron Microscopy and Fourier transform infrared spectrometer were investigated at the National Research Center; UV and DLS were conducted at the Nawah Scientific Center.

Isolation and purification of the causal pathogen

Tomato plant leaves exhibiting typical symptoms of early blight from three different governorates: El-Minia, Al-Sharqiyah, and Qalyubia, were collected. The infected tissues were cut into small pieces before being surface sterilized using a 0.5% solution of sodium hypochlorite for 2-3 minutes. Afterwards, the tissues were washed repeatedly using sterilized distilled water. Small fragments from the sterilized pieces' edges were dried into sterilized filter paper, transferred straight to the PDA medium in 9 cm Petri dishes, and then incubated 27± ºC at 12 hr. of light and 12 hr. of darkness according to Naik *et al*. (2010). Pure cultures were kept on PDA slants and kept between 5 and 10 degrees Celsius in the refrigerator using the descriptions of Singh (1982), Barnett and Hunter (1987). Tomato plants of the super strain B were placed in plastic pots with a diameter of 30 cm. Each pot was filled with sterilized soil, and three seedlings were transplanted into each pot. Three replicates were employed, each of which was inoculated using an atomizer with 30 ml of spore suspension. As a control, plants were sprayed with an equal amount of distilled water. Following inoculation, the plants were placed under polyethylene bags for 48 hr. to promote spore germination by increasing the relative humidity. The bags were then removed, and the plants were kept in greenhouse conditions. Pots were arranged in a completely randomized design, disease severity was measured using a scale with six categories ranging from 0 to 5 (0 = not infected, 1 = sporadic places of infection less than 10% of the leaf area, 2 = higher than 10% >20%, 3 = 20% >30%, 4 = 30% >40%, and 5 = 40% of the leaf area). The created formula was used to determine the disease's severity:

DS (%) = summation of (nv)/NV×100

As: N being the category with the highest rating, n is the degree of infection as measured by the scale, v is the number of samples evaluated for every category, and V is the total number of samples (Townsend and Heuberger, 1943).

Green synthesis

Ten grams of neem and lemon leaves were cut into small pieces. Every plant was transferred to a beaker with 50 ml of deionized water and boiled one hour in an 80 º C water bath to prepare the extract. After that, the produced extract was permitted to cool before being filtered by dry, spotless Whatman paper. The extracted filtrate is collected in a beaker and used synthesizes silver nanoparticles 1 mM $AgNO_3$ each the plant extract was added. Then blended in a 150 milliliter Erlenmeyer flask, then incubated 24 hours at room temperature. A change in color was periodically observed, this indicates the formation of AgNPs.

Characterization

UV and DLS were investigated at the Nawah Scientific Center. TEM and FTIR were conducted at the National Research Center in Egypt. Jenway 6305 was used to record the UV-Vis spectra of AgNPs as a function of wavelength-Vis is a spectrophotometer with a wavelength range of 300 to 800 nm.

Dynamic light scattering

The size range and particle size on the average were determined by Zetasizer (Nano-ZS).

Fourier transform infrared spectrometer

FTIR the observations were done in order to obtain information about the chemical groups present around AgNPs for their stabilization and conclude the transformation of functional groups due to the reduction process. The FT-IR VERTEX 80 Germany was used for the measurements. The N-H stretching band was measured at 3324.11 cm1 (Wei *et al*., 2009).

Transmission electron microscopy

The nanoparticles' size and shape were measured using TEM (JEOL, JEM-2100, Tokyo, Japan).

Radiation source

The process of irradiation was carried out at the Nuclear Research Center. The silver nanoparticles were exposed to gamma irradiation using a Cobalt 60 source (Gamma cell) in a cyclotron project at certain doses of 1.5, 3, 6, 12, and 24 kGy.

Antifungal test

Silver nanoparticles had been evaluated *in vitro* for their efficacy against the tested virulent *A. solani* isolate; each flask contained 500 L/L 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).

Field experiment

Two experiments were undertaken in the field at the experimental Farm of Research Plants, Nuclear Research Centre, Egyptian Atomic Energy Authority, during two seasons (from August to November 2018 and 2019), Plots in the field (3 x 3.5 m^2). A complete randomized block design with 3 rows and 4 plants per row was used. Three plots were utilized as replications for each of the treated plants and the control. Super Strain B tomato seedlings were transplanted at 28 days of age and given all advised farming techniques, including fertilization and irrigation. The seedlings were subsequently sprayed three times with ten days interval as part of the experimental treatment. Disease severity was assessed after 5 days from spraying as mentioned before. The study consisted of 18 treatments that were each replicated three times

in a field experiment. Neem AgNPs, neem AgNPs +1.5 kGy, neem AgNPs +3 kGy, neem AgNPs +6 kGy, neem AgNPs +12 kGy, neem AgNPs +24 kGy, lemon AgNPs, lemon AgNPs +1.5 kGy, lemon AgNPs +3 kGy, lemon AgNPs +6 kGy, lemon AgNPs +12 kGy, lemon AgNPs +24kGy, control (infected), healthy plant, $AgNO_3$+ neem, lemon, and Mancozeb 200 g/100 L.

Determination of chlorophyll

After 45 days, samples of tomato leaves were collected. Chlorophyll was extracted and a spectrophotometer reads according to Arnon, (1949) and Lorenzen, (1967).

Total chlorophyll (mg/g F.W) is equal to (20.2 A_{645} + 8.02 A_{663}) x W/1000 x V, where V is the amount of the filtrate and W is the tissue weight as Arnon (1949).

Determining enzyme activities

Forty-five days after transplanting, samples were taken and pulverized. A mortar and pestle to turn it into a fine powder was used. The extraction buffer phosphate (pH 6.0) and one gram of pulverized tissues were combined. The samples were spun for 25 minutes at 8000 rpm. at 4°C, the supernatant was kept clear. at 20 °C (Bollage *et al.,* 1996).

Peroxidase activity measurement

Absorbance at 425 nm, the activity of peroxidase (PO) was measured using the spectrophotometric method of Allam and Hollis (1972).

Determination of polyphenol oxidase activity

Polyphenol oxidase (PPO) was estimated at an absorption of 420 nm using a spectrophotometric approach (Ishaaya, 1971).

Determination of catalase

Catalase (CAT) activity was measured by observing the oxidation of H_2O_2 at 240 nm for 15 minutes. At 25 °C, the catalase activity of a reaction mixture that contains 100 mM potassium phosphate solution in 1 ml (pH 7.5) and 25 L of 30% solution H_2O_2 was measured spectrophotometrically (Nogueirol *et al.*, 2015).

Statistical analyses

Using SPSS 14.0, the results were statistically examined (ANOVA). According to Duncan's multiple range tests, the means of all values and comparisons were set at ($P \leq 0.05$) (Gomez and Gomez., 1984).

Results

Characterization of silver nanoparticles

Data display in Fig. (1) illustrate the silver nanoparticles' characteristics prepared by green synthesis using neem extract, conducted by using a UV spectrophotometer, DLS, FTIR, and TEM. Fig. 1 (A, B, C, D): using gamma radiation to enhance silver nanoparticles (A): measuring the size of neem AgNps without gamma radiation by DLS. (B): using DLS to measure the size of neem AgNps at 1.5kGy. (C): Fourier transform infrared (FTIR) spectroscopy for neem AgNps. (D): Transmission electron microscope images of lemon AgNPs extract. (E): UV images for preliminary confirmation of lemon AgNPs. Results show that a peak was seen between 410 and

450 nm after the color of the lemon leaf extract yellow to yellow-dark changed after the addition of $AgNO_3$.

Dynamic light scattering

Dynamic light scattering was used to produce graphs showing the typical particle size of AgNPs. Neem AgNPs are depicted as a curve in Figure (1-A). Peak had a maximal diameter of less than 100 nm, according to the distribution of the hydrodynamic diameter of the nanoparticles. The nanoparticles were monodispersed without aggregation indicating stabilization of the nanoparticles by a protein capping agent. The size was decreased neem AgNps +1.5 kGy (Fig. 1-B). Dynamic light scattering was used to calculate average dimensions of particles of AgNPs from DLS graphs. AgNPs solution graph in Fig. 1 (A) AgNPs without gamma irradiation the distribution of the hydrodynamic diameter of the nanoparticles reveals a peak with a maximum diameter of less than 100 nm. Fig. (1-B) shows that the size was reduced by 1.5 neem AgNPs and 1.5 kGy compared with neem AgNPs (Fig. 1-A).

Fourier transform infrared

The reduction of the Ag^+ ions and the protein molecules that serve as capping agents are analyzed using FT-IR data. The FT-IR spectrum of silver nanoparticles is displayed in Figure (1-C).

Transmission electron microscope

Lemon AgNPs measured by TEM analysis of the solution containing these particles revealed particles in the nano range of less than 100 nm (Fig. 1–D).

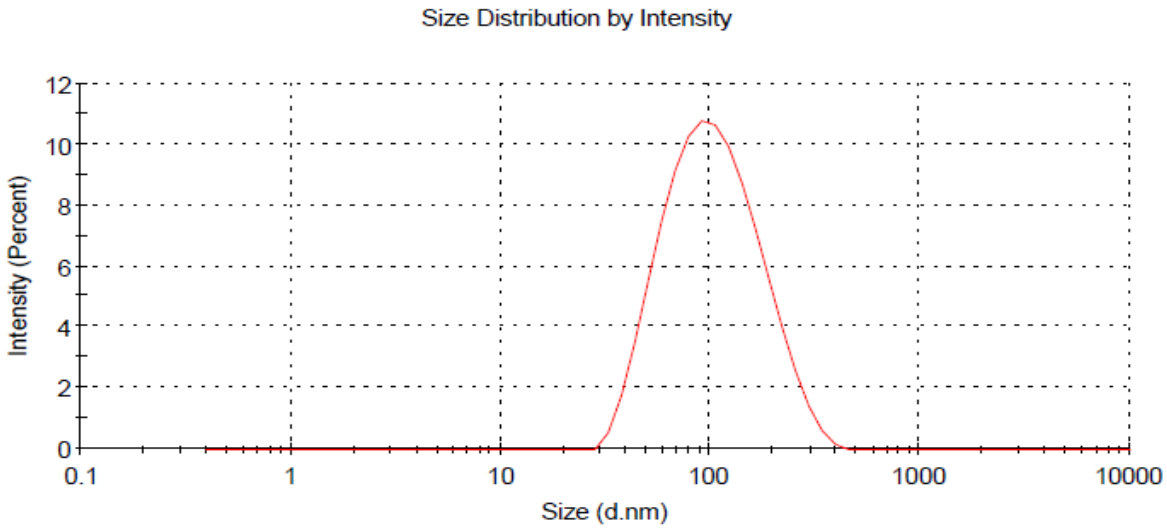

Figure **1** A**.** Measuring the size of neem AgNps without gamma radiation by DLS.

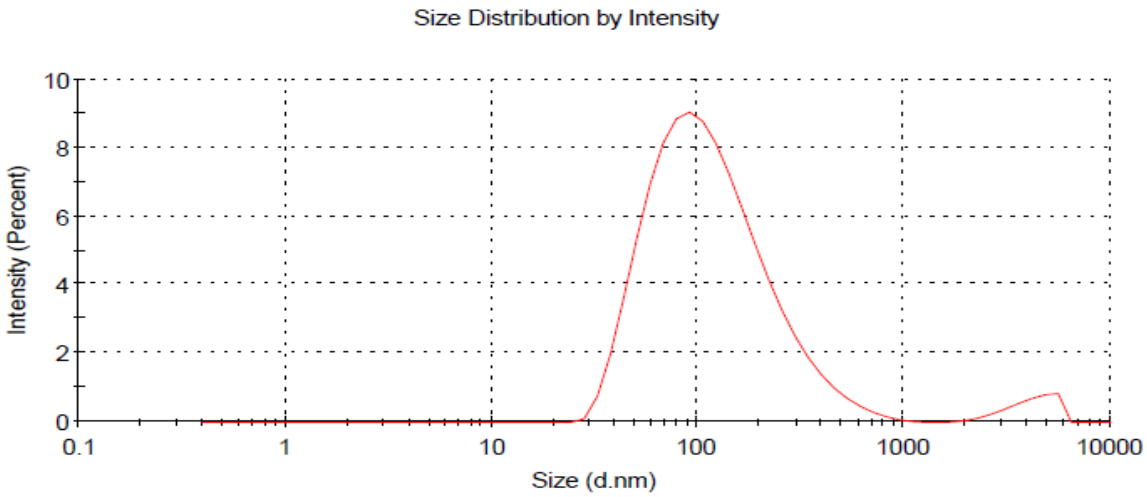

Figure 1B. Using DLS to measure neem AgNps exposed to 1.5 kGy of gamma irradiation.

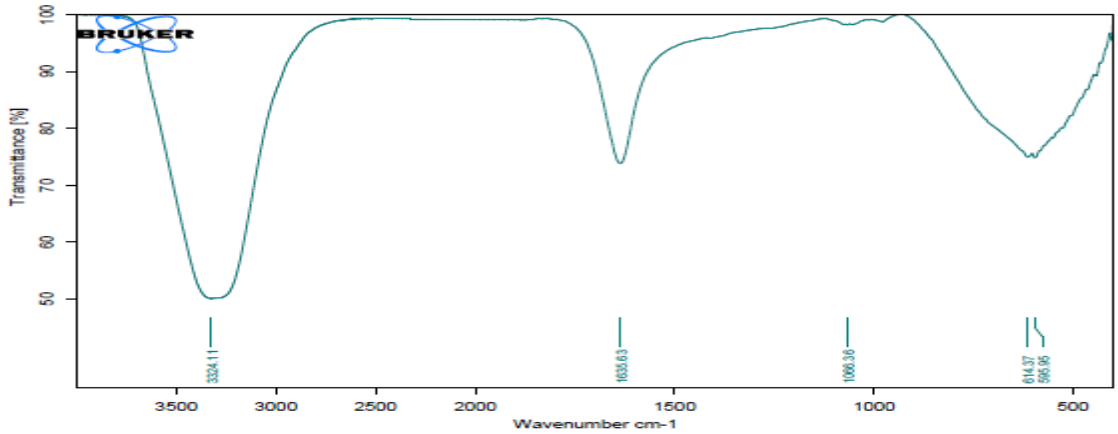

Figure 1C. Fourier transform infrared spectroscopy (FTIR) for neem AgNps without gamma irradiation.

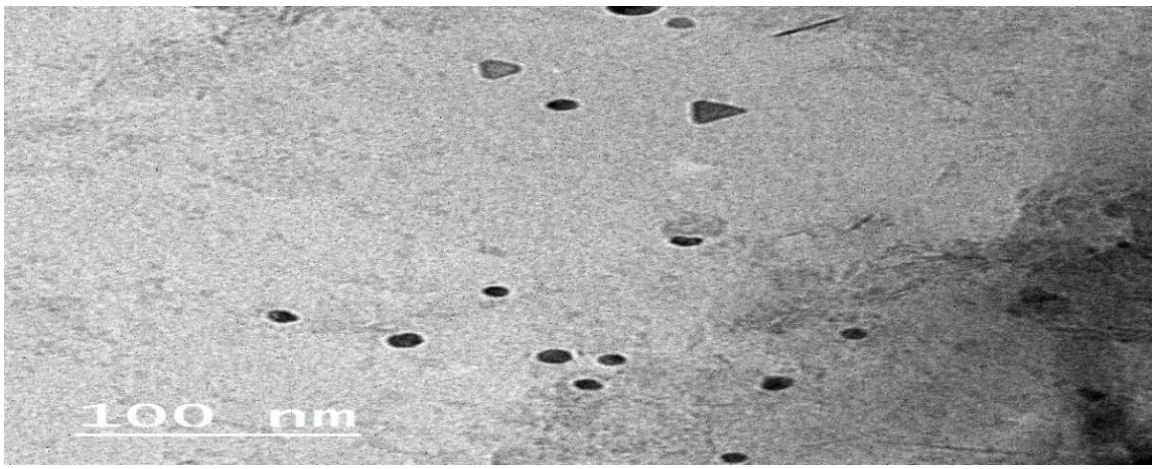

Figure 1D. Transmission electron microscope image of lemon AgNPs (E) UV image for preliminary confirmation of lemon AgNPs.

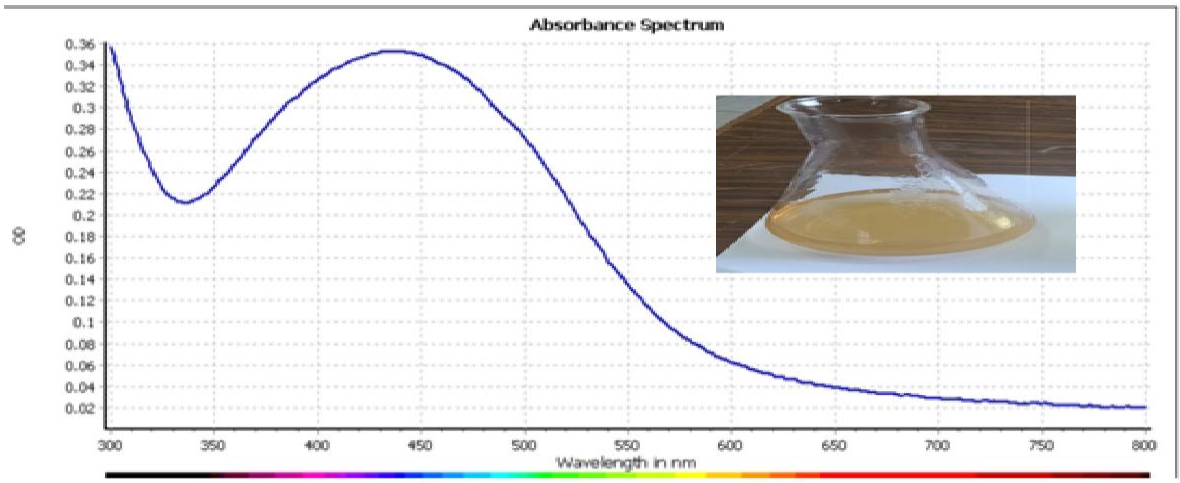

Figure 1D.Transmission electron microscope image of lemon AgNPs (E) UV image for preliminary confirmation of lemon AgNPs.

Effect of irradiated and non-irradiated silver nanoparticles on mycelium growth

Data in Table (1) and Fig. (2) show that neem AgNPs +3 kGy and neem AgNPs +24 kGy caused the highest reduction of the mycelial growth of *A. solani* (100% both of them), followed by lemon AgNPs +24 kGy, lemon AgNPs +3 kGy, and Mancozeb (95.06%, 93.82% and 91.35% respectively,). However, neem extract inhibited the pathogen's mycelium growth the least (1.42%) followed by lemon extract and $AgNO_3$ (7.40% and 11.11% respectively).

Table1. Effect of neem AgNps, lemon AgNps at certain doses of gamma radiation on linear growth of *Alternaria solani* *in vitro.*

Treatment	Linear growth (LG)	Reduction %
Mancozeb 200g/100 L	**7**	**91.35**
Neem AgNPs	**64**	**20.98**
Neem AgNPs+1.5 kGy	**54**	**33.33**
Neem AgNPs +3 kGy	**0**	**100**
Neem AgNPs + 6 kGy	**22**	**72.83**
Neem AgNPs + 12 kGy	**14**	**82.71**
Neem AgNPs + 24 kGy	**0**	**100**
Lemon AgNPs	**60**	**25.92**
Lemon AgNPs + 1.5 kGy	**57**	**29.62**
Lemon AgNPs + 3 kGy	**5**	**93.82**
Lemon AgNPs + 6kGy	**20**	**75.30**
Lemon AgNPs + 12kGy	**12**	**85.18**
Lemon AgNPs + 24kGy	**4**	**95.06**
$AgNO_3$	**72**	**11.11**
Lemon extract	**75**	**7.40**
Neem extract	**70**	**1.42**

control	**81**	**0**

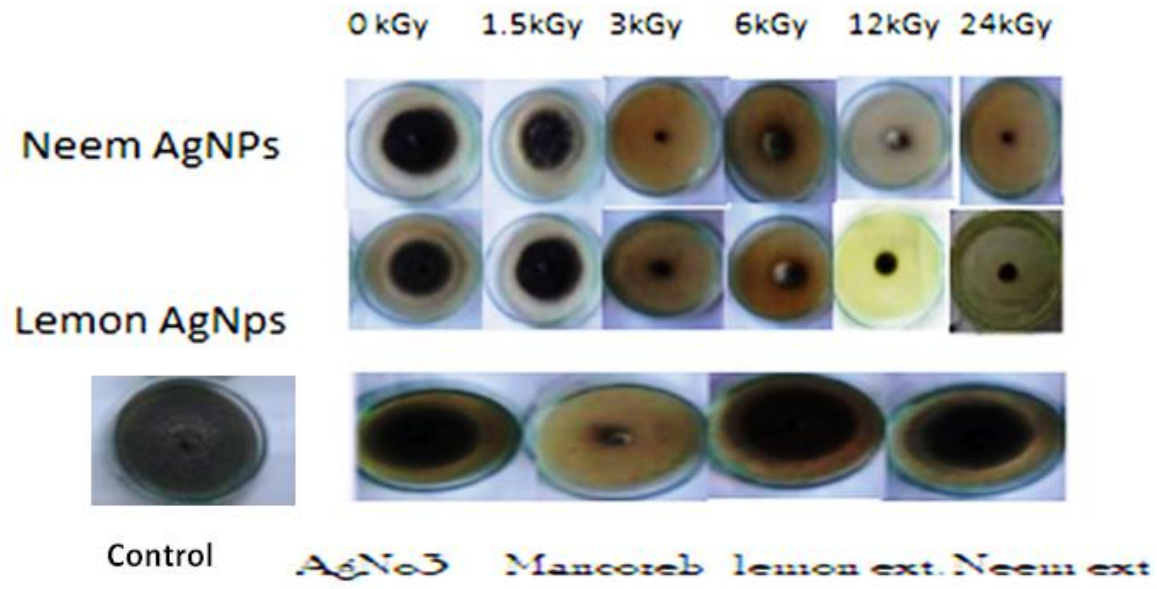

Fig. 2 Effect of AgNPs at certain doses of gamma irradiation on linear growth of *Alternaria solani*.

Effect of irradiated and non-irradiated silver nanoparticles on shoot fresh and dry weight

Results in Table (2) show the impact of for "green" synthesis of silver nanoparticles using neem and lemon extracts than silver nanoparticles irradiated with doses of gamma radiation (1.0, 1.5, 3, 6, 12, and 24) kilo gray (kGy) on a shoot fresh weight and dry weight of tomato plants during both seasons of 2018 and 2019. In comparison with untreated plants, all silver nanoparticles both irradiated and non-irradiated increased shoot fresh and dry weight. The highest shoot fresh weight for an average of two seasons was obtained by utilizing irradiated silver nanoparticles synthesized by lemon extract with doses at 12 and 24 kGy, respectively. On the other hand, the maximum shoot dry weight was achieved by neem extract AgNPs and lemon extract AgNPs irradiated by 24 kGy average of two seasons with synthetic lemon extract.

Efficacy of irradiated and non-irradiated silver nanoparticles on the disease severity of tomato early blight

The results in Table (3) show that silver nanoparticles using neem and lemon extracts irradiated with doses of gamma radiation (1.0, 1.5, 3, 6, 12, and 24) kilo gray or not irradiated significantly reduced disease severity compared to control treatment. The results clearly showed that in the first season, no significant difference between mancozeb, neem AgNPs + 6 kGy, and lemon AgNPs + 12 kGy applications which provided the least early blight severity and recorded 8.94, 9.6,7 and 9.69% respectively compared with 44.11 in the control treatment. As for the second season, the insignificant difference between Mancozeb, Neem AgNPs + 6 kGy, lemon AgNPs + 12 kGy, and lemon AgNPs + 24 kGy treatments which recorded the lowest early blight

severity 9.37, 9.99, 9.37, and 10.88 respectively compared with 45.33 in the control treatment. Generally, irradiation of silver nanoparticles increased the efficiency of silver nanoparticles. The most effictive irradiation dose of Neem AgNPs was 6 kGy whereas the best irradiation dose of lemon AgNPs was 12 kGy.

Table 2. Effect of irradiated and non-irradiated silver nanoparticles and some foliar applications on shoot fresh and dry weight.

Silver Nanoparticles	Gamma (KGy)	Shoot Fresh Weight (g)		Mean	Shoot Dry Weight (g)		Mean
		First season	Second season		First season	Second season	
Neem AgNPs	0	126.06 b	116.90 b	121.48	89.73 c	93.80 b	91.76
	1.5	152.50 de	155.33 d	153.92	98.50 d	108.43 c	103.46
	3	149.43 d	154.13 d	151.78	104.66 e	115.10 d	109.88
	6	141.33 c	146.16 c	143.75	120.00 f	124.56 d	122.28
	12	158.16 ef	158.13 de	158.15	123.36 fg	133.36 f	128.36
	24	170.00 g	172.50gh	171.25	141.10 h	146.53 g	143.81
Lemon AgNPs	0	125.67 b	113.46 b	119.75	83.83 b	91.00 b	87.41
	1.5	158.50 ef	163.80 ef	161.15	104.50 e	107.80 c	106.15
	3	157.93 ef	166.03 fe	161.98	108.26 e	117.96 d	113.11
	6	160.00 f	156.93 de	158.47	124.16 fg	130.16 f	127.16
	12	168.50 g	176.20 h	172.35	129.20 g	141.60 g	135.40
	24	171.56 g	178.40 h	174.98	140.26 h	151.80 h	145.03
Neem extract	0	142.5 c	145.22 c	143.86	120.33 f	133.33 f	126.83
Lemon extract	0	126.06 b	117.1 b	121.58	85.10 b	94.33 b	89.72
$AgNO_3$	0	141.05 c	144.33 c	142.96	123.33 fg	130.50 f	126.92
Mancozeb 200g/100L	0	170.33 g	175.2 h	172.77	138.03 h	148.30 h	143.17
Control	Without Gamma Irradiation	112.20 a	101.30 a	106.75	60.20a	64.83 a	60.52

According to Duncan's multiple range tests, the values assigned to similar letters are not substantially different (*P≤0.05*).

Table 3. Effect of irradiated and non-irradiated silver nanoparticles on the severity of tomato early blight.

Treatment	Disease Severity							
	First Season			Mean	Second Season			Mean
	1st Spray After 25 days	2nd Spray After 35 days	3rd Spray After 45 days		1st Spray After 25 days	2nd Spray After 35 days	3rd Spray After 45 days	
$AgNo_3$	22.33 g	24.33 h	26.33 h	24.33 g	23.66 h	26.33 g	27.00 i	25.66 g
Neem ext.	24.00 g	26.00 i	27.33 h	25.77 g	24.66 h	27.00 g	28.00 i	26.55 g
Neem AgNPs	11.83 de	15.46 e	17.53 e	15.00 e	14.00 e	17.33 e	18.23 f	16.52 de
Neem AgNPs +1.5kGy	11.06 cd	13.00 cd	15.00 d	13.02 bc	14.33 e	15.16 d	16.00 e	15.16 cd
Neem AgNPs +3 kGy	10.66 bc	14.13 de	15.66 d	13.48 bc	14.00 e	15.33 d	16.66 e	15.22 cd
Neem AgNPs + 6 kGy	9.60 ab	8.93 a	10.50 ab	9.67 a	8.33 a	10.33 ab	11.33ab	9.99 a
Neem AgNPs +12 kGy	11.00 cd	11.33 bc	12.50 c	11.61 ab	9.66 ab	11.00 bc	13.33 d	11.33 ab
Neem AgNPs +24 kGy	12.33 e	15.06 e	16.00 d	14.46 de	12.66 de	15.50 d	16.66 e	14.94 cd
Lemon ext.	19.33 f	21.00 g	22.33 g	20.88 f	19.33 g	22.00 f	23.33 h	21.55 f
Lemon AgNPs	12.00 e	14.00 de	15.33d	13.77 cd	12.00 cd	14.66 d	16.00 e	14.22 bc
Lemon AgNPs+1.5kGy	18.00 f	18.33 f	20.00 f	18.77 f	16.00 f	18.00 e	20.66 g	18.22 e
Lemon AgNPs +3 kGy	11.66 de	11.30 bc	12.33 c	11.76 ab	9.66 ab	11.66 bc	12.66cd	11.32 ab
Lemon AgNPs +6 kGy	10.00 bc	11.33 bc	12.66 c	11.33 ab	10.66 bc	12.33 c	13.33 d	12.10 ab
Lemon AgNPs+12kGy	9.00 ab	9.43 a	10.66 ab	9.69 a	8.00 a	10.66 ab	11.00ab	9.88 a
Lemon AgNPs+24kGy	10.66 bc	10.00 ab	11.33 bc	10.66 ab	9.33 ab	11.00 bc	12.33bc	10.88 a
Mancozeb 200g/100L	8.10 a	9.00 a	9.73 a	8.94 a	8.36 a	9.33 a	10.43 a	9.37 a
Control (untreated)	42.00 h	44.00 j	46.33 i	44.11 h	43.00 j	46.00 h	47.00 j	45.33 h
Mean	14.91	16.27	18.13	16.31	15.15	17.27	18.46	17.83

According to Duncan's multiple range tests, the values assigned to similar letters are not substantially different (*P≤0.05*). Disease severity was detected 5 days after each spray.

Efficacy of irradiated and non-irradiated silver nanoparticles and certain foliar applications on the severity and yield of tomato

The results in Table (4) show that all treatments specifically containing AgNPs achieved an increase in tomato yield compared to the control. In the first seasons, the highest yield was achieved by Mancozeb followed by Neem AgNPs +24 kGy which recorded 44379 and 44219 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 Neem AgNPs +24 kGy followed by Mancozeb. The least effective treatment in increasing yield was Lemon extract.

Table 4. Effect of irradiated and non-irradiated silver nanoparticles and some application on yield.

Treatment	Yield kg fed^{-1}		Mean	
	First season	Second Season	Yield kg fed^{-1}	Yield kg ha^{1}
$AgNo_3$	33206 ^{d}	32114 ^{d}	32660	77761.9
Neem ext.	28653 ^{b}	27302 ^{b}	27977.5	66613.1
Neem AgNPs	39513 ^{g}	39704 ^{h}	39608.5	94306
Neem AgNPs +1.5 kGy	41210 ^{k}	41208 ^{ij}	41209	98116.7
Neem AgNPs +3 kGy	43308 ^{m}	44115 ^{lm}	43711.5	104075
Neem AgNPs + 6 kGy	39751^{n}	38902 ^{g}	39326.5	93634.5
Neem AgNPs +12 kGy	38106 ^{e}	38059 ^{f}	38082.5	90672.6
Neem AgNPs +24 kGy	44219 ^{o}	45209 ^{n}	44714	106462
Lemon ext.	24104 ^{a}	23584 ^{a}	23844	56771.4
Lemon AgNPs	32607 ^{c}	31305 ^{c}	31956	76085.7
Lemon AgNPs +1.5kGy	43759 ^{n}	43783 ^{l}	43771	104217
Lemon AgNPs +3 kGy	41503 ^{i}	41706 ^{j}	41604.5	99058
Lemon AgNPs +6 kGy	40707 ^{J}	40704 ^{i}	40705.5	96917.9
Lemon AgNPs +12 kGy	39201^{f}	37308 ^{e}	38254.5	91082.1
Lemon AgNPs +24 kGy	40604 ^{i}	42041^{k}	41322.5	98386.9
Mancozeb 200g/100L	44379 ^{p}	44517 ^{m}	44448	10582.9
Control (untreated)	24102 ^{a}	23524 ^{a}	23813	56697.6
Mean	37584.23	37357.94	36882.85	83614.19

According to Duncan's multiple range tests, the values assigned to similar letters are not substantially different (*P≤0.05*).

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

The data in Table (5**)** illustrate that the effect of all treatments on both healthy and infected plants led to an increase in the total chlorophyll in all treatments compared with the infected control (untreated). The highest total chlorophyll contents were observed by non-infected (healthy plants) (26 mg/g fresh weight) followed by Neem AgNps + 6

kGy and Neem AgNPs+1.5 kGy (23.1 and 22 mg/g fresh weight, respectively).

Table 5. Effect of irradiated and non-irradiated silver nanoparticles as foliar applications on total chlorophyll.

Tretement	Total Chlorophyll mg g^{-1} FW	Efficacy
Healthy plant	26 [j]	1138.1
Control infected	2.1 [a]	0
Mancozeb 200g/100 L	15.2 [c]	623.81
Neem AgNPs	19.3 [f]	819.04
Neem AgNPs+1.5 kGy	22 [h]	947.62
Neem AgNPs +3 kGy	18.7 [e]	790.48
Neem AgNPs + 6 kGy	23.1 [i]	1004.76
Neem AgNPs + 12 kGy	18.6 [e]	790.48
Neem AgNPs + 24 kGy	19.3 [f]	819.05
Lemon AgNPs	15.3 [c]	628.57
Lemon AgNPs + 1.5 kGy	16.6 [d]	690.48
Lemon AgNPs + 3 kGy	21.6 [g]	928.57
Lemon AgNPs + 6kGy	19.3 [f]	819.05
Lemon AgNPs + 12kGy	15.9 [c]	657.14
Lemon AgNPs + 24kGy	16.5 [d]	685.71
$AgNO_3$	5.3 [b]	152.38
Lemon extract	6 [b]	185.71
Neem extract	6.4 [b]	204.76

According to Duncan's multiple range tests, the values assigned to similar letters are not substantially different (*P≤0.05*).

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

Results in Table (6) show that all treatments increased the oxidative enzymes activity compared with the control infected. The highest increase of Peroxidase activity was achieved with Neem AgNPs + 3 kGy (39) followed by Neem AgNPs + 12 kGy (38) compared with 20 in the infected control. However, $AgNO_3$ (21) had the lowest value. As for polyphenol oxidase, all treatments increased the polyphenol oxidase activity. The maximum value was obtained by Neem AgNPs +1.5 kGy (35) followed by Neem AgNPs (31.33) and Neem AgNPs +3 kGy (33). The lowest value was obtained by Neem extract (15.6) while the control infected was 16. Meanwhile, the highest value of catalase was achieved by Lemon AgNPs+ 6kGy (31) followed by Lemon AgNPs (30.6) and Mancozeb (30). However, the lowest values were achieved by Lemon AgNPs +3 kGy (20). Whereas,the infected control was 11.

Table 6. Effect of irradiated and non-irradiated silver nanoparticles as foliar applications on oxidative enzymes activity

Treatment	Peroxidase	Polyphenol oxidase	Catalase	Efficacy		
				Peroxidase	Polyphenol oxidase	Catalase
Healthy plant	11 a	10 a	8 a	-45	-37.5	-27.27
Control infected	20 b	16 b	11 b	0	0	0
Mancozeb 200g/100 L	36 fg	27 gh	30 ij	+80	+68.75	+172.73
Neem AgNPs	36 fg	33 jk	28.6 hi	+80	+106.25	+160.00
Neem AgNPs +1.5 kGy	35 f	35 k	25 de	+75	+118.75	+127.27
Neem AgNPs +3 kGy	39 h	31.33 ij	25.6 ef	+95	+95.81	+132.73
Neem AgNPs + 6kGy	33 e	21.66 de	23.3 d	+65	+35.38	+111.82
Neem AgNPs + 12kGy	38 g	24.66 fg	25.6 ef	+90	+54.13	+132.73
Neem AgNPs + 24kGy	33 e	27gh	24 de	+65	+68.75	+118.20
Lemon AgNPs	29 d	23.33 ef	30.6 j	+45	+45.81	+178.20
Lemon AgNPs +1.5kGy	36 fg	25.66 f g	26.6 fg	+80	+60.38	+141.82
Lemon AgNPs +3 kGy	31 de	29.66 hi	20 c	+55	+85.38	+81.82
Lemon AgNPs + 6kGy	32 e	26.33 gh	31 k	+60	+64.56	+181.82
Lemon AgNPs + 12kGy	29 d	26.66 g	28 hi	+45	+66.63	+154.55
Lemon AgNPs + 24kGy	30 de	21.33 de	28.3 hi	+50	+33.31	+157.27
$AgNO_3$	21 bc	19.33 cd	20 c	+5	+20.81	+81.82
Lemon extract	23 c	17 bc	12 b	+15	+6.25	+9.10
Neem extract	23 c	15.6 b	18.6 c	+15	-0.03	-27.27

* According to Duncan's multiple range tests, the values assigned to similar letters are not substantially different ($P \leq 0.05$).

*Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

* Polyphenol oxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/ minutes.

* Catalase activity was expressed as the increase in absorbance at 240 nm/gram fresh weight/15 minutes.

DISCUSSION

The result data showed two FT-IR spectra for AgNPs and the creation of a peak at 614.37 and 595.95 cm-1, which could be attributed to one of the Ag - O vibration modes (Roy *et al.,* 2007). 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. The metal NPs can easily infiltrate the cell wall due to their nano-sizes and very precise surface areas, interacting with the interior biomolecules of the cell (Razack *et al.,* 2016). These improved variables have an impact on how silver nanoparticles develop and the production of silver nanoparticles is impacted by several factors

(Vanmathi and Sivakumar, 2012). Through the obtained results, the peEak was noticed between 410 and 450 nm after the color changed from a pale yellow (from the plant extract) to a dark yellow after adding $AgNO_3$. According to Zaheer and Rafiuddin, (2012). Lemon AgNPs, a peak between 410 and 450 nm has been noted. mong the many chemical and physical parameters, metal ion concentration, pH, incubation time, and temperature are just a few instances of the several chemical and physical parameters that affect how nanoparticles are formed. Silver nanoparticles reduced the mycelial growth of *A. solani*. Neem AgNPs +3 kGy and Neem AgNPs +24 kGy caused the highest reduction. These results concur with those attained by Gomaa *et al.*, (2021). They found that Neem AgNPs inhibited completely the mycelium growth and sporulation of *Fusarium oxysporum* f. sp*. lycopersici.* Treating tomato plants with silver nanoparticles increases the activity of peroxidase, polyphenol oxidase, and catalase enzymes. These results are in harmony with the results of *Ashraf et al*., (2020) who found that treating tomato plants with MLE-AgNPs increases the phenolic contents and activities of PO, PPO, and PAL enzymes compared with control. Silver nanoparticles improved plant growth. Also, significantly decreased the severity of early blight disease and increased tomato fruit yield. Different factors that influence the effectiveness of the fungicidal effect, such as particle size and particle concentration, are particularly effective in halting fungal development. The extreme toxicity of silver nanoparticles to microbes is well recognized. According to several studies, silver nanoparticles may adhere to the cell membrane's surface, impairing the cell's ability to breathe and maintain permeability. In addition to the membrane's surface, silver nanoparticles may potentially interact with the interior of the organism (Srivastava*. et al.,* 2011). Similarly, AgNps have the capacity to adsorb to various cellular organelles and increase reactive oxygen species (ROS), which obstruct cellular metabolic reactions (Miao *et al.,* 2010) The AgNPs may interfere with cellular phosphate control due to their capacity to adhere to cell membranes as opposed to permeability or ion transport capabilities. After then, the hydrogen bonding is broken, which prevents DNA synthesis from occurring. This also causes ribosomes to become denaturized, which renders enzymes and proteins inactive by occupying their active sites (Dakal *et al.,* 2016). The present investigation regarding Mancozeb agrees with Tofoli *et al.,* (2003) who evaluated the effectiveness of fungicides for controlling *Alternaria solani* as well as their effects on tomato fruit yield. They reported that the highest level of disease control, quality, and increase in fruit yields were obtained with Azoxystrobin followed by Mancozeb. It can be said that by increasing the radiation dose, the particle size decreases, resulting in the mean diameter of Ag-NPs These results agree with Saleh *et al.,* (2021).

Conclusion

The present study established that irradiated silver nanoparticles significantly inhibited the pathogenic tomato fungus *A. solani*. AgNPs can therefore be employed as antimicrobial treatments to efficiently

prevent tomato early blight disease. Neem and lemon extract can synthesize silver nanoparticles alternative to chemical materials. Gamma radiation can be used to decrease the silver nanoparticle size. AgNPs Instead of using fungicides made of chemicals that are readily accessible on the market but are more toxic to humans.

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Conceptualization, ASF, AMM, GME, AAA, GAA; data curation, ASF, AMM, GAA; AA formal analysis, AMM, AAA Investigations, ASA, AMM, GAA, AAA Methodology, ASF, AMMM, GAA writing original drafts, and writing and editing ASF.AMMM, AAA, GA All authors have read and agreed to the purplish version of the manuscript.

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