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ORIGINAL PAPER

Evaluation of Some Nanoparticles Against *Sclerotium rolfsii* the Cause of Root and Crown Rots in Common Beans

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ABSTRACT

Fungal diseases have a significant role in the low productivity of common bean crops. The use of fungicides is the most effective solution to control fungal pathogens, but excessive and indiscriminate fungicide use causes negative effects on the plant and the ecosystem. The quick advancement of nanotechnology seems to offer a novel solution to controlling phytopathogens. Different concentrations of three nanoparticles, Ag_2O-NPs, CuO-NPs, and CaO-NPs were tested individually against *Sclerotium rolfsii*, the cause of root and crown rots. A significant effect was observed at 50 µg/mL. Our findings showed that the commercial fungicide Rizolex-T 50 WP and CaO-NPs did not perform as well as Ag_2O-NPs and CuO-NPs in reducing disease incidence and severity caused by *S. rolfsii*. However, the three tested nanoparticles improved crop yield, defense enzymes (catalase, peroxidase, and polyphenol oxidase), and characteristics of plant development.

Keywords: Common bean, *Phaseolus vulgaris*, root rot, crown rot, *Sclerotium rolfsii*, nanoparticles, Ag_2O-NPs, CuO-NPs, CaO-NPs

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INTRODUCTION

One of the most valuable grain legumes for human consumption worldwide is the common bean, *Phaseolus vulgaris* L., which is a significant source of valuable plant proteins and other nutrients (Los *et al*., 2015). Beans are regarded as a premium crop for economic production worldwide. Additionally, the common bean has an economic influence worldwide and plays a key role in sustainable improvements to the environment through nitrogen fixation that has a positive impact on the soil (Rondon *et al*., 2007 and Uebersax *et al*., 2022). Plant pathogens cause severe risks to agricultural production, resulting in huge economic losses of 20-40% each year (Worrall *et al*., 2018). Fungal species are responsible for nearly 70% of all main crop diseases and induced damage in various crop species (Patel *et al*., 2014). *Sclerotium rolfsii* is one of the dangerous necrotrophic soil-borne pathogens that commonly spread in warm temperate areas such as the tropics and subtropics, causing severe damage to more than 500 important plant species, including dicotyledonous plants and crops (Mishra *et al*., 2017). Furthermore, it produces abundant hyphae and sclerotia that allow the pathogen to survive without a host or during a stressful period until conditions become more favorable for germination and development (Parikh and Jha, 2012). It is capable to infect any plant part, such as roots, shoots, leaves, petioles, flowers, and fruits. Symptoms include seed rot or pre-emergence damping-off; yellowing and drooping of the leaves; grey water-soaked lesions on the stem; and stem, root, and pod rots (Eid, 2014 and Mishra *et al*., 2017). Consequently, the disease can cause a 76% loss in common bean productivity (Paparu *et al*., 2018). The traditional management strategy for this disease depends on removing and destroying diseased plants that act as inoculum sources, the use of

fungicides, soil solarization (Flores-Moctezuma *et al*., 2006), and the cultivation of resistant cultivars (Woodward *et al*., 2008).

Fungicide use has many advantages, including availability, efficacy, and speed of action. However, there are drawbacks, including adverse effects on healthy plants, the emergence of resistant strains, and increased virulence of pathogens (Yadav *et al*., 2020). Additionally, a significant portion of fungicides is lost in the soil, leading to the cumulative effect of fungicides causing toxicity in the soil. This has led to the evident appearance of environmental risks brought on by the overuse of fungicides, and there have been numerous attempts to find alternative solutions in recent years. Therefore, in order to maintain and safeguard global food security and avoid economic losses, agricultural scientists are interested in promising recent breakthroughs and are looking for solutions that can reduce the use of fungicides. Nanotechnology is considered an effective tool in the search for solutions to many agricultural challenges, such as disease diagnosis and management, boosting productivity, and the sustainable use of chemical inputs. This would have a significant positive impact on the challenges of food production and climate change (Gogos *et al*., 2012).

Nanoparticle materials have distinctive physicochemical characteristics that are not found in their bulk counterparts, which improve their ability to interact with microbes and perform a variety of antimicrobial actions (Chen *et al*., 2013 and Boxi *et al*., 2016). They have small sizes ranging from 1 to 100 nm and high surface area to volume ratios pore sizes, in addition to surface charge density, crystalline and amorphous structures, spherical and cylindrical shapes, and environmental sensitivity. The antifungal activities of nanoparticles are represented by their ability to cause physical and mechanical damage to the cell walls and membranes as a result of their strong adhesion to the external surface of the fungus and the ease of penetration and deposition into fungal cells; modulation of the cellular level signaling by dephosphorylating putative key peptide substrates, which are critical for cell viability and cell division; increasing the membrane permeability, blocking the water channels; inactivating microbial enzymes; facilitating the production of reactive oxygen species; and arresting the respiration and other metabolic pathways that all lead to the fatality of the fungi (Shrivastava *et al*., 2007; Allahverdiyev, 2011 and Wang *et al*., 2014). Under normal and stressful conditions, nanoparticles can perform hormone-like functions, e.g., promoting cell divisions, callus proliferation, root structure, the length of shoots, the number of leaves, and the total amount of biomass in different plant species (Gohari *et al*., 2020).

Silver, copper, and calcium nanoparticles have made their way into the field of controlling plant diseases (Chu *et al*., 2012; Abou-Salem *et al*., 2022 and Nazir *et al*., 2022). In order to enhance the effectiveness, reactivity, and characteristics of metal-based nanoparticles, metal oxides were created (Mansoor *et al*., 2021), which are characterized by their stability, robustness, and long shelf life (Roy *et al*., 2013). The current study aims to evaluate the ability of some nanoparticles to suppress *S. rolfsii*, enhance common bean defensive mechanisms, and influence plant growth parameters.

MATERIALS AND METHODS

2.1. Source of bean seeds:

Seeds of two common bean susceptible cultivars; Giza 12 and Alpha were obtained from the Department of Vegetables Production Research, Horticultural Research Institute, Agricultural Research Center, Egypt.

2.2. Nanoparticles and fungicide sources:

Three different nanoparticles were obtained from Sigma Aldrich and used as antifungal substances against *S. rolfsii*. Silver oxide nanoparticles [Ag_2O-NPs] (nanopowder, <100 nm particle size, contains PVP as a dispersant agent, CAS No. 7440-22-4), copper oxide nanoparticles [CuO-NPs] (nanopowder, <50 nm particle size (TEM), CAS No. 1317-38-0) and calcium oxide nanoparticles [CaO-NPs] (nanopowder, <160 nm particle size (BET), 98%, CAS No. 1305-78-8). A commercial fungicide product (Rizolex-T 50 WP) containing the active ingredient tolcofos-methyl was obtained from Sumitomo Chemical Co., Ltd., Osaka, Japan. All stock solutions were prepared using sterilized distilled water. Prior to incorporation into a sterilized growth medium or addition directly to the soil, nanoparticle suspensions were subjected to sonication for 30 min using a Transonic 420 (Elma, Germany) sonicator.

2.3. Isolation and identification of the associated fungi:

Common bean plants with typical symptoms of crown rot and/or root rot diseases were collected from Giza and Menoufia governorates, Egypt. The infected samples were first cut into

small pieces (5 mm sections) and washed under running tap water, air dried, surface disinfected by dipping in a 3% sodium hypochlorite solution for 2 minutes, washed twice with sterilized distilled water, and dried using sterilized filter papers. The sterilized plant pieces were transferred under aseptic conditions to 9-cm Petri dishes containing 20 mL of potato dextrose agar (PDA) supplemented with ampicillin antibiotic (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The plates were incubated at 25 ± 2 °C and visually inspected every day for 1 week. The emerging fungi were picked up and transmitted onto a new PDA medium for purification using the hyphal tip technique (Paparu *et al*., 2018). The pure cultures of isolated fungi were examined and identified using a light microscope (Leica DM1000) at the Mycological Research and Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt. The percentage of colonies frequency was calculated based on the number of the pure fungal colonies of the isolated fungi according to the following equation:

$$\textbf{The frequency (\%)} = \frac{\mathbf{n}}{\mathbf{N}} \times \mathbf{100}$$

Where:

n = the number of colonies for each pathogen

N = the total number of colonies

The most frequently isolated fungus was maintained on PDA slants and kept at 4 °C for further studies.

2.4. Pathogenicity test of isolated *S. rolfsii*:

The isolated *S. rolfsii* was tested against common bean plants under greenhouse conditions in the Vegetables Disease Research Department, Plant Pathology Research Institute, Giza governorate. Twenty isolates of *S. rolfsii* were tested for their pathogenicity against common bean plants. Isolates were grown on PDA at 25±2 °C for 7 days. Mycelial disks (5 mm) of each tested isolate were placed in bottles containing sterilized sand-corn medium (25 g washed sand, 75 g corn, and 80 mL water). The bottles were plugged with cotton wool and aluminium foil then incubated and shacked regularly to encourage the fungus colonization at 25 ±2 °C for 21 days (Sennoi *et al*., 2010). 25 cm diameter sterilized pots were filled with 3 kg/pot of sterilized sandy loam soil using 5% formalin and left for 7 days to ensure complete formalin evaporation. Three weeks post-incubation, the inoculum was transferred to the sterilized pots. Five healthy seeds of the common bean Giza 12 and Alpha cultivars were planted in the infested plastic pots; each pot served as one replicate, and six replicates were used for each isolate. Five seeds of common bean were planted in non-infested pots.

Disease assessment:

Plants of both cultivars were evaluated based on the pathological behavior of *S. rolfsii* in infecting common bean plants, which starts with pre- and post-emergency damping-off and ends with root rot.

The pre- and post-emergency damping-off percentages were assessed 15 and 30 days after the sowing date, respectively, using the following formulas:

$$\textbf{Pre-emergency damping-off (\%)} = \frac{\textbf{Dead seeds or seedlings before emergence from soil}}{\textbf{Total number of planted seeds}} \times \mathbf{100}$$

$$\textbf{Post-emergency damping-off (\%)} = \frac{\textbf{Dead seedlings after emergence from soil}}{\textbf{Total number of planted seeds}} \times \mathbf{100}$$

The percentages of root rot disease incidence and the efficacy of treatments were assessed 45 days after the sowing date using the following formulas:

$$\textbf{Root/crown rot (\%)} = \frac{\textbf{Number of infected plants}}{\textbf{Total number of planted seeds}} \times \mathbf{100}$$

$$\textbf{Plant survival (\%)} = \frac{\textbf{Total number of planted seeds - (number of pre and post emergence damping-off + number of infected plants)}}{\textbf{Total number of planted seeds}} \times \mathbf{100}$$

The disease severity was evaluated 45 days after sowing depending on the progress of symptoms according to a 0-5 scale developed by Kator *et al*. (2015).

Where:

0 = no symptoms, **1** = > 0 – 20%, **2** = > 20 – 40%, **3** = > 40 – 60%, **4** = > 60 – 80%, and **5** = > 80 – 100% of damaged plant tissue.

The percentage of disease severity was calculated using the following formula:

$$\textbf{Disease severity (\%)} = \frac{\sum(\mathbf{n} \times \mathbf{r})}{\mathbf{5N}} \times \mathbf{100}$$

Where:

n = number of plants in each numerical rate

r = rating category

N = total number of plants

5 = the maximum numerical rate

2.5. Effect of nanoparticles on mycelial growth of *S. rolfsii in vitro*:

Nanoparticles (Table 2) were used to evaluate their impact on the radial growth of *S. rolfsii* at four different concentrations: 10, 25, 50, and 75 µg/mL compared to the recommended dose of the fungicide Rizolex-T 50 WP using a toxic food technique (Dhingra and Sinclair, 1985). The different concentrations

of nanoparticles or the fungicide were separately amended with PDA and poured into 9 cm diameter plates. A 5 mm disc of *S. rolfsii* was cut from a 7 days-old culture and placed in the center of the plate. A PDA medium free of the tested substances was used as a control. Plates were incubated at 25 ± 2 °C. The fungal growth was measured when the linear growth of the control colony had been completed. Three replicates were conducted for each treatment. The efficiency of nanoparticles on mycelial growth inhibition was calculated based on the following formula:

$$\textbf{Nanoparticles efficiency (\%)} = \frac{\mathbf{X - Y}}{\mathbf{X}} \times \mathbf{100}$$

Where:

X = mycelial growth of *S. rolfsii* grown in control plates

Y = mycelial growth of *S. rolfsii* grown in the desired treatment plates

The effect of nanoparticles on sclerotia was estimated using ImageJ software.

2.6. Microscopic Analysis:

To study the effect of nanoparticles on hyphae and sclerotia morphology of *S. rolfsii*, Petri plates containing 40-day-old cultures of *S. rolfsii* on PDA amended with 50 µg/mL of Ag_2O, CuO, and CaO nanoparticles, each alone were examined individually under a scanning electron microscope, JEOL JSM 6510 Iv, British University, Egypt. (El-Argawy *et al*., 2016).

2.7. Evaluation of nanoparticles against *S. rolfsii* under greenhouse conditions:

The effect of nanoparticles was tested in controlling common bean root and crown rots caused by *S. rolfsii* under greenhouse conditions in the Vegetables Disease Research Department, Plant Pathology Research Institute, Giza governorate, and Mit Khalaf Agricultural Research Station, Shebin Elkom, Menoufia governorate. Five agar disks (5mm) of the most aggressive *S. rolfsii* isolate were transferred to an autoclaved 500 mL bottles containing sand-corn medium. Three weeks post-incubation, the inoculum was transferred to 25 cm diameter sterilized pots. The preceding inoculum was added to each pot at a rate of 3% w/w to carry out the soil infestation. The pots were watered every 2 days for one week to ensure the establishment of *S. rolfsii* in the soil. Seeds of common bean cultivars Giza 12 and Alpha were directly sown in the infested pots (5 seeds per pot). At the same time, 50 µg/mL of each nanoparticle material and 3 g/L of fungicide were added to the pots each alone. Six pots were used for each treatment. Pots free of the nanomaterials and the fungicide served as controls. The addition of all treatments was repeated at 7 days after sowing. Both cultivars' disease assessments were assessed as previously described.

2.8. Biochemical assay:

Enzyme activity assays of catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO) were conducted for common bean plants treated with nanoparticles and infected with *S. rolfsii*. 1 g of fresh treated common bean leaves were homogenized in 5 mL of 50 mM Tris buffer (pH 7.8), containing 1mM EDTA-Na_2 and 7.5% polyvinylpyrrolidone at 4 °C. The homogenates were centrifuged for 20 min at 12,000 rpm (4 °C). The enzymatic activity was measured using the model UV-160A spectrophotometer at 25 °C according to Malik and Singh (1980); Hammerschmidt *et al*. (1982) and Aebi (1984).

2.9. Statistical analysis:

ANOVA was performed on the collected data using a randomized complete block design (RCBD). The WASP statistical software was used for dataset analysis. Table 6 and 7 were analyzed based on the three factor factorial experiments. The least significant difference (LSD) was utilized to compare mean differences (Hoshmand, 2006).

RESULTS

3.1. Frequency of the isolated fungi from diseased common bean plants:

Fungi isolated from diseased common bean roots were purified and identified according to their cultural and morphological characters as *Sclerotium rolfsii* Sacc., *Rizctonia solani* Kühn, *Fusarium solani* (Mart.) Sacc., and *Macrophomina* phaseolina (Tassi) Goid. It is evident that the total number of isolates obtained from Giza governorate (24 isolates) was higher than those isolated from Menoufia governorate (18 isolates). *S. rolfsii* showed the highest frequencies in the two governorates. The highest frequency rate of colonies was recorded by *S. rolfsii* (20 colonies), compared to 9 for *Rizctonia solani*, 7 for *Fusarium solani*, and 6 for *Macrophomina phaseolina* (Table 1).

3.2. Pathogenicity and virulence of *Sclerotium rolfsii* isolates:

All the tested isolates caused significant damping-off and root rot symptoms in common bean plants under greenhouse conditions. Degrees of disease incidence and severity varied from weak to severe with significant differences. The isolate that recorded the highest percentages of disease incidence and severity was selected for further experiments.

Table (1): Occurrence and frequency of fungi isolated from the roots and crowns of common bean plants suffered from root rot and crown rot.

Isolated fungi	Inspected governorates				Total No. of isolates
	Menoufia		Giza		
	No. of fungal colonies	Frequency (%)	No. of fungal colonies	Frequency (%)	
S. rolfsii	9.0 a	50.00	11.0 a	45.83	20.0
R. solani	4.0 b	22.22	5.0 b	20.83	9.0
F. solani	3.0 c	16.67	4.0 b	16.67	7.0
M. phaseolina	2.0 d	11.11	4.0 b	16.67	6.0
Total	18.0	100.00	24.0	100.00	42.0

There is no significant difference between values in each column that have the same letter at $P \leq 0.05$.

3.3. Effect of nanoparticles on mycelial growth of *S. rolfsii*:

Three nanoparticles (Ag_2O-NPs, CuO-NPs, and CaO-NPs) and one fungicide (Rizolex-T 50 WP) were tested against *S. rolfsii* growth at different concentrations. The significant inhibition was recorded with Ag_2O-NPs (94.4%), followed by CuO-NPs (88.9%) and the fungicide Rizolex-T 50 WP (84.4%) at 50 µg/mL, while CaO-NPs showed negligible inhibition in comparison with the control (Fig. 1, Supporting Information Table S1). The rate of inhibition and the treatment concentration, up to 50 µg/mL, are directly correlated. The 75 µg/mL concentration had the same impact as the 50 µg/mL concentration.

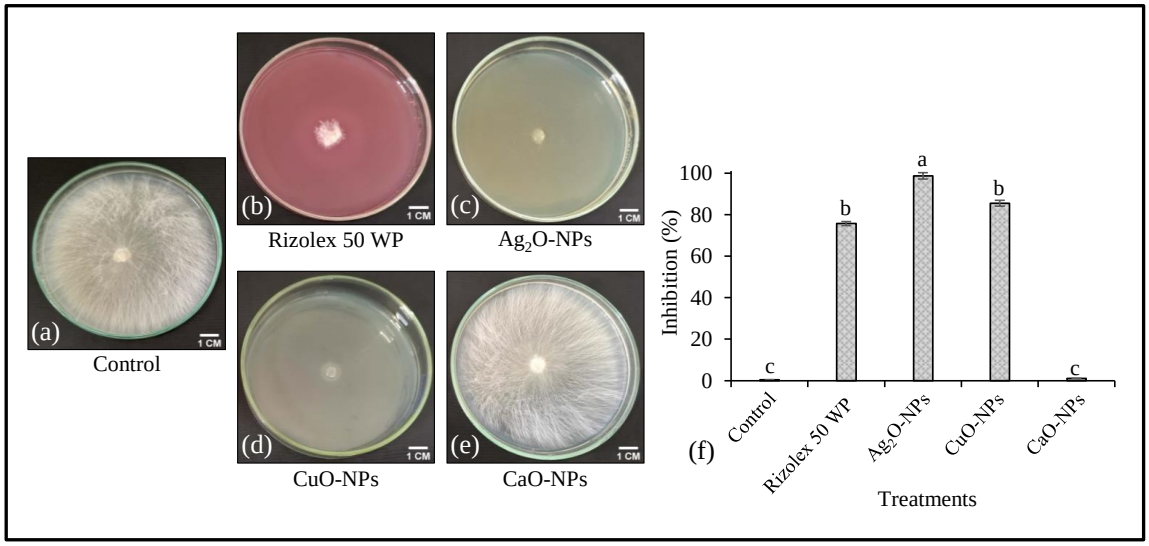

Figure (1): Effect of different treatments on the linear growth of *S. rolfsii* at a concentration of 50 µg/mL. (a) control, (b) Rizolex-T 50 WP, (c) Ag_2O-NPs, (d) CuO-NPs, (e) CaO-NPs, and (f) inhibition percentage of each treatment. The standard deviation of the three biological replicates is represented by error bars. Significant differences are denoted by letters at $p \leq 0.01$.

3.4. Effect of nanoparticles on sclerotia of *S. rolfsii*:

Sclerotia of *S. rolfsii* were highly affected by all tested treatments (Table 2). Although all treatments inhibited the formation of sclerotia when compared to the control, each treatment had a different effect. Rizolex-T 50 WP gave the highest inhibition. The number of sclerotia formed in the presence of Ag_2O-NPs was higher than the rest of the treatments, but the sclerotia formed were mostly decomposed. The sclerotia formed with CuO-NPs were more in number than the Rizolex-T 50 WP treatment, but they delayed maturation. The CaO-NPs formed relatively large sclerotia in size (Fig. 2). Additionally, the diameter measurement of sclerotia was reduced in Ag_2O-NPs (0.63 mm), followed by CuO-NPs (0.86 mm), and Rizolex-T 50 WP (1.1 mm), while the diameter measurement was increased in CaO-NPs (2.9 mm) compared to the control (2.1 mm) (Table 2).

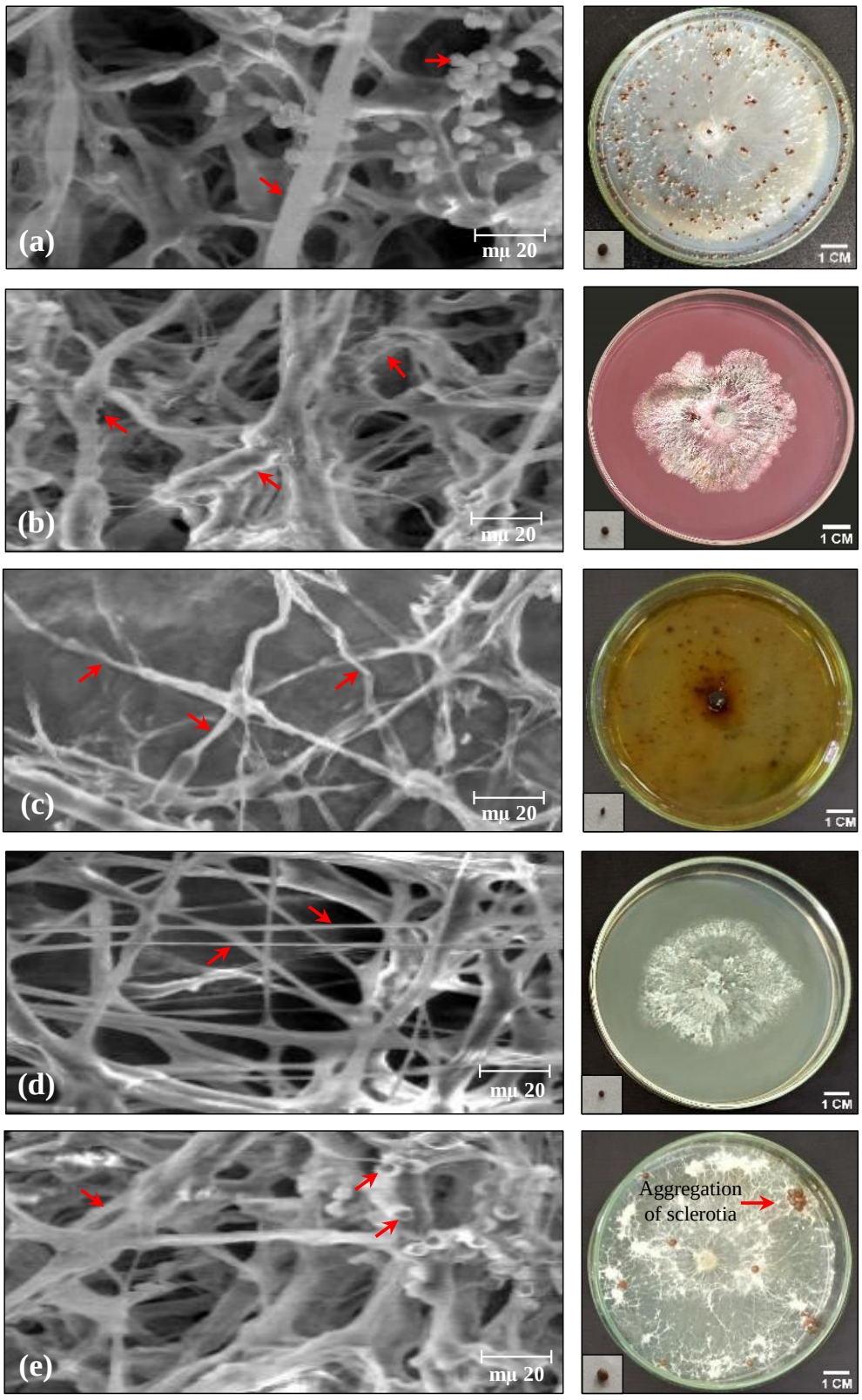

Figure (2): The effect of nanoparticles on *S. rolfsii* and representative scanning electron micrographs compared to control and Rizolex-T 50 WP after 40 days of incubation at 25 °C. Left panel: scanning electron micrograph showing morphological changes of *S. rolfsii* mycelium as denoted by red arrows. Right panel: mycelial fungal growth on PDA amended with nanoparticles, with the enlarged view of sclerotia depicted in the inset. (a) control, (b) Rizolex-T 50 WP, (c) Ag_2O-NPs, (d) CuO-NPs, and (e) CaO-NPs.

Table (2): Number of formed sclerotia on PDA after 40 days of incubation at 25 °C.

Treatments	No. of sclerotia	Reduction of sclerotia (%)	Diameter of sclerotia (mm)
Ag_2O-NPs	35.0 b	87.1	0.63 d
CuO-NPs	18.0 d	93.4	0.86 d
CaO-NPs	25.0 c	90.8	2.9 a
Rizolex-T	12.0 e	95.6	1.1 c
Control	273 a	0	2.1 b

There is no significant difference between values in each column that have the same letter at $P \leq 0.05$.

3.5. Scanning electron microscope (SEM) examination:

The results of the scanning electron microscope examination revealed abnormal growth and thickening in the mycelium after treatment with Rizolex-T 50 WP; distortion and erosion of the mycelium after the treatment by Ag_2O-NPs; shrinkage and atrophy of the mycelium after the treatment by CuO-NPs; and mycelial lumps and thickening with the appearance of cavities in the sclerotia after treatment with CaO-NPs (Fig. 2).

3.6. Evaluation of nanoparticles on controlling *S. rolfsii* damping-off and crown rot:

The effectiveness of nanoparticles and fungicide (50 µg/mL) against damping-off caused by *S. rolfsii* was assessed on treated common bean cultivars Giza 12 and Alpha under greenhouse conditions (Tables 3, 4). The three nanoparticles reduced the pre- and post-emergence damping-off in the two locations in comparison with the infested control. Ag_2O-NPs showed a more effective impact than the other nanoparticles. The effects of the Ag_2O-NPs were relatively close to the effect of Rizolex 50 WP in controlling damping-off.

Furthermore, Ag_2O-NPs and Rizolex-T 50 WP recorded the highest decrease in root and crown rot severity, followed by CuO-NPs and CaO-NPs in Giza governorate (Fig. 3a, Supporting Information Table S2), and Menoufia governorate (Fig. 3c, Supporting Information Table S3). Additionally, the results showed that the best efficacy in decreasing root rot incidence and disease severity was recorded by Rizolex-T 50 WP and Ag_2O-NPs, respectively, followed by CuO-NPs, while CaO-NPs recorded the lowest efficacy compared with control in both locations (Tables 3, 4 and Fig. 3b, d).

Table (3): Effect of nanoparticles on damping-off and root rot of common beans caused by *S. rolfsii* grown under greenhouse located at Giza governorate.

Treatments	Cultivar Giza 12				Cultivar Alpha			
	Damping-off		Root/ crown rot	Plant Survival (%)	Damping-off		Root/ crown rot	Plant Survival (%)
	Pre-emergence	Post-emergence			Pre-emergence	Post-emergence		
Ag_2O-NPs	10.00 d	6.66 c	10.00 c	73.33 b	6.66 d	13.33 c	13.33 b	66.66 b
CuO-NPs	23.33 c	13.33 b	6.66 d	56.66 c	26.66 c	23.33 b	6.66 c	50.00 c
CaO-NPs	33.33 b	26.66 a	13.33 b	26.66 d	30.00 b	33.33 a	20.00 a	23.33 d
Rizolex-T	6.66 e	6.66 c	10.00 c	76.66 b	10.00 e	16.66 c	16.66 b	66.66 b
Control (infested)	46.66 a	30.00 a	16.66 a	6.66 e	43.33 a	26.66 b	20.00 a	10.00 e
Control (untreated)	0.00 f	0.00 d	0.00 e	100 a	0.0 f	0.0 d	0.0 d	100 a

There is no significant difference between values in each column that have the same letter at $P \leq 0.05$.

Table (4): Effect of nanoparticles on damping-off and root rot of common beans caused by *S. rolfsii* grown under greenhouse located at Menoufia governorate.

Treatments	Cultivar Giza 12				Cultivar Alpha			
	Damping-off		Root/ crown rot	Plant Survival (%)	Damping-off		Root/ crown rot	Plant Survival (%)
	Pre-emergence	Post-emergence			Pre-emergence	Post-emergence		
Ag_2O-NPs	6.66 d	6.66 c	6.66 c	80.00 b	6.66 c	10.00 c	10.00 c	73.33 b
CuO-NPs	16.66 c	16.66 b	6.66 c	60.00 c	23.33 b	13.33 b	6.66 d	56.66 c
CaO-NPs	30.00 b	26.66 a	13.33 b	30.00 d	26.66 b	26.66 a	20.00 a	26.66 d
Rizolex-T	6.66 d	3.33 d	6.66 c	83.33 b	6.66 c	6.66 d	13.33 b	73.33 b
Control (infested)	46.66 a	26.66 a	20.00 a	6.66 e	40.00 a	26.66 a	20.00 a	13.33 e
Control (untreated)	0.00 e	0.00 e	0.00 d	100 a	0.00 d	0.00 e	0.00 e	100 a

There is no significant difference between values in each column that have the same letter at $P \leq 0.05$.

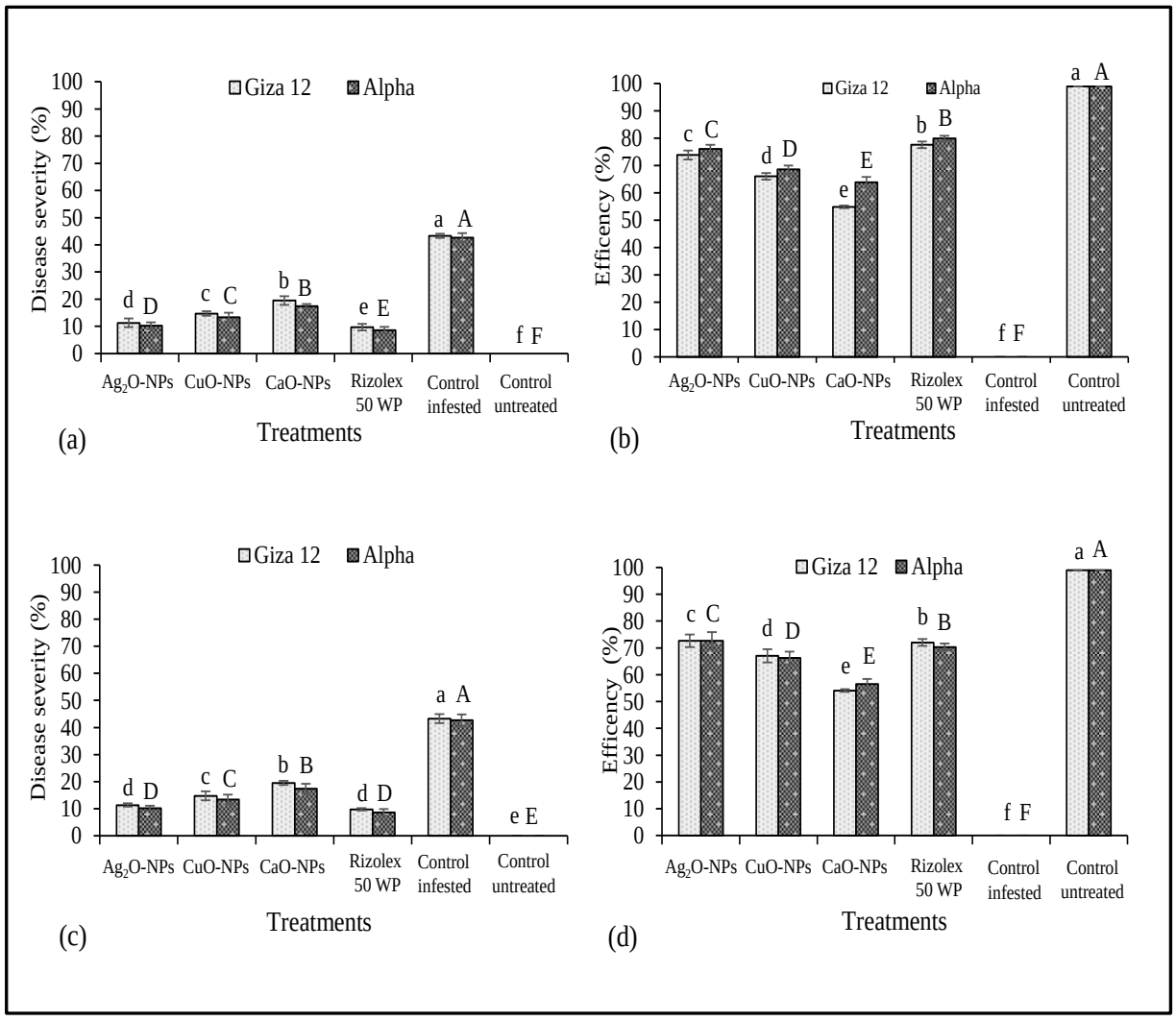

Figure (3): Evaluation of nanoparticles in controlling root and crown rot caused by *S. rolfsii* under greenhouse conditions. Effect of nanoparticles on the disease severity of common bean root and crown rot in (a) Giza, and (c) Menoufia governorates. The efficacy of various treatments in reducing common bean crown rot in (b) Giza and (d) Menoufia governorates. Small letters denote Giza 12 cultivar whereas Capital letters denote Alpha cultivar. The standard deviation of the three biological replicates is represented by error bars.

3.7. Effect of nanoparticles on plant growth and yield parameters:

Application of nanoparticles increased the growth parameters of both bean cultivars, Giza 12 and Alpha. The Ag_2O-NPs and CuO-NPs treatments had the greatest impact on increasing stem and root length and fresh and dry weights, followed by CaO-NPs (Table 5). Furthermore, the impact of nanoparticles extended beyond the increase in plant growth parameters to include increasing the number of pods and seeds and the weight of seeds per plant for both cultivars compared to Rizolex-T 50 WP and control, which had a negative effect on the plants of both cultivars (Table 6).

3.8. Effect of nanoparticles on the activity of defense enzymes in common bean plants:

Ag_2O-NPs, CuO-NPs, and CaO-NPs demonstrated outstanding effects in enhancing the activities of the defense-related enzymes, catalase, peroxidase, and polyphenol oxidase. The responses of the two common bean cultivars (Giza 12 and Alpha) to the tested nanoparticles were almost the same. Although the effect of Rizolex-T 50 WP on the pathogen was effective, its effect on stimulating enzymes inside the plant was slight compared to the nanoparticles materials (Fig. 4, Supporting Information Table S4).

Table (5): Effect of nanoparticles on plant growth parameters of common bean grown in Giza and Menoufia governorates under greenhouse conditions.

Treatments (A)	Cultivars (B)	Shoot length (cm)		Mean (AB)	Mean (A)	Root length (cm)		Mean (AB)	Mean (A)	Fresh weight (g)		Mean (AB)	Mean (A)	Dry weight (g)		Mean (AB)	Mean (A)
		Location (C)				Location (C)				Location (C)				Location (C)			
		Giza	Menoufia			Giza	Menoufia			Giza	Menoufia			Giza	Menoufia		
Ag_2O-NPs	Giza 12	57.3	59.7	58.5	64.7	6.2	7.1	6.65	10.1	74.8	77.4	76.1	78.3	17.4	19.8	18.6	19.8
	Alpha	69.3	72.3	70.8		12.8	14.1	13.5		79.3	81.8	80.6		20.4	21.4	20.9	
Mean (AC)		63.3	66	-	-	9.5	10.6	-	-	77.1	79.6	-	-	18.9	20.6	-	-
CuO-NPs	Giza 12	53.0	54.8	53.9	60.9	5.8	6.8	6.3	9.38	61.3	64.8	63.1	70.3	14	16.3	15.2	16.6
	Alpha	66.5	69.3	67.9		11.7	13.2	12.5		76.8	78.3	77.6		17.5	18.4	18.0	
Mean (AC)		59.8	62.1	-	-	8.75	10	-	-	69.1	71.6	-	-	15.8	17.4	-	-
CaO-NPs	Giza 12	40.2	58.4	49.3	54.3	5.5	6.4	5.95	8.43	57.2	61.4	59.3	65.5	13.3	15.9	14.6	15.4
	Alpha	57.1	61.6	59.4		9.9	11.9	10.9		70.7	72.6	71.7		15.7	16.8	16.3	
Mean (AC)		48.7	60.0	-	-	7.7	9.15	-	-	64.0	67.0	-	-	14.5	16.4	-	-
Rizolex-T	Giza 12	47.8	56.8	52.3	52.5	3.4	4.2	3.8	6.55	40.5	43.2	41.9	47.9	10.2	11.1	10.7	12.8
	Alpha	47.1	58.1	52.6		8.7	9.9	9.3		52.9	54.8	53.9		14.6	15.4	15.0	
Mean (AC)		47.5	57.5	-	-	6.05	7.0	-	-	46.7	49	-		12.4	13.3	-	-
Control (infested)	Giza 12	32.4	41.2	36.8	37.7	2.5	3.1	2.8	3.98	28.4	31.9	30.2	32.3	6.1	9.2	7.65	9.23
	Alpha	33.5	43.7	38.6		4.8	5.5	5.1		33.5	35.4	34.5		10.3	11.3	10.8	
Mean (AC)		33.0	42.5	-	-	3.65	4.3	-	-	31.0	33.7			8.2	10.3	-	-
Control (untreated)	Giza 12	61.3	63.5	62.4	67.9	6.7	8.2	7.4	11.1	77.3	79.9	78.6	80.7	20.1	21.4	20.8	22.1
	Alpha	70.4	76.3	73.4		14.1	15.3	14.7		80.4	85.3	82.9		23.7	23.2	23.5	
Mean (AC)		65.9	69.9	-	-	10.4	11.8	-	-	78.9	82.6	-	-	21.9	22.3	-	-
Overall mean	Giza 12	48.6	55.7	52.2	-	5.0	5.9	5.4	-	56.5	59.8	58.1	-	13.5	15.6	14.6	-
	Alpha	57.3	63.5	60.4		10.3	11.6	10.9		68.0	59.8	63.9		17.0	17.8	17.4	
	Mean (C)	52.9	59.6	-		7.6	8.8	-		62.3	59. 7	-		15.3	16.7	-	
LSD at 0.05	A	1.362				0.475				3.293				0.800			
	B	0.860				0.298				2.089				0.513			
	C	0.860				0.298				2.089				0.513			
	A × B	1.931				0.662				4.652				1.142			
	A × C	1.931				0.662				4.652				1.142			
	B × C	1.220				0.426				2.949				0.728			
	A×B×C	2.735				0.940				6.586				1.610			

Table (6): Effect of nanoparticles on yield parameters of common bean grown in Giza and Menoufia governorates under greenhouse conditions.

Treatments (A)	Cultivars (B)	No. of pods per plant		Mean (AB)	Mean (A)	No. of seeds per pod		Mean (AB)	Mean (A)	No. of seeds per plant		Mean (AB)	Mean (A)	Weight of 100 seeds (g)		Mean (AB)	Mean (A)
		Location (C)				Location (C)				Location (C)				Location (C)			
		Giza	Menoufia			Giza	Menoufia			Giza	Menoufia			Giza	Menoufia		
Ag_2O-NPs	Giza 12	7	7.4	7.2	7.475	6.5	6.4	6.45	6.525	49.4	47.4	48.4	49.775	47.2	48.3	47.75	47.275
	Alpha	7.7	7.8	7.75		6.6	6.6	6.6		50.8	51.5	51.15		46.3	47.3	46.8	
Mean (AC)		7.35	7.6	-	-	6.55	6.5	-	-	50.1	49.45	-	-	46.75	47.8	-	-
CuO-NPs	Giza 12	6.3	6.6	6.45	6.5	5.8	5.7	5.75	5.8	36.5	37.6	37.05	37.675	44.5	45.2	44.85	45.3
	Alpha	6.4	6.7	6.55		5.8	5.9	5.85		37.1	39.5	38.3		45.1	46.4	45.75	
Mean (AC)		6.35	6.65	-	-	5.8	5.8	-	-	36.8	38.55	-	-	44.8	45.8	-	-
CaO-NPs	Giza 12	6.5	6.3	6.4	6.4	4.5	5.3	4.9	5	29.3	33.4	31.35	32	39.8	40.5	40.15	41.05
	Alpha	6.3	6.5	6.4		5	5.2	5.1		31.5	33.8	32.65		40.7	43.2	41.95	
Mean (AC)		6.4	6.4	-	-	4.75	5.25	-	-	30.4	33.6	-	-	40.25	41.85	-	-
Rizolex-T	Giza 12	6.7	6.6	6.65	6.525	4.3	5.4	4.85	5.175	28.8	35.6	32.2	33.675	42.3	45.1	43.7	44.075
	Alpha	6.6	6.2	6.4		5.4	5.6	5.5		35.6	34.7	35.15		43.5	45.4	44.45	
Mean (AC)		6.65	6.4	-	-	4.85	5.5	-	-	32.2	35.15	-	-	42.9	45.25	-	-
Control (infested)	Giza 12	3.7	3.2	3.45	3.45	3.2	3.3	3.25	3.35	19.2	17.4	18.3	18.725	29.7	30.6	30.15	31
	Alpha	3.4	3.5	3.45		3.4	3.5	3.45		19.5	18.8	19.15		31.2	32.5	31.85	
Mean (AC)		3.55	3.35	-	-	3.3	3.4	-	-	19.35	18.1	-	-	30.45	31.55	-	-
Control (untreated)	Giza 12	7.8	8.1	7.95	8.2	7.2	6.8	7	7.05	52.2	50.3	51.25	53	50.8	51.4	51.1	50.7
	Alpha	8.4	8.5	8.45		7.3	6.9	7.1		55.3	54.2	54.75		50.5	50.1	50.3	
Mean (AC)		8.1	8.3	-	-	7.25	6.85	-	-	53.75	52.25	-	-	50.65	50.75	-	-
Overall mean	Giza 12	6.3	6.3	6.30	-	5.2	5.4	5.30	-	35.9	36.9	36.40	-	42.3	43.5	42.9	-
	Alpha	6.4	6.5	6.45		5.5	5.6	5.55		38.3	38.7	38.50		42.8	44.1	43.45	
	Mean (C)	6.35	6.40	-		5.35	5.50	-		37.10	37.80	-		42.55	43.8	-	
LSD at 0.05	A	0.463				0.427				1.224				1.326			
	B	0.271				0.251				0.792				0.811			
	C	0.271				0.251				0.792				0.811			
	A × B	0.632				0.613				1.823				1.892			
	A × C	0.632				0.613				1.823				1.892			
	B × C	0.416				0.401				1.213				1.302			
	A×B×C	0.927				0.911				2.534				2.613			

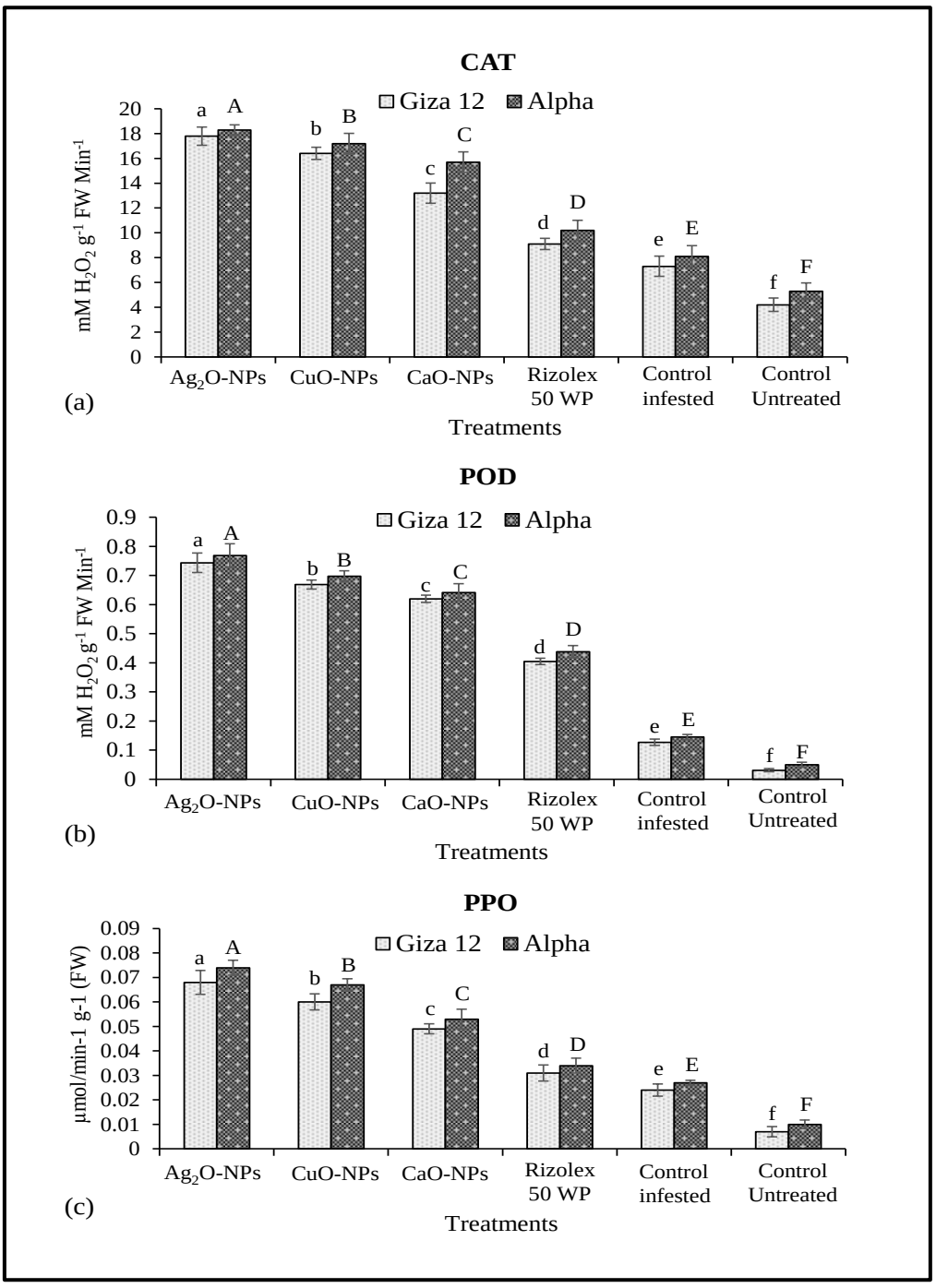

Figure (4): Effect of nanoparticles on the activities of defense related enzymes in common bean plants. (a) catalase (CAT), (b) peroxidase (POD), and (c) polyphenol oxidase (PPO). Small letters denote Giza 12 cultivar whereas Capital letters denote Alpha cultivar. The standard deviation of the three biological replicates is represented by error bars.

DISCUSSION

4.1. Mechanism of nanoparticles against phytopathogens:

The mechanisms underlying the antimicrobial activity of nanoparticles (Fig. 5) may be due to: (1) the physical contact between nanoparticles and fungal propagules that allows nanoparticles to bind with sulfur-containing proteins, inhibits their proper function in the membrane causing membrane potential collapse, and affects cell permeability; (2) the interruption in electron transport causing protein oxidation; (3) the genotoxic ions that can damage DNA causing cell death; (4) the interference with the intake of nutrients; and (5) the release of Reactive Oxygen Species (ROS) by the disruption of ROS-scavenging defense mechanisms causes damage to the biomolecules, leading to cell death (Lemire *et al*., 2013; Sun *et al*., 2018).

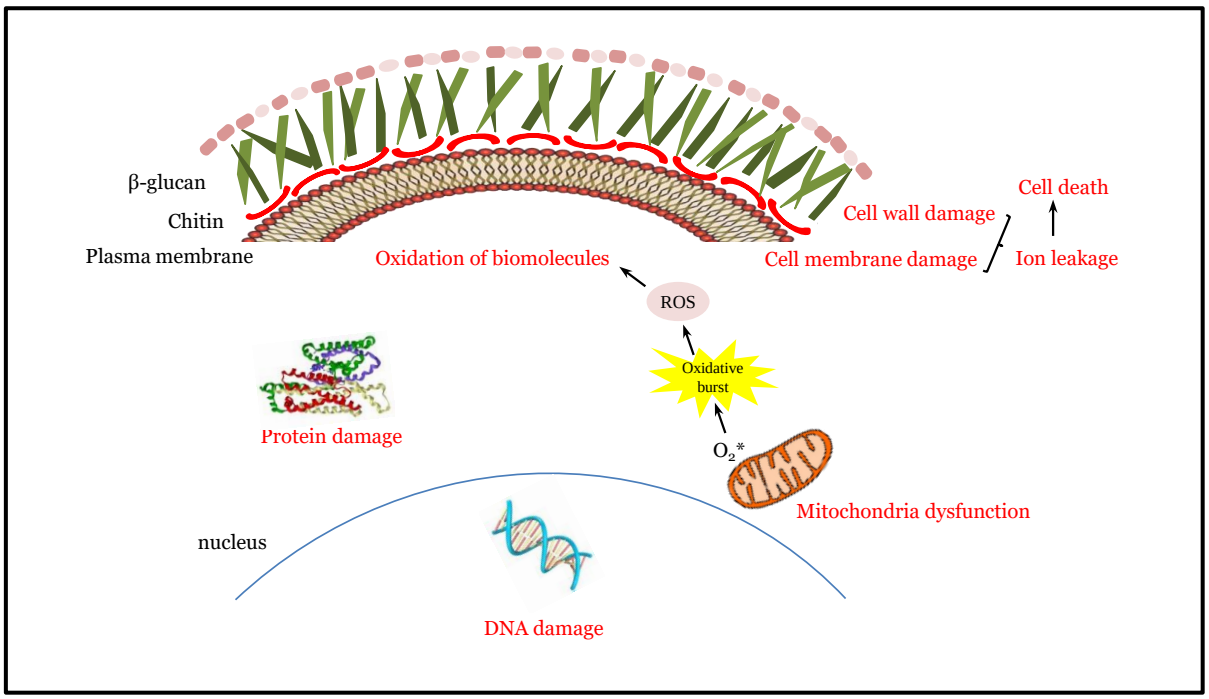

Figure (5): The toxic impact of nanoparticles on *S. rolfsii*.

Our results showed that nanoparticles cause physiological, morphological, and structural changes in mycelium and sclerotia, which were emphasized by scanning electron microscope results. This may be due to their ability to penetrate the walls of mycelium and sclerotia and their ease of permeability due to the precision of their size compared to the fungicide used (Nel *et al*., 2006). This explains nanoparticles superiority over fungicide in changing sclerotia measurements and destroying mycelium.

4.2. Effects of Ag_2O-NPs on Fungal Growth:

Silver NPs have gained more attention compared to other NPs due to their unique properties, many studies have demonstrated that silver nanoparticles have a potent inhibitory effect against a variety of plant pathogenic fungi, including *Colletotrichum gloeosporioides*, *Bipolaris sorokiniana*, *Alternaria alternata*, *Sclerotinia minor* and *Fusarium oxysporum* (Kim *et al*., 2008, Min *et al*., 2009 and Pandey *et al*., 2018). They mentioned that silver nanoparticles caused extensive damage that started with breaking in the hyphal wall and cell membrane and extended internally, causing hyphal death. The fungal growth and sclerotial germination of *R. solani* and *S. sclerotiorum* were significantly reduced by silver nanoparticles (Min *et al*., 2009).

The obtained results demonstrated that Ag_2O-NPs and CuO-NPs are the most effective NPs against *S. rolfsii* and have performed better than CaO-NPs and the commercial fungicide Rizolex-T 50 WP. Moreover, Ag_2O-NPs displayed stronger overall inhibition than CuO-NPs.

This may be due to the highest dual impact of Ag_2O-NPs in the inhibition of the fungus, which was simultaneous with the enhancement of plant defense enzymes. These results are in agreement with those reported by Aleksandrowicz-Trzcinska *et al*. (2018) against *R. solani*, *F. oxysporum*, and *F. redolens*. Ag_2O-NPs treated plates showed abnormal sclerotial formation, this result agrees with the findings of Kumar *et al*. (2015). Furthermore, Al-Othman *et al*. (2014) reported that silver nanoparticles reduced *A. flavus* spore number. Additionally, Elamawi and Al-Harbi, (2014) reported that Fusarium disease incidence was reduced by silver nanoparticles to 5% compared with 100% for the untreated control in tomatoes. Also, Jo *et al*. (2009) found that silver nanoparticles reduced the disease severity of *Bipolaris sorokiniana* the cause of spot blotch and common root rot on gramineous species and *Magnaporthe grisea* the cause of blast on rice.

4.3. Effects of CuO-NPs on Fungal Growth:

Copper nanoparticles were used against various fungal phytopathogens belonging to various genera, such as *Alternaria*, *Macrophomina*, *Fusarium*, *Penicillium*, *Colletotrichum*, *Rhizoctonia*, *Phytophthora*, and *Botrytis* (Gunalan *et al*., 2012; Sankar *et al*., 2014; Ismail, 2021). Rubina *et al*. (2017) reported cytoplasmic loss, cytoplasmic coagulation, distortion, and destruction of fungal

hyphae in *R. solani* and *S. rolfsii* after copper nanoparticle application. Hermida-Montero *et al*. (2019) reported that CuO nanoparticles were implicated in the suppression of fungal radial growth, alterations in the shape of the hypha, ROS production and membrane damage in *F. oxysporum*.

4.4. Effects of CaO-NPs on Fungal Growth:

Our results demonstrated that CaO-NPs have no significant effect on the hyphal growth of *S. rolfsii*, while the effect of CaO-NPs was limited to the aggregation of sclerotia, which decreased sclerotial germination. This was in agreement with Wang *et al*. (2014), who reported that CaO-NPs caused sclerotia aggregation.

Although calcium nanoparticles have no significant effect on fungal growth, many studies have reported their wide range on controlling plant bacteria. The broad-spectrum antibacterial efficiency of CaO-NPs was demonstrated by Roy *et al*. (2013) against *Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida tropicalis.* The antimicrobial activity of CaO-NPs is due to enhanced ROS release. ROS interacts with the carbonyl group present in bacterial cell wall peptide linkages and polyunsaturated phospholipids in the plasma membrane, resulting in protein degradation and bacterial cell wall destruction (Gedda *et al*., 2015).

4.5. Nanoparticles enhanced plant growth and increased yield:

Silver and copper nanoparticles showed high effectiveness in reducing the number of damping-off plants compared to the control and calcium nanoparticles due to their direct effect on the pathogen and the positive effect on the improvement rate of seed germination. Moreover, the root uptake of silver, copper, and calcium nanoparticles increased all growth criteria (shoot & root length and fresh & dry weight) of common bean plants compared with fungicide-treated plants and control in both cultivars (Fig. 6). Silver nanoparticles had a stimulating effect on the growth of common bean and corn plants (Salama, 2012), wheat plants (Latif *et al*., 2017), and fenugreek plants (Sadak, 2019). Silver nanoparticles significantly promote photosynthesis by increasing chlorophyll content and nitrogen metabolism, which in turn increases the weight and growth of plants (Farghaly and Nafady, 2015; Latif *et al*., 2017).

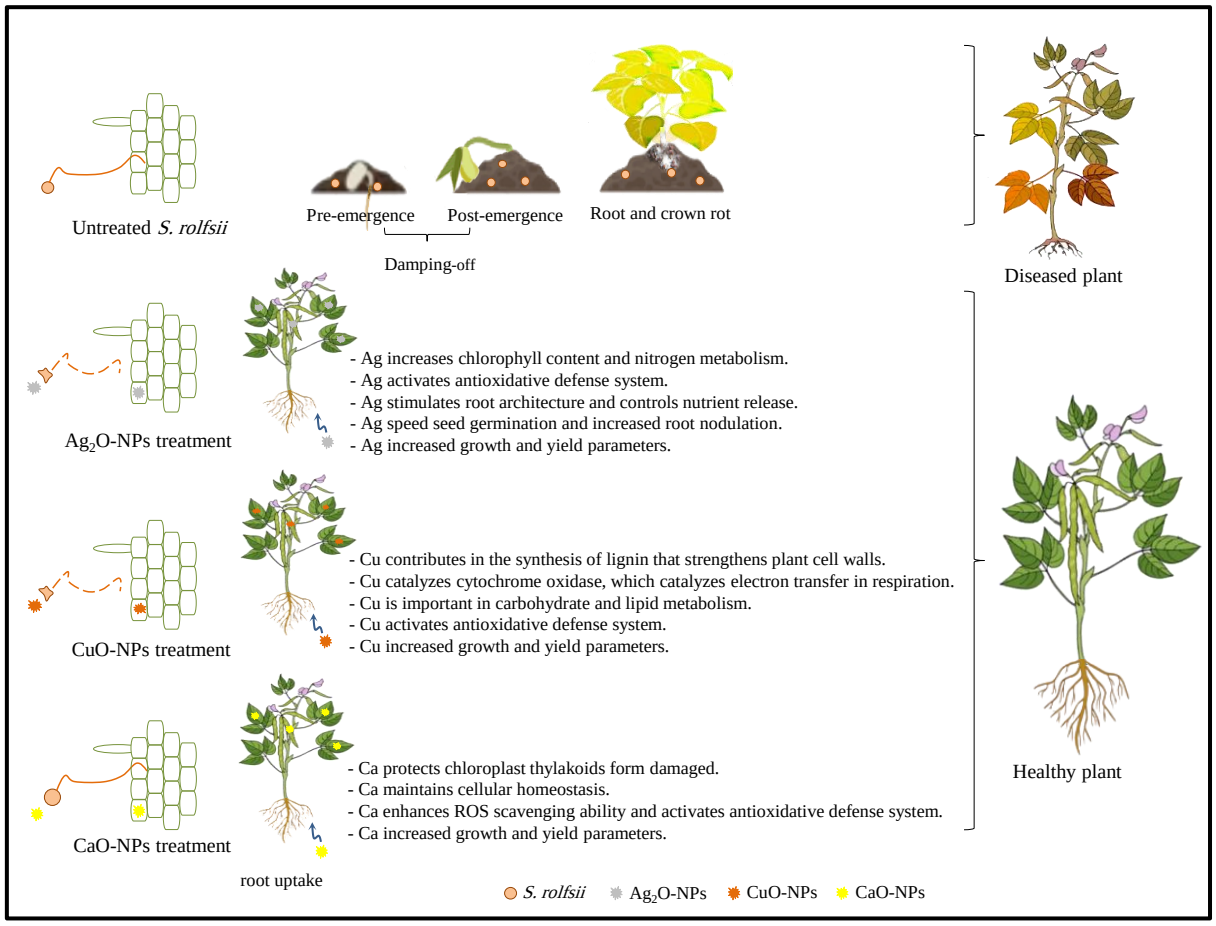

Figure (6): Model of nanoparticle effects on *S. rolfsii* root colonization ability and improving plant characterization.

Many studies have reported the importance of silver nanoparticles in increasing yield production of mung beans (Najafi and Jamei, 2014) and wheat (Razzaq *et al*., 2016) and they have attributed this to the increase in growth parameters, photosynthetic pigments, and Indole-3-acetic acid. CuO nanoparticle-treated maize plants outperformed control plants in terms of anthocyanin, chlorophyll, and carotenoid content, as well as leaf water content and biomass (Nguyen *et al*., 2022). CaO nanoparticles exhibit unique structural properties for common beans, so they can be exploited for soil improvement and plant fertilization. The positive effects of CaO-NPs on the growth of lettuce and zucchini (Meier *et al*., 2020), rice (Syu *et al*., 2020), chickpea (Gandhi *et al*., 2021), and barley (Nazir *et al*., 2022) have been reported. According to Gandhi *et al*. (2021), CaO-NPs may prevent chloroplast thylakoids from being damaged and can keep cellular homeostasis during abiotic stress, which can enhance the photosynthetic activity in plants.

Our results showed that calcium nanoparticles did not have a significant effect on the fungal growth of *S. rolfsii* in laboratory experiments, but they achieved an impressive effect in inhibiting it inside the plant. This may be due to the fact that Ca^{2+} is the essential regulator in many developmental and adaptive mechanisms in plants since it is involved in several biological activities, including cell proliferation, intracellular signaling, resisting abiotic stress, and plant-pathogen interactions (Yazıcılar *et al*., 2021).

4.6. Nanoparticles enhanced plant defense enzymes activities:

Common bean plants cultivars Giza 12 and Alpha had a great chance of benefiting from nanoparticles by increasing the enzymatic activity of various plant enzymes such as catalase, peroxidase, and polyphenol oxidase, which was positively reflected in the physiological state and the defense system of the plant against the pathogen. Peroxidase and polyphenol oxidase increase lignification and suberization, which protect plant cell walls and limit the spread of pathogens (Passardi *et al*., 2004). Furthermore, polyphenol oxidase has a role in the oxidation of plant phenolic compounds that increases the content of oxidized quinone and its derivatives, causing a delay in pathogen progress and increasing plant resistance to pathogen invasion (Tyagi *et al*., 2000). Silver nanoparticles controlled soft rot disease in sugar beet and increased peroxidase and polyphenol oxidase enzymes (Ghazy *et al*., 2021). According to Elmer *et al*. (2018), nanoparticles serve a special function in the direct uptake and accumulation of silica, which promotes leaf erectness and improves the defense response to fungal infections. Catalase activity of cowpea plant was significantly impacted by copper nanoparticles (Ogunkunle *et al*., 2018). The use of calcium nanoparticles enhanced peroxidase and catalase activities in barley seedlings, which are self-defense responses to oxidative stress (Nazir *et al*., 2022).

CONCLUSION

The soil-borne fungus *S. rolfsii* causes severe damage to different vegetable crops and is difficult to control by traditional methods. This prompted us to use new alternatives to the fungicide that are more effective and easier to penetrate into the sclerotia of the fungus. The Ag and Cu nanoparticles achieved a significant reduction of *S. rolfsii* both *in vitro* and *in vivo*. Additionally, Ag, Cu, and Ca nanoparticles improved plant growth and crop production. Furthermore, the nanoparticles enhanced plant defense enzymes compared with the fungicide-treated plants and controls. Despite the fact that the effect of nanomaterials on fungal reduction, plant growth promotion, and productivity improvement is to some extent acceptable, no study has confirmed the safety of using nanomaterials. In the future study we will attempt to analyze the proteins and study their involved pathways inside common bean plants after using nanomaterials in order to have a complete overview of nanomaterial behavior inside plant cells and fully ensure the safety of nano-treated plants for humans and animals.

AUTHOR CONTRIBUTIONS

Kamel, S.M. conceived the experiments. Elgobashy, S.F.; Shebl, A.M. and Kamel, S.M. designed the experiments. Taha, A.A. brought nanomaterials. Elagamey, E.; Elgobashy, S.F.; Shebl, A.M.; Taha, A.A.; Arafa, R.A. and Kamel, S.M. performed the experiments. Elgobashy, S.F. and Kamel, S.M. carried out the data analysis. Elagamey, E. illustrated, graphically represented and designed figures. Elagamey, E. discussed the study and wrote the article. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

Abou-Salem, E.; Ahmed, A.R.; Elbagory, M. and Omara, A.E.D. 2022. Efficacy of biological copper oxide nanoparticles on controlling damping-off disease and growth dynamics of sugar beet (*Beta vulgaris* L.) Plants. Sustainability, 14(19): 12871.

Aebi, H. 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.

Aleksandrowicz-Trzcinska, M.; Szaniawski, A.; Olchowik, J. and Drozdowski, S. 2018. Effects of copper and silver nanoparticles on growth of selected species of pathogenic and wood-decay fungi in vitro. For Chron., 94 (2): 109-116.

Allahverdiyev, A.M.; Emrah, S.A.; Malahat, B. and Miriam, R. 2011. Antimicrobial effects of TiO_2 and Ag_2O nanoparticles against drug-resistant bacteria and *leishmania* parasites. Future Microbiol., 6: 933-940.

Al-Othman, M.R.; Abd El-Aziz, A.R.M.; Mahmoud, M.A.; Eifan, S.A.; El-Shikh, M.M. and Majrashi, M. 2014. Application of silver nanoparticles as antifungal and antiaflatoxin B1 produced by *Aspergillus flavus*. Dig. J. Nanomater Biostruct., 9: 151-157.

Boxi, S.S.; Mukherjee, K. and Paria, S. 2016. Ag doped hollow TiO_2 nanoparticles as an effective green fungicide against *Fusarum solani* and *Venturia inaequalis* phytopathogens. Nanotechnol., 27: 085103.

Chen, J.; Wang, X. and Han, H. 2013. A new function of graphene oxide emerges: inactivating phytopathogenic bacterium, *Xanthomonas oryzae* pv. *oryzae*. J. Nanopart. Res., 15(5): 1658.

Chu, H.; Kim, H.J.; Kim, J.S.; Kim, M.S.; Yoon, B.D.; Park, H.J. and Kim, C.Y. 2012. A nanosized Ag-silica hybrid complex prepared by γ-irradiation activates the defense response in Arabidopsis. Radiation Physics Chemist., 81(2): 180-184.

Dhingra, O.D. and Sinclair, J.B. 1985. Basics plant pathology methods. CRC Press. Inc. Boca. Raton. Florida., 13-44.

Eid, K.E. 2014. Biological control of Bean damping-off caused by *Sclerotium rolfsii*. Egypt. J. Phytopathol., 42(1): 179-191.

Elamawi, R.M. and Al-Harbi, R.E. 2014. Effect of biosynthesized silver nanoparticles on *Fusarium oxysporum* fungus the cause of seed rot disease of faba bean, tomato and barley. J. Plant Protect. Pathol. Mansoura Univ., 5(2): 225-237.

El-Argawy, E.; Rahhal, M.M.H.; El-Korany, A.; Elshabrawy, E.M. and Eltahan, R.M. 2016. Efficacy of some nanoparticles to control damping-off and root rot of sugar beet in El-Behiera Governorate. Asian J. Plant Pathol., 11(1): 35-47.

Elmer, W.; De La Torre-Roche, R.; Pagano, L.; Majumdar, S.; Zuverza-Mena, N.; Dimpka, C.; Gardea-Torresdey, J. and White, J.C. 2018. Effect of metalloid and metallic oxide nanoparticles on Fusarium wilt of watermelon. Plant Dis., 102(7): 1394-1401.

Farghaly, F.A. and Nafady, N.A. 2015. Green synthesis of silver nanoparticles using leaf extract of *Rosmarinus officinalis* and its effect on tomato and wheat plants. J. Agric. Sci., 7(11): 277-287.

Flores-Moctezuma, H.E.; Montes-Belmont, R.; Jiménez-Pérez, A. and Nava-Juárez, R. 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. Crop Prot., 25: 195-201.

Gandhi, N.; Shruthi, Y.; Sirisha, G. and Anusha, C.R. 2021. Facile and eco-friendly method for synthesis of calcium oxide (CaO) nanoparticles and its potential application in agriculture. Saudi J. Life Sci., 6: 89-103.

Gedda, G.; Pandey, S.; Lin, Y.C. and Wu, H.F. 2015. Antibacterial effect of calcium oxide nano-plates fabricated from shrimp shells. Green Chemist., 17(6): 3276-3280.

Ghazy, N.A.; El-Hafez, A.; Omnia, A.; El-Bakery, A.M. and El-Geddawy, D.I. 2021. Impact of silver nanoparticles and two biological treatments to control soft rot disease in sugar beet (*Beta vulgaris* L). Egypt. J. Bio. Pest Con., 31(1): 1-12.

Gogos, A.; Knauer, K. and Bucheli, T.D. 2012. Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. J. Agric. Food Chem., 60(39): 9781-9792.

Gohari, G.; Mohammadi, A.; Akbari, A.; Panahirad, S.; Dadpour, M.R.; Fotopoulos, V. and Kimura, S. 2020. Titanium dioxide nanoparticles (TiO_2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. Sci. Rep., 10(1): 1-14.

Gunalan, S.; Sivaraj, R. and Venckatesh, R. 2012. *Aloe barbadensis* Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: Optical properties. Spectrochim. Acta A. Mol. Biomol. Spectrosc., 97: 1140-1144.

Hammerschmidt, R.; Nuckles, E. and Kuć, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol., 20: 73-82.

Hermida-Montero, L.A.; Nicolaza, P.A.I; Mtz-Enriquez, G.C.; Paraguay-Delgado, F. and Greta R. S. 2019. Aqueous-phase synthesis of

nanoparticles of copper/copper oxides and their antifungal effect against *Fusarium oxysporum*. J. Hazard Mater, 380: 120850.

Hoshmand, A.R. 2006. Design of Experiments for Agriculture and the Natural Sciences. 2nd Ed. Chapman and Hall, New York. 456 pp.

Ismail, A.M., 2021. Efficacy of copper oxide and magnesium oxide nanoparticles on controlling black scurf disease on potato. Egypt. J. Phytopathol., 49(2): 116-130.

Jo, Y.K.; Kim, B.H. and Jung, G. 2009. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis., 93:1037-1043.

Kator, L.; Oche, O.D. and Hosea, Z.Y. 2015. Incidence and severity of *Sclerotium rolfsii* disease on tomato farms in Chile Island (Makurdi), Benue State, Nigeria. J. Agric. Vet. Sci., 8(11): 97-103.

Kim, K.J.; Sung, W. S.; Moon, S. K; Choi, J.S.; Kim, J.G. and Lee, D.G. 2008. Antifungal effect of silver nanoparticles on dermatophytes. J. Microbiol. Biotechnol., 18: 1482-1484.

Kumar, G.D.; Natarajan, N. and Nakkeeran, S. 2015. Synthesis and characterization of silver (Ag) nanoparticles and its antifungal activity against *Sclerotium rolfsii* in chilli (*Capsicum annum* L.). Int. J. Agric. Sci. Res., 5: 211-218.

Latif, H.H.; Ghareib, M. and Abu Tahon, M. 2017. Phytosynthesis of silver nanoparticles using leaf extracts from *Ocimum basilicum* and *Mangifira indica* and their effect on some biochemical attributes of *Triticum aestivum* Gesunde Pflanzen, 69: 39-46.

Lemire, J.A.; Harrison, J.J. and Turner, R.J. 2013. Antimicrobial activity of metals: Mechanisms, molecular targets and applications. Nat. Rev. Microbiol., 11: 371-384.

Los, F.G.B.; Zielinski, A.A. F.; Wojeicchowski, J.P.; Nogueira, A. and Demiate, I.M. 2018. Beans (*Phaseolus vulgaris* L.): whole seeds with complex chemical composition. Current Opinion in Food Sci., 19: 63-71.

Malik, C.P. and Singh, M.B. 1980. Plant Emynology and Histo-enzymology, Kalyani Publishers. Indian and Printed in Navin, Shanndara. Delhi, pp. 54-56.

Mansoor, S.; Zahoor, I.; Baba, T.R.; Padder, S.A.; Bhat, Z.A.; Koul, A.M. and Jiang, L. 2021. Fabrication of silver nanoparticles against fungal pathogens. Front. Nanotechnol., 67.

Meier, S.; Moore, F.; Morales, A.; González, M. E.; Seguel, A. and Meriño-Gergichevich, C. 2020. Synthesis of calcium borate nanoparticles and its use as a potential foliar fertilizer in lettuce (*Lactuca sativa*) and zucchini (*Cucurbita pepo*). Plant Physiol. Biochem., 151: 673-680.

Min, J.S.; Kim, S.W.; Kim, J.H.; Jung, K.S. and Lamsal, K. 2009. Effects of colloidal silver nanoparticles on Sclerotium-forming phytopathogenic fungi. Plant Pathol. J., 25(4): 376-380.

Mishra, S.; Singh, B.R.; Naqvi, A.H. and Singh, H.B. 2017. Potential of biosynthesized silver nanoparticles using *Stenotrophomonas* sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens. Sci. Rep., 7: 1-15.

Najafi, S. and Jamei, R. 2014. Effect of silver nanoparticles and Pb $(NO3)_2$ on the yield and chemical composition of mung bean (*Vigna radiata*). J. Stress Physiol. Biochem., 10(1): 316-325.

Nazir, M.M.; Li, Q.; Noman, M.; Ulhassan, Z.; Ali, S.; Ahmed, T.; Zeng, F. and Zhang, G. 2022. Calcium oxide nanoparticles have the role of alleviating arsenic toxicity of barley. Front Plant Sci., 13: 83-95.

Nel, A.; Xia, T.; Mädler, L. and Li, N. 2006. Toxic potential of materials at the nanolevel. Science, 311(5761): 622-627.

Nguyen, D.V.; Nguyen, H.M.; Le, N.T.; Nguyen, K.H.; Nguyen, H.T.; Le, H.M.; Nguyen, A.T.; Dinh, N.T.T.; Hoang, S.A. and Ha, C.V. 2022. Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions. J. Plant Growth Regul., 41: 364-375.

Ogunkunle, C.O.; Jimoh, M.A.; Asogwa, N.T.; Viswanathan, K.; Vishwakarma, V.; Fatoba, P.O. 2018. Effects of manufactured nanocopper on copper uptake, bioaccumulation and enzyme activities in cowpea grown on soil substrate. Ecotoxicol. Environ. Saf., 155: 86-93.

Pandey, S.; Giri, K.; Kumar, R.; Mishra, G. and Raja, R.R. 2018. Nanopesticides: opportunities in crop protection and associated environmental risks. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci., 88(4): 1287-1308.

Paparu, P.; Acur, A.; Kato, F.; Acam, C.; Nakibuule, J.; Musoke, S.; Nkalubo, S. and Mukankusi, C. 2018. Prevalence and incidence of four common bean root rots in Uganda. J. Exp. Agric., 54: 888-900.

Parikh, K. and Jha, A. 2012. Biocontrol features in an indigenous bacterial strain isolated from agricultural soil of Gujarat India. J. Soil Sci. Plant Nutr., 12: 249-256.

Passardi, F.; Penel, C. and Dunand, C. 2004. Performing the paradoxal: how plant peroxidases modify the cell wall. Trends Plant Sci., 9: 534-540.

Patel, N.; Desai, P.; Patel, N.; Jha, A. and Gautam, H.K. 2014. Agro nanotechnology for plant fungal disease management. Int. J. Curr. Micobiol. App. Sci., 3(10): 71-84.

Razzaq, A.; Ammara, R.; Jhanzab, H.M.; Mahmood, T.; Hafeez, A. and Hussain, S. 2016. A noval nanomaterial to enhance growth

and yield of wheat. J. Nanosci. Technol., 2(1): 55-58.

Rondon, M.A.; Lehmann, J.; Ramírez, J. and Hurtado, M. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biol. Fertil. Soils, 43: 699-708.

Roy, A.; Gauri, S.S.; Bhattacharya, M. and Bhattacharya, J. 2013. Antimicrobial activity of CaO nanoparticles. J. Biomed. Nanotechnol., 9(9): 1570-1578.

Rubina, M.S.; Vasil'kov, A.Y.; Naumkin, A.V.; Shtykova, E.V.; Abramchuk, S.S.; Alghuthaymi, M.A. and Abd-Elsalam, K.A. 2017**.** Synthesis and characterization of chitosan-copper nanocomposites and their fungicidal activity against two sclerotia-forming plant pathogenic fungi. J. Nanostruct. Chem., 7: 249-258.

Sadak, M.S. 2019. Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*). Bull. Natl. Res. Cent., 43(38): 1-6.

Salama, H.M.H. 2012. Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). Int. Res. J. Biotechnol., 3(10): 190-197.

Sankar, R.; Maheswari, R.; Karthik, S.; Shivashangari, K.S. and Ravikumar, V. 2014**.** Anticancer activity of *Ficus religiosa* engineered copper oxide nanoparticles. Mater. Sci. Eng. C Mater. Biol. Appl., 44: 234-239.

Sennoi, R.; Jogloy, S.; Saksirirat, W. and Patanothai, A. 2010. Pathogenicity test of *Sclerotium rolfsii*, a causal agent of Jerusalem artichoke (*Helianthus tuberosus* L.) stem rot. Asian J. Plant Sci., 9(5): 281-284.

Shrivastava, S.; Bera, T.; Roy, A.; Gajendra, S.; Ramachandrarao, P. and Dash, D. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnol., 18(22).

Sun, Q.; Li, J. and Le, T. 2018. Zinc oxide nanoparticle as a novel class of antifungal agents: current advances and future perspectives. J. Agric. Food Chem., 66(43): 11209-11220.

Syu, C.H.; Yu, C.H. and Lee, D.Y. 2020. Effect of applying calcium peroxide on the accumulation of arsenic in rice plants grown in arsenic-elevated paddy soils. Environ. Pollut., 266: 115-140.

Tyagi, M.; Arvind, M.K. and Sinha, B. 2000. The role of peroxidase and polyphenol oxidase isozymes in wheat resistance to *Alternaria triticina*. Biol. Plant, 43(4): 559-562.

Uebersax, M.A.; Cichy, K.A.; Gomez, F.E.; Porch, T.G.; Heithol, J.; Osorno, J.M.; Kamfwa, K.; Snapp, S.S. and Bales, S. 2022. Dry beans (*Phaseolus vulgaris* L.) as a vital component of sustainable agriculture and food security. Legume Sci., 155.

Wang, X.P.; Liu, X.Q.; Chen, J.N. Han, H.Y. and Yuan, Z.D. 2014. Evaluation and mechanism of antifungal effects of carbon nanomaterials in controlling plant fungal pathogen. Carbon, 68: 798-806.

Woodward, J.E.; Brenneman, T.B.; Kemerait, R.C.; Smith, N.B.; Culbreath, A.K. and Stevenson, K.L. 2008. Use of resistant cultivars and reduced fungicide programs to manage peanut diseases in irrigated and nonirrigated fields. Plant Dis., 92: 896-902.

Worrall, E.A.; Hamid, A.; Mody, K.T.; Mitter, N. and Pappu, H.R. 2018. Nanotechnology for plant disease management. Agronomy, 8(12): 285-308.

Yadav, R.K.; Singh, N.B.; Singh, A.; Yadav, B.; Bano, C.; Khare, S. and Niharika, 2020. Expanding the horizons of nanotechnology in agriculture: recent advances, challenges and future perspectives. Vegetos., 33: 203-221.

Yazıcılar, B.; Böke, F.; Alaylı, A.; Nadaroglu, H.; Gedikli, S. and Bezirganoglu, I. 2021. In vitro effects of CaO nanoparticles on *Triticale* callus exposed to short and long-term salt stress. Plant Cell Rep., 40(1): 29-42.