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Metallic oxide nanoparticles enhance chickpea resistance to root rot and wilt

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Summary. Antifungal properties of nanoparticles (NPs) of copper oxide (CuO), titanium dioxide (TiO_2), and silica dioxide (SiO_2) were compared to the fungicide thiophanate-methyl for controlling root rot and wilt of chickpea, caused by, respectively, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *ciceris*. Different concentrations (10, 20, or 40 ppm) of the NPs were assessed for their ability to inhibit fungal growth *in vitro*. All the nanoparticles had antifungal activity, with greatest effects at 40 ppm. CuO NPs at 40 ppm gave 61% reduction for Rhizoctonia rot and 65% reduction for Fusarium wilt. Alterations in the ultrastructure of the fungal mycelia were observed in response to treating with CuO NPs. No differences in *in vivo* tests were observed between CuO NPs and thiophanate-methyl for reducing root rot or wilt. Applications of CuO NPs also enhanced growth and yield of chickpea plants. CuO NPs had antifungal properties, increased activities of peroxidase and polyphenol oxidase in chickpea plants, and increased plant phenol contents. These results indicate that CuO NPs have potential as effective, eco-friendly alternatives to conventional fungicides for controlling of root rot and wilt of chickpea.

Keywords. *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *ciceris*.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a highly nutritious legume, which is grown in more than fifty countries and is as a major source of proteins and carbohydrates for human and animal consumption. Through biological nitrogen fixation, chickpea plants also improve soil fertility (Jukanti *et al*., 2012).

Chickpea plants are susceptible to wilt, caused by *Fusarium oxysporum* Schlecht emend. Snyd. & Hans. f. sp. *ciceris* (Padwick) Snyd. & Hans., and root rot caused by *Rhizoctonia solani* J.G. Kühn (Choudhary *et al*., 2013; Zian

et al., 2023). These diseases may adversely affect chickpea growth metrics and crop productivity.

Using fungicides for control of fungal diseases offers several benefits, including availability, effectiveness, and fast action. However, the compounds have disadvantages, including potential harmful impacts on human and animal health, and development of fungicide resistant pathogen races (Yadav *et al*., 2020). Environmental hazards resulting from fungicide use have also been demonstrated, and recent studies have been conducted to find alternative treatments (Al-Askar *et al*., 2014; Rashad *et al*., 2018; Rashad *et al*., 2020a; Rashad *et al*., 2022; Zian *et al*., 2024). Novel approaches have therefore been assessed to managed fungal diseases, and safeguard and fortify worldwide food security while mitigating financial setbacks, by seeking methods to limit the use of fungicide compounds.

Nanotechnology can enhance crop production by boosting input efficiency and reducing losses. The most important benefit of using active nanomaterials is the large specific surface area they provide to fertilizers and pesticides. Furthermore, nanomaterials are can be carriers of agrochemicals, providing precise and targeted delivery of nutrients, leading to enhanced plant protection. Gogos *et al*. (2012) indicated that nanotechnology can alleviate issues related to food production as well as climate change by benefiting the environment and aiding in disease management (Worrall *et al*., 2018). Nanoparticle materials have unique physicochemical features, including surface and quantum effects, that distinguish them from bulk materials. These effects enhance their ability to interact with fungi and perform therapeutic functions (Boxi *et al*., 2016). Nanoparticles have minute diameters (1 to 100 nm). They adhere to fungal surfaces and penetrate and deposit into fungal cells, and have been shown to exhibit antifungal properties. These features include capacity to mechanically and physically harm cell walls and membranes, and to alter cellular signals by dephosphorylating potential integral peptide substrates that are essential for cell survival and division. Permeability of membranes is also increased, water channels are obstructed, and microbial enzymes are deactivated, reactive oxygen species effectively produced, respiration is halted, and other metabolic pathways are modified. All of these actions contribute to inhibition of fungal growth (Allahverdiyev *et al*., 2011; Wang *et al*., 2014). Nanoparticles can stimulate cell division, host callus development, enhance root structure, increase shoot length, leaf numbers, and overall biomass in many plant species under stress and also in normal conditions (Gohari *et al*., 2020).

Silicon (Si) NPs can prevent pathogen infections by enhancing host plant disease resistance. Because plants transpire less, those with nanosilicon coatings have protection from high temperatures (Rastogi *et al*., 2019; Rashad *et al*., 2021). Plant growth and yield can be also be enhanced by Si NPs (Siddiqui and Al-Whaibi 2014; Suriyaprabha *et al*., 2014). White rot was shown to be less common in garlic and onion plants when exogenous Si and silicate salts enhanced the action of host systemic defense enzymes (Elshahawy *et al*., 2021). Silica treatment triggered potato resistance against late blight by improving the ethylene and jasmonic acid metabolism in separated foliage and in whole plants (Xue *et al*., 2021).

Due to their photocatalytic and antibacterial characteristics, nanoparticles of titanium dioxide (TiO_2 NPs) and of other metal oxides have promise as agricultural additives. TiO_2 NPs increased photosynthetic activity of cucumber and reduced infections caused by *Pseudomonas syringae* pv. *lachrymans* and *Pseudoperonospora cubensis* in field research conducted by Cui *et al*. (2009). Servin *et al*. (2015) demonstrated how systemic acquired resistance or direct suppression of disease-causing organisms by nanoparticles of TiO_2, CuO, and ZnO could enhance in disease control programs.

Studies have demonstrated antifungal action of copper oxide nanoparticles (CuO NPs) on *F. oxysporum* that causes tomato wilt (Kanhed *et al*., 2014), and *R. solani* (El-Shewy *et al*., 2019; Ismail, 2021). Abou-Salem *et al*. (2022) also tested CuO NPs against *F. oxysporum, Macrophomina phaseolina,* and *Pectobacterium carotovorum*, which cause root rot in sugar beet. That study showed that CuO NPs at 150 μg mL^{-1} was an effective treatment, reducing disease incidence while enhancing vegetative growth, improving physiological traits, and boosting antioxidant enzyme activity. Elmer and White (2016) found when eggplant and tomato plants were cultivated in soil inoculated with *F. oxysporum* f. sp. *lycopersici* and *Verticillium dahliae*, CuO was more effective than other tested nanomaterials, including MnO, ZnO, NiO, TiO, FeO, and AlO.

Systemic resistance induced by abiotic agents has been extensively studied, since it enhances plant defense against a wide range of phytopathogens. Triggered systemic resistance involves several processes, including generation of salicylic acid and antibiotics, and expression of the antioxidant enzymes peroxidase (POD), polyphenol oxidase (PPO), and other phenolic compounds. Copper has many functions in plant physiological and biochemical processes as an enzyme activator, and is integral to numerous enzymes. Copper is also essential for plant growth and nutrition (Bowler *et al*., 1992; Kasana and AliNiazee, 1997). Previous studies Nair and

Chung (2014) showed that treatments with copper oxide nanoparticles (CuO NPs) enhanced activities of ascorbate peroxidase (APX) and peroxidase (POD). Sarkar *et al.* (2020) demonstrated that lentil plants treated with CuO NPs had high activity of APX and superoxide dismutase (SOD). Potato plants treated with CuO NPs had enhanced PPO and POD activities (Ismail, 2021).

The present study aimed to investigate the efficacy of nanoparticles of CuO, TiO_2, and SiO_2 nanoparticles in comparison with the fungicide thiophanate-methyl, for suppressing *R. solani* and *F. oxysporum* f. sp. *ciceris*, enhancing chickpea defense responses, affecting plant growth parameters. Effects of CuO NPs on chickpea ultrastructure were also assessed.

MATERIALS AND METHODS

Chickpea seeds

Chickpea seeds (cv. Giza-2) were obtained from the Legume Research Department, Field Crops Research Institute, ARC, Giza, Egypt.

Nanoparticles and fungicide

The three tested nanomaterials were purchased from Nanotechnology & Advanced Nano-Materials Laboratory (NANML), Plant Pathology Research Institute (PPRI), Giza, Egypt. These were: CuO NP (25 ± 5 nm particle size (Supplementary data), and previously characterized by Ismail (2021); Ti_2O NP (45 ± 5 nm particle size (Supplementary data); and SiO_2 NP (50 ± 23 nm particle size) (Supplementary data). Sterilized distilled water was used to produce all stock solutions. A Transonic 420 sonicator (Elma) was used to sonicate nanoparticle suspensions for 30 min before they were applied as seed soakings or included in sterilized plant growth medium. The chemical fungicide (thiophanate-methyl (Topsin-M70® 70% WP, Cairo Chemical Company, Egypt) was used for comparisons with the three nanomaterials.

Chickpea root rot and wilt pathogens

Two virulent isolates (Zian *et al.*, 2023), one of *R. solani* (accession No. OR074128) and the other of *F. oxysporum* f. sp. *ciceris* (accession No. OR074126), were used in this study. These isolates were previously obtained from chickpea fields in the Ismailia governorate, Egypt, where they had caused symptoms of wilt and root rot.

Screening the tested nanoparticles for antifungal activity

CuO NP, TiO_2 NP, and SiO_2 NP were each assessed at 10, 20, and 40 ppm against *R. solani* and *F. oxysporum* f. sp. *ciceris* and compared to thiophanate-methyl. Initial stock solutions of nanoparticles were prepared at concentrations of 1000 ppm, and were diluted with sterile deionized water to obtain these concentrations. Before being utilized in experiments, the solutions were maintained at 4°C. The agar dilution procedure reported by Fraternale *et al.* (2003) was used for *in vitro* assays. The prepared concentrations of nanoparticles and thiophanate-methyl at 200 ppm were added to sterilized potato dextrose agar (PDA) before solidification, and the amended agar was poured into Petri dishes (9 cm diam.). Agar discs (5 mm diam.) were taken from the margins of 7-d-old cultures of the test fungi and placed individually in the center of each plate. PDA plates without nanoparticle or fungicide amended media and inoculated with the assessed fungi served as experimental controls. The plates were then incubated at 25 ± 2°C for 7 d, and radial growth of the fungi was measured after 4 and 7 d of incubation. Each treatment was applied in four replicates, and the assay was repeated twice. Inhibition of mycelium growth of the two tested fungi was calculated using the procedure of Kaur *et al.* (2012).

The equation used for calculating inhibition percentage of the fungi was:

$$\text{Inhibition \%} = \frac{C - T}{C} \times 100$$

where C = radial growth in control plates, and T = radial growth in the treatment.

Transmission electron microscopy studies

Effects of CuO NP on the ultrastructure of the *R. solani* and *F. oxysporum* f. sp. *ciceris* were assessed by taking samples from cultures used in the nanoparticle antifungal assessments (above), after 3 d incubation. Morphological modifications in the hyphal cell structures of the two fungi were assessed using transmission electron microscopy (TEM). Specimens (1 mm^2 each) were taken from each treated colony. The samples were added to phosphate buffer and then washed in a 3% glutaraldehyde solution, and then fixed in potassium permanganate solution for 5 min. The specimens were then dehydrated for 30 min using absolute ethanol, with immersion in nine ethanol concentrations (10 to 90%), for 15 min in each concentration. The samples were then treated in a graded epoxy resin and acetone series, fol-

lowed by immersion in pure wax, then loaded onto copper grids, followed by staining in uranyl acetate and lead citrate. A JEOL JEM 1010 transmission electron microscope running at 70 kV was used to observe the stained sections (Amin, 2016; Amin *et al*., 2020; Amin *et al*., 2022).

Cytotoxicity bioassay

Cytotoxicity bioassays were carried out using the cell line WI-38, ATCC® CCL-75 (normal human fetal lung fibroblasts). For preparation of the cell monolayer, the cells were grown on fetal bovine serum (FBS) medium and adjusted at 5×10^5 cell mL^{-1}. In a 96 well plate, 200 µL of cell culture was added to each well, except the peripheral wells which were used as blanks. The plate was then incubated at 37°C for 24 h. To investigate CuO NP cytotoxicity, the nanoparticles were serially diluted in culture medium. The test was carried out in three replicates, with three plates (media only) as experimental controls. After 24 h, the plate was assessed using MTT assay on a microplate ELISA reader (M965 Mate 2.0). Inhibition percentages were calculated for each nanoparticle each concentration (Rashad *et al.,* 2021). The half-maximum inhibitory concentrations (IC_{50}s) were determined using the Graph Pad Prism program.

Hemolytic activity assay

This assay was carried out using 2 mL capacity tubes, and the procedure of Farias *et al*. (2013), with minor adjustments. CuO NP was serially diluted in 0.9% NaCl solution. 100 µL of 1% rabbit red blood cell solution was combined with 900 µL of the each nanoparticle dilution in each microtube, and the microtubes were incubated at 37°C for 1 h and then centrifuged for 5 min at 3000 rpm. Supernatant was then poured into a 96-well plate (200 µL per well), and the absorbance of the supernatant was determined (at 540 nm) using a spectrophotometer. Experimental controls each used 900 µL of 0.9% NaCl combined with 100 µL of the red blood cell suspension. The CuO NP concentration required to achieve a 50% inhibition of red blood cell hemolysis was used to obtain the IC_{50} value for the tested nanoparticles.

Pot experiments

For inoculum preparation, Inoculum of the two fungal pathogens (*F. oxysporum* and *R. solani*) was prepared after the fungi were cultured on sorghum/sand (2:1 v/v) medium. This sterilized at 121°C and 1.5 air pressure for 20 min. Agar discs (5 mm diam.) from 4-d-old *R. solani* culture (mycelium) or 7-d-old *F. oxysporum* culture (mycelium and spores) were used to inoculate the sterilized media. The bottles containing the inoculated media for soil inoculation in pot tests were then incubated at 25 ± 2°C for 15 d.

Pot experiments were carried out under natural conditions to compare effects of CuO, TiO_2, or SiO_2 NPs with thiophanate-methyl for effects on root rot and wilt of chickpea. The two pathogens were separately cultured in glass bottles on sorghum/sand (3:1 w/w) medium for 15 d at 25 ± 2°C. Each plastic pot (approx. 30 cm diam.) was filled with 6 kg of soil (clay plus sand, 1:1 v/v) which had been disinfected with formalin. Fungal inoculum (mycelium and sclerotia of *R. solani*, mycelium and conidia of *F. oxysporum*) was added at 2% of the soil weight, as by Papavizas and Devey (1962). Inoculated soil was watered and then allowed to rest for 1 week to allow proliferation of the fungi. The Nanoparticles of CuO, TiO_2 or SiO_2 were prepared at 40 ppm, along with 0.2% Tween 80, and were each used as chickpea seed soaks for 2 h before planting. The fungicide thiophanate-methyl was applied at 3 g kg^{-1} seeds. The treated seeds were then planted in the pots (five seeds per pot), and the pots were regularly watered and fertilized. Each experimental treatment was applied to four replicate pots, and additional pots, seeded in sterile soil, were used as non-inoculated controls for comparisons with the treated pots containing the pathogens. The pots were arranged in completely randomize experimental design, and the experiment was repeated twice.

Assessments of damping-off and root rot of the chickpea plants were carried out at 30 and 90 d after planting. Numbers of plants affected were assessed as percentages, respectively, of early and late wilt, in a similar manner, at 30 an 90 days using the following equations:

$$\text{Damping-off \%} = \frac{\text{No. of un-germinated seeds after 15 days + no.of dead seedlings after 30 days}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Root rotted plants \%} = \frac{\text{No. of rotted seedlings after 90 days}}{\text{Total No. of sown seeds}} \times 100$$

$$\text{Early wilt \%} = \frac{\text{No. of wilted plants after 30 days}}{\text{Total No. of sown seed}} \times 100$$

$$\text{Late wilt \%} = \frac{\text{No. of wilted plants after 90 days}}{\text{Total No. of sown seed}} \times 100$$

$$\text{Survived plants \%} = \frac{\text{No. of survival plants after 90 days}}{\text{Total No. of sown seeds}} \times 100$$

The percentage that was reduced or increased above the infested control was also determined using the subsequent calculation:

$$\text{Reduction or Increasing \%} = \frac{\text{DI of Control} - \text{DI of treatment}}{\text{DI of Control}} \times 100$$

Effects of nanoparticles on the antioxidant enzymes and total phenol contents

Fresh leaves were picked from chickpea plants 15 d after seed sowing, and extracts were made from the leaves. The peroxidase (POD) and total phenol contents (PPO) in these extracts were assessed (four replicates each). POD activity in the chickpea leaf extracts was assayed using the procedure of Chakraborty and Chatterjee (2007). PPO was extracted and assayed based on the protocol of Sadasivam and Manickam (1996), and total phenol content in the plants was determined as described by Zilesin and Ben-Zaken (1993).

Field experiments

Field experiments were carried out at the Sers El-Layian and Etai El-Baroud farms of the Agricultural Research Station during 2021/2022, assess the efficacy of CuO, TiO_2 and SiO_2 nanoparticles for managing chickpea root rot and wilt. Three replicates of each treatment were used a randomized block design field trial. Each replicate comprised four rows (each 3 m long and 50 cm wide, covering 6 m^2 (2 × 3 m). Before planting, chickpea seeds were soaked for 2 h in the same nanoparticle treatments used in the pot experiment (described above). In each plot, one seed per hill was planted on either side of the row ridge, 25 cm between each seed. The experimental control treatment comprised soaking seeds in distilled water for 2 h.. At both sites, all normal agricultural practices were followed during the trials. Proportions of damping-off, rotten seedlings, wilted plants, and surviving plants were recorded. At harvest, measurements of plant height, number of branches, number of capsules, seed weight, weights of 100 seeds and total seed yield were determined.

Statistical analyses

The statistical program COSTAT version 6.4 was used for statistical analyses of data. Mean values from different treatments were compared using Duncan's multiple-range test, and significance standards for comparisons of means were indicated as $P \leq 0.01$ and $P \leq 0.05$.

RESULTS

Effects of nanoparticles on the fungal growth

As indicated in Table 1, all the assessed nanoparticles (CuO, TiO_2 and SiO_2) at all tested concentrations decreased mycelium growth of the two fungi. CuO NPs

Table 1. Mean colony diameters of *Rhizoctonia solani* and *Fusarium oxysporum* f. sp *ciceris* in agar amended with different concentrations of three nanoparticles, or the fungicide thiophanate-methyl.

Treatment	Concentration (ppm)	*R. solani*		*F. oxysporum* f. sp. *ciceris*	
		Mean fungal growth (mm)	% reduction	Mean fungal growth (mm)	% reduction
Control	-	90.0 ± 0 [a]	0.0	90.0 ± 0 [a]	0.0
CuO-NPs	10	71.0 ± 1.0 [d]	21.2	70.2 ± 0.2 [d]	22.0
	20	56.1 ± 1.2 [f]	37.6	54.4 ± 0.7 [f]	39.6
	40	35.4 ± 0.5 [h]	60.7	31.1 ± 0.9 [i]	65.3
TiO_2-NPs	10	81.8 ± 1.2 [b]	09.0	75.8 ± 0.8 [b]	15.7
	20	61.4 ± 0.6 [e]	31.7	59.1 ± 0.9 [e]	34.4
	40	40.8 ± 0.7 [g]	54.6	38.3 ± 0.7 [g]	57.3
SiO_2-NPs	10	74.8 ± 0.7 [c]	16.8	72.7 ± 0.6 [c]	19.1
	20	57.8 ± 0.7 [f]	35.7	55.1 ± 0.8 [f]	38.8
	40	39.5 ± 1.3 [g]	56.2	36.4 ± 0.5 [h]	59.4
Thiophanate-methyl	200	00.0 ± 0 [i]	100.0	00.0 ± 0 [j]	100.0

Means in each column accompanied by different letters are different ($P \leq 0.01$), as indicated by Duncan's multiple-range test. Standard deviations of the means are also indicated.

at 40 ppm gave the greatest antifungal effects, by 61.7% for *R. solani* and 65.3% for *F. oxysporum* f. sp. *ciceris*. SiO_2 and TiO_2 NPs at 40 ppm also reduced growth of these fungi. TiO_2-NP at 10 ppm gave the least suppression of mycelium growth for both fungi. Inhibition of fungal growth decreased with reductions in nanoparticle concentrations, and *F. oxysporum* was less sensitive to nanoparticles than *R. solani*. The fungicide thiophanate-methyl completely suppressed growth of both fungi.

TEM observations

Figure 1 contains transmission electron micrographs of transverse sections of *R. solani* hyphae. A normal and organized cell (Figure 1a) is enclosed by a thin electron-dense cell wall and a thin electron-lucent plasma membrane. The cytoplasm contains a normal nucleus, vacuoles, and electron-lucent glycogen granules. In contrast, *R. solani* hyphae treated with CuO NPs (Figure 1b) had abnormal disorganized cells with cytoplasmic granulation, numerous mitochondria and electron-dense bodies that were enclosed by electron-dense cell walls.

Figure 2 a shows a normal untreated cell of *F. oxysporum* f. sp. *ciceris*. The cell has a thin cell wall, a thin plasma membrane, a normal nucleus, and mitochondria in the cytoplasm. Numerous electron-lucent glycogen bodies were also present. Mycelium of *F. oxysporum,* treated with CuO NPs had many ultrastructural alterations (Figure 2b). The treated cell was irregular with thickening of the cell wall and plasma membrane. Cytoplasmic granulation, an irregular large vacuole and many electron-dense particles were also present.

Cytotoxicity of CuO-NPs

The CuO NPs had toxic effects on human lung fibroblast cells (WI-38), particularly at high concentrations. Cytotoxicity of CuO NPs increased with increasing concentration, reaching complete mortality at 255 ppm (Figure 3a). At 40 ppm, the CuO NPs gave 80% cytotoxicity, while its IC_{50} was 25.2 ppm. In contrast, the hemolytic activity assay showed that CuO NPs did not have substantial adverse effects on red blood cells (no toxicity at 100 ppm; Figure 3b). At 40 ppm, the CuO NPs gave 8% hemolytic activity of red blood cells, and the IC_{50} for the CuO NPs was 440.1 ppm.

Pot experiments

All the treatments (nanoparticles or thiophanate-methyl) reduced damping-off, root rot and wilt diseases (Table 2). Thiophanate-methyl exhibited the greatest efficacy. Least occurrences of root rot and wilt were recorded after treatments with CuO NPs. Plants treated with

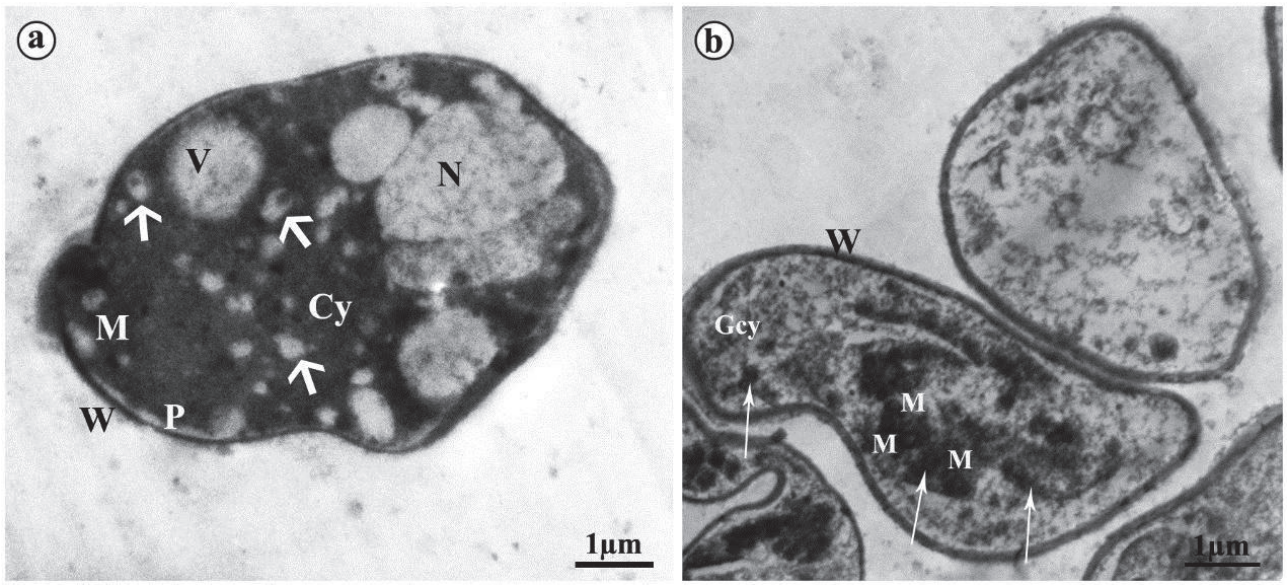

Figure 1. Transmission electron micrographs transverse section of hyphae of *Rhizoctonia solani*. a) An untreated hypha has a thin electron-dense cell wall (W) and a thin electron-lucent plasma membrane (P). The cytoplasm (Cy) has a normal nucleus (N), vacuoles (V), and electron-lucent glycogen granules (arrowheads). b) A hypha of *R. solani* which was treated with CuO NPs has granulated cytoplasm (Gcy), many mitochondria (M), and electron-dense bodies (arrows) that are enclosed by an electron-dense cell wall (W).

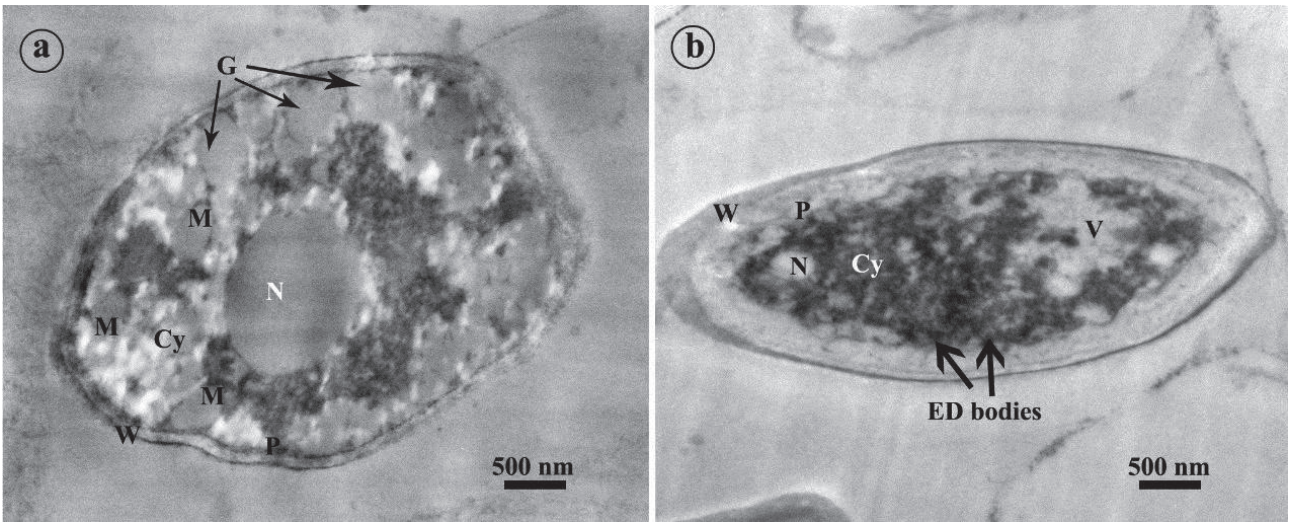

Figure 2. Transmission electron micrographs of transverse sections of hyphae of *Fusarium oxysporum* f. sp. *ciceris*. a) An untreated hypha with a thin cell wall (W), a thin plasma membrane (P), a normal nucleus (N), and mitochondria (M) in the cytoplasm (Cy). Numerous electron-lucent glycogen bodies (G) were also observed. b) A hypha of *F. oxysporum* which was treated with CuO-NPs had a thickened cell wall (W) and plasma membrane (P). Cytoplasmic granulation (Cy), an irregular large vacuole (V) and many electron-dense particles were also observed.

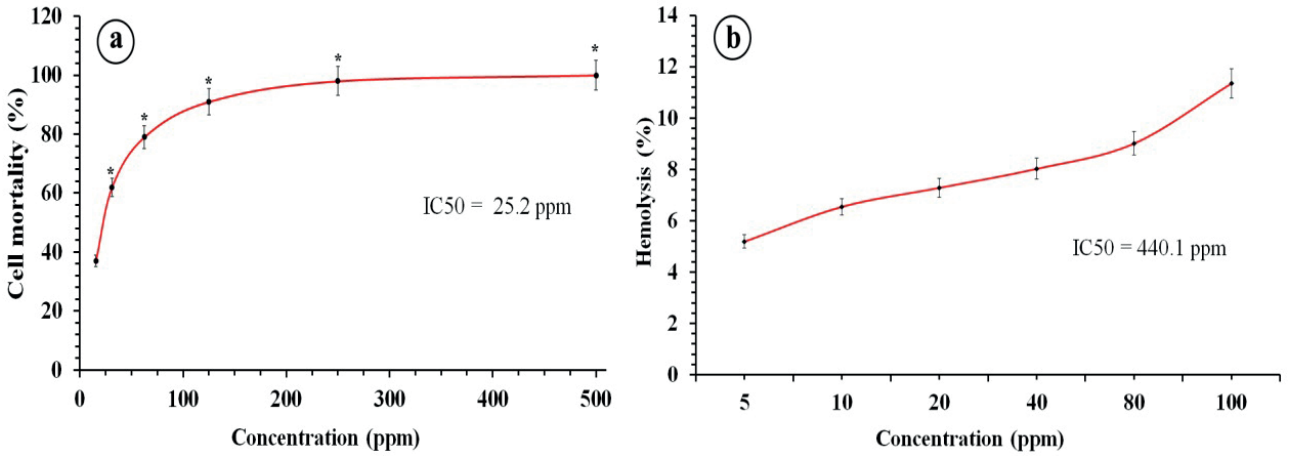

Figure 3. Cytotoxicity bioassay of CuO-NPs. Bioassay carried out at exposure time of 24 h on (a) human fetal lung fibroblasts (Vero), plus (b) lung epithelial cells (BEAS-2B). Each value is the mean of three replicates (± standard error). IC_{50} = the half-maximal inhibitory dose. * = significant at $P \leq 0.05$, compared with experimental controls.

SiO_2 or TiO_2 NPs also had reductions in these diseases, but to lesser extents than from CuO NPs. When the plants were inoculated with either *R. solani* or *F. oxysporum* f. sp. *ciceris,* greatest plant survival occurred after treatments with CuO NPs.

Effects of nanoparticles on the antioxidant enzymes and total phenol contents

Applications of the different nanoparticles increased oxidative enzyme activity and total phenol contents in chickpea plants (Table 3). For inoculations with *R. solani*, CuO NPs gave the greatest POD activity, followed by TiO_2 NPs and thiophanate-methyl. PPO activity also increased in response to CuO NP treatment, thiophanate-methyl, or SiO_2 NP treatments. In the plants inoculated with *F. oxysporum* f. sp. *ciceris*, the CuO NP treatments resulted in the greatest activity levels of both POD and PPO.

The chickpea plants treated with nanoparticles had high total phenolic contents. The greatest total phenolic content was recorded for plants treated with CuO NPs. Total phenol contents increased after treatments with

Table 2. Mean disease parameters in pot trials, where chickpea plants were exposed to *Rhizoctonia solani* (A) or *Fusarium oxysporum* (B) after treatments with different nanoparticles, or the fungicide thiophanate-methyl.

(A) *Rhizoctonia solani*:

Treatment	Damping-off %		Rotted seedlings %		Surviving plants %	% increase
	Incidence %	% reduction	Incidence %	% reduction		
CuO NPs	5.0 ± 10 ^{cd}	80.0	10.0 ±11.5 ^{b}	50.0	85.0 ± 10 ^{bc}	54.5
TiO_2NPs	15.0 ± 10 ^{b}	40.0	10.0 ± 11.5 ^{b}	50.0	75.0 ± 10 ^{d}	36.3
SiO_2 NPs	10.0 ± 11.5 ^{bc}	60.0	10.0 ± 11.5 ^{b}	50.0	80.0 ± 0 ^{cd}	45.4
Thiophanate-methyl	5.0 ± 10 ^{cd}	80.0	5.0 ± 10 ^{bc}	75.0	90.0 ± 11.5 ^{b}	63.6
Inoculated control	25.0 ± 10 ^{a}	-	20.0 ± 0 ^{a}	-	55.0 ± 10 ^{e}	-
Non-inoculated control	0.0 ± 0 ^{d}	-	0.0 ± 0 ^{c}	-	100.0 ± 0 ^{a}	-

(B) *Fusarium oxysporum* f. sp. *ciceris*:

Treatment	Wilted plants %				Surviving plants %	% increase
	Early wilt		Late wilt			
	Incidence %	% reduction	Incidence %	% reduction		
CuO-NPs	5.0 ± 10 ^{bc}	66.7	5.0 ± 10 ^{b}	80.0	90.0 ± 11.5 ^{bc}	50.0
TiO_2-NPs	5.0 ± 10 ^{bc}	66.7	10.0 ± 11.5 ^{b}	60.0	85.0 ± 10 ^{cd}	41.7
SiO_2 -NPs	10.0 ± 11.5 ^{ab}	33.4	10.0 ± 11.5 ^{b}	60.0	80.0 ± 0 ^{d}	33.4
Thiophanate-methyl	0.0 ± 0 ^{c}	100.0	5.0 ± 10 ^{b}	80.0	95.0 ± 10 ^{ab}	58.4
Inoculated control	15.0 ± 10 ^{a}	-	25.0 ± 10 ^{a}	-	60.0 ± 0 ^{e}	-
Non-inoculated control	0.0 ± 0 ^{c}	-	0.0 ± 0 ^{b}	-	100.0 ± 0 ^{a}	-

Means in each column accompanied by different letters are different ($P \leq 0.05$), as indicated by Duncan's multiple-range test. Standard deviations of the means are also indicated.

CuO NPs, TiO_2 NPs, and thiophanate methyl, although this effect was least for the fungicide. However, in comparison to the control, the use of SiO_2 NP treatments had the least effects on overall phenol contents.

Field experiments

All the experimental treatments decreased root rot and wilt of field-grown chickpea plants. Thiophanate-methyl gave the greatest disease reductions at Etai El-Baroud (Table 4), followed by treatments with CuO or TiO_2 NPs, which also reduced disease incidence. The SiO_2 NP treatment gave the least reduction in incidence of the two diseases. At Sers El-Layian, thiophanate-methyl was again the most effective treatment, giving the greatest reduction in disease incidence. CuO and SiO_2 NP treatments also had disease reduction efficacy, but to a lesser extent than thiophanate-methyl. However, The TiO_2 NP treatment was the least effective of the tested treatments

Effects of nanoparticles on the chickpea growth parameters in field conditions

After the nanoparticle treatments, chickpea growth and yields were increased at both field trial sites (Table 5). Applications of thiophanate-methyl or CuO NP treatments increased plant heights. All the treatments increased chickpea branch numbers compared to the experimental control. Thiophanate-methyl was most effective treatment at Etai El-Baroud experiment, followed by CuO NP and TiO_2 NP treatments. At the Sers El-Layian Agricultural Research Station, thiophanate-methyl was again the most effective treatment, followed by treatments with CuO NPs or SiO_2 NPs.

In contrast to the control treatment, all yield parameters were increased by the different experimental treatments, including 100 seed weights, numbers of capsules per plant, and plant seed weights. The treatments with thiophanate-methyl or CuO NPs at both trial sites produced the greatest yield parameters. Thiophanate-methyl gave

Table 3. Mean peroxidase and polyphenoloxidase activities, and total phenols, in chickpea plants receiving different nanoparticle and fungicide treatments, and inoculations with either *Rhizoctonia solani* (A) or *Fusarium oxysporum* f. sp. *ciceris* (B).

Treatment	Peroxidase (unit/mg protein/min)		Polyphenoloxidase (unit /mg protein/min)		Total phenols (mg/g fresh leave weight)	
	Activity	% increase	Activity	% increase	Activity	% increase
(A) *Rhizoctonia solani*						
CuO-NPs	1.46 ± 0.03 [a]	217.3	1.48 ± 0.02 [a]	139.4	4.24 ± 0.03 [a]	100.7
TiO_2-NPs	1.12 ± 0.04 [b]	142.6	1.13 ± 0.03 [c]	82.4	3.54 ± 0.02 [b]	67.7
SiO_2-NPs	0.83 ± 0.1 [c]	79.8	1.28 ± 0.02 [b]	107.4	2.64 ± 0.01 [c]	24.9
Thiophanate-methyl	1.33 ± 0.02 [a]	188.5	1.42 ± 0.02 [a]	128.9	2.68 ± 0.02 [c]	27.1
Inoculated control	0.46 ± 0.01 [d]	-	0.62 ± 0.01 [d]	-	2.11 ± 0.01 [d]	-
Non-inoculated control	0.36 ± 0.01 [d]	-	0.51 ± 0.01 [e]	-	1.93 ± 0.02 [e]	-
(B) *Fusarium oxysporum* f. sp. *ciceris*						
CuO-NPs	1.49 ± 0.03 [a]	208.8	1.57 ± 0.02 [a]	114.8	4.25 ± 0.02 [a]	97.5
TiO_2-NPs	1.21 ± 0.01 [c]	150.1	1.16 ± 0.01 [c]	58.9	3.78 ± 0.02 [b]	75.8
SiO_2-NPs	0.93 ± 0.02 [d]	92.3	1.32 ± 0.02 [b]	79.9	2.88 ± 0.02 [d]	34.0
Thiophanate-methyl	1.37 ± 0.01 [b]	182.6	1.52 ± 0.02 [a]	107.5	2.96 ± 0.01 [c]	37.6
Inoculated control	0.48 ± 0.01 [e]	-	0.73 ± 0.02 [d]	-	2.15 ± 0.02 [e]	-
Non-inoculated control	0.36 ± 0.01 [f]	-	0.51 ± 0.01 [e]	-	1.93 ± 0.02 [f]	-

Means in each column accompanied by different letters are different ($P \leq 0.01$), as indicated by Duncan's multiple-range test. Standard deviations of the means are also indicated.

Table 4. Mean incidences of damped-off, rotted chickpea seedlings, or and wilted plants in two field trials (A and B) carried out during the 2021/2022 growing seasons.

Treatment	Damping- off		Rotted seedlings		Wilted plant		Surviving plants %	% increase
	Incidence %	% reduction	Incidence %	% reduction	Incidence %	% reduction		
(A) Etai El-baroud Agricultural Research Station								
CuO-NPs	3.7 ± 1.5 [b]	66.3	4.0 ± 1.0 [b]	58.7	4.0 ± 0 [b]	69.2	88.3 ± 2.5 [ab]	33.2
TiO_2-NPs	4.0 ± 2.0 [b]	63.6	3.7 ± 1.5 [b]	61.8	6.0 ± 1.0 [b]	53.8	86.3 ± 1.5 [bc]	30.1
SiO_2-NPs	5.0 ± 1.0 [b]	54.5	5.4 ± 1.1 [b]	44.3	6.0 ± 1.0 [b]	53.8	83.6 ± 1.5 [c]	26.0
Thiophanate-methyl	2.7 ± 0.5 [b]	75.4	3.0 ± 0 [b]	69.0	4.0 ± 1.0 [b]	69.2	90.3 ± 0.5 [a]	36.2
Control	11.0 ± 1.0 [a]	-	9.7 ± 1.5 [a]	-	13.0 ± 2.6 [a]	-	66.3 ± 2.5 [d]	-
(B) Sers El-layian Agricultural Research Station								
CuO-NPs	2.7 ± 0.5 [c]	66.2	3.0 ± 0 [cd]	55.2	4.3 ± 1.1 [bc]	70.5	90.0 ± 1.7 [a]	27.3
TiO_2-NPs	5.0 ± 1.0 [b]	37.5	4.7 ± 0.5 [b]	29.8	6.7 ± 0.5 [b]	54.1	83.6 ± 0.5 [b]	18.2
SiO_2-NPs	4.7 ± 1.1 [b]	41.2	4.0 ± 1.0 [bc]	40.3	6.0 ± 1.7 [b]	58.9	85.3 ± 3.7 [b]	20.6
Thiophanate-methyl	2.4 ± 1.1 [c]	70.0	2.0 ± 1.0 [d]	70.1	2.6 ± 1.1 [c]	82.2	93.0 ±1.0 [a]	31.5
Control	8.0 ± 1.0 [a]	-	6.7 ± 0.5 [a]	-	14.6 ± 1.1 [a]	-	70.7 ± 0.5 [c]	-

Means in each column accompanied by different letters are different ($P \leq 0.05$), as indicated by Duncan's multiple-range test. Standard deviations of the means are also indicated.

the greatest yield increases at Etai El-Baroud, followed by treatments with CuO NPs and TiO_2 NPs. The greatest seed production obtained at Sers El-Layian was from treatments with thiophanate-methyl, CuO NPs or SiO_2 NPs.

DISCUSSION

Plant disease control can be difficult in crop production, and increases in fungicide resistance in phytopath-

Table 5. Mean chickpea plant parameters at two field sites after applications of different treatments in two field trials (A and B) in the 2021/2022 growing season.

Treatment	Plant height (cm)	Number of branches/plant	Number of capsules/plant	Weight (g) of seeds/plant	Weight (g) of 100 seeds	Seed yield (kg/ feddan)
(A) Etai El-baroud Agricultural Research Station						
CuO NPs	81.4 ± 5.5 [a]	4.8 ± 1.3 [a]	26.6 ± 1.0 [a]	11.7 ± 0.8 [b]	29.0 ± 1.1[b]	1110.3 ± 1.5 [b]
TiO_2 NPs	81.0 ± 2.5 [a]	4.3 ± 0.3 [ab]	25.4 ± 1.2 [ab]	11.3 ± 0.8 [bc]	27.9 ± 0.5 [b]	1098.0 ± 23.4 [b]
SiO_2 NPs	77.4 ± 3.0 [a]	3.6 ± 1.0 [b]	23.8 ± 1.3 [b]	10.5 ± 0.1 [c]	27.4 ± 0.5 [b]	1065.0 ± 7.2 [c]
Thiophanate-methyl	82.3 ± 5.9 [a]	4.9 ± 0.6 [a]	27.1 ± 1.6 [a]	12.7 ± 0.2 [a]	32.0 ± 2.0 [a]	1204.3 ± 13.6 [a]
Control	67.0 ± 1.7 [b]	1.8 ± 0.2 [c]	19.4 ± 0.8 [c]	7.0 ± 0.2 [d]	18.5 ± 0.7 [c]	720.0 ± 12.4 [d]
(B) Sers El-layian Agricultural Research Station						
CuO-NPs	104.3 ± 4.1[a]	5.4 ± 0.5 [a]	27.1 ± 1.4 [ab]	13.4 ± 0.1 [b]	31.4 ± 3.2 [ab]	1167.4 ± 26.6 [b]
TiO_2 NPs	89.6 ± 4.0 [bc]	4.0 ± 1.7 [ab]	25.5 ± 1.0 [c]	11.7 ± 0.2 [c]	27.7 ± 0.6 [b]	1092.0 ± 10.1 [c]
SiO_2 NPs	94.0 ± 3.4 [b]	4.5 ±1. [a]	26.4 ± 1.1 [bc]	12.3 ± 0.1 [c]	28.3 ± 0.7 [b]	1110.7 ± 1.5 [c]
Thiophanate-methyl	106.3 ± 3.0 [a]	5.5 ± 1.3 [a]	28.3 ± 0.8 [a]	14.5 ± 0.9 [a]	34.7 ± 4.5 [a]	1213.4 ± 18.9 [a]
Control	81.6 ± 5.7 [c]	2.3 ± 0.05 [b]	20.1 ± 0.5 [d]	8.6 ± 0.4 [d]	20.4 ± 0.5 [c]	780.4 ± 5.5 [d]

Means in each column accompanied by different letters are different ($P \leq 0.05$), as indicated by Duncan's multiple-range test. Standard deviations of the means are also indicated

ogenic fungi have become important. Fungicides are frequently used to manage fungal infections and protect crops, and frequent and inappropriate use of these compounds has induced resistance in many economically important fungi (Goffeau, 2008).

Significant effort has been applied to create non-hazardous disease management practices. Nanoparticles have been proposed as possible pesticide substitutes for managing diseases caused by pathogenic bacteria. Nanoparticles can also have antifungal capabilities, are environmentally benign, and are also cost-effective (Gupta and Gupta, 2005; Nel, *et al*., 2006).

The present study has demonstrated that nanoparticles of CuO, TiO_2, and SiO_2, at different concentrations, reduced the fungal growth of *F. oxysporum* f. sp. *ciceris* and *R. solani*, which are important pathogens of chickpea. CuO NPs at 40 ppm had the greatest inhibitory effects on these fungi, with growth reduction of 35% for *R. solani* and 31% for *F. oxysporum* f. sp. *ciceris*. These results are similar to those from previous studies. Hermida-Montero *et al*. (2019) showed that CuO NPs contributed to development of reactive oxygen species (ROS) and caused membrane damage in *F. oxysporum*. They also reported decreases in fungal radial growth and alterations in hyphal morphology of this fungus. El-Shewy *et al*. (2019), using CuO NPs at 200 µL L^{-1}, decreased growth of *R. solani* by 55%. Oussou-Azo *et al*. (2020) noted that Cu NPs at 200 mg mL^{-1} suppressed growth of *Colletotrichum gloeosporoides* by 77%. Kanhed *et al*. (2014) recorded strong activity of CuO NPs against *F. oxysporum, Alternaria alternata, Phoma distructiva* and *Curvularia lunata*.

Nanoparticles can physically and mechanically damage fungal cell walls and membranes, give these materials their antifungal properties. They can penetrate and accumulate within fungal cells, and can influence cellular signaling at the molecular level by dephosphorylating peptide substrates that are essential for cell viability and division. They can also enhance membrane permeability, block water channels, inhibit enzymes, and increase production of reactive oxygen species produced. These activities can disrupt essential metabolic pathways leading to the death or inhibition of fungi (Allahverdiyev *et al*., 2011; Wang *et al*., 2014). Perez-de-Luque and Rubiales (2009) also reported that extracellular enzymes and metabolites may be released as a result of using nanoparticles.

Transmission electron micrographs of hyphae of *F. oxysporum* f. sp. *ciceris* and *R. solani* verified the damage caused by CuO NP treatments. Treating *R. solani* mycelium with CuO NPs gave abnormal and disorganized cells with cytoplasm granulation, numerous mitochondria, and electron-dense bodies enclosed by electron-dense cell walls. Treated cells of *F. oxysporum* were irregular with thickened cell walls and plasma membranes. Cytoplasmic granulation, irregular large vacuoles and many electron-dense particles were also observed. El-Shewy *et al*. (2019) and Ismail (2021) observed similar effects to those observed in the present study.

Results from the cytotoxicity assay showed that CuO NPs were toxic to the test cells (IC_{50} = 25.2 ppm). At 40

ppm, CuO NPs were toxic to WI-38 human lung fibroblast cells, giving 80% cell mortality. This corroborated the conclusions of Katsumiti *et al.* (2018), who reported high toxicity for CuO NPs towards human lung epithelial cells (TT1 cells), with LC_{50} of 9.05 ppm. Cytotoxicity can be caused by generation of reactive oxygen species which cause oxidative stress to human cells and increases in ROS production as the mechanisms of cytotoxicity of CuO NPs. However, these cytotoxic effects depend on numerous factors, including surface functionalization, type of the tested cells, and concentration, size, shape, exposure time, and dose of CuO NPs (Naz *et al.* 2020). In contrast, no hemolytic effects were recorded from CuO NPs on red blood cells (IC_{50} = 440.1 ppm).

The pot and field experiments of the present study supported the findings from the *in vitro* experiments, demonstrating that the nanoparticles reduced development of root rot and wilt in chickpea plants. Among the nanoparticles assessed, CuO NPs had the greatest disease reduction efficacy. CuO NPs had effects comparable with the commercial fungicide thiophanate -methyl, with CuO NPs and the fungicide giving the least root rot and wilt incidences, compared to experimental controls. This result is similar to those of El-Shewy *et al.* (2019) who reported complete elimination of black scurf of potato in field trials using CuO-NPs. Copper-based nanoparticles have also been shown to be efficacious against fungal diseases, including tomato late blight caused by *Phytophthora infestans* (Giannousi *et al.*, 2013), and Fusarium wilt and Verticillium wilt of tomato (Elmer and White, 2016). The study by Servin *et al.* (2015) showed that nanoparticles of zinc oxide, titanium dioxide, and copper oxide provided effective disease management by direct inhibition of pathogens or stimulation of systemic acquired host resistance.

Previous research has demonstrated the mechanisms of action of nanomaterials towards phytopathogens. The biocidal effects of CuO NPs can be attributed to their direct impacts, or through release of copper ions. Because of the extensive surface area of CuO NPs, they can strongly attach to microbial cells, releasing essential cellular components and disrupting cell permeability (Raffi *et al.,* 2010). Oussou-Azo *et al.* (2020) showed that copper interacts with microbes through permeabilization of cell membranes, lipid peroxidation, alteration of proteins, and nucleic acid denaturation, leading to cell death.

The present study has shown that the assessed nanoparticles, particularly CuO NPs, have the ability to activate host plant defense systems against infections caused by *R. solani* and *F. oxysporum* f. sp. *ciceris.* Activity levels of POD and PPO enzymes, and total phenols were enhanced by all experimental nanoparticle treatments compared to the untreated experimental controls. CuO NPs exhibited greatest stimulation of these defense mechanisms among the experimental treatments applied. Previous investigations have given similar results, where copper-based nanoparticles augmented antioxidant mechanisms in plants, including the actions of SOD as well as PPO enzymes, and total antioxidant levels (Regier *et al*., 2015; Singh *et al*., 2017). El-Shewy *et al.* (2019) reported that potato plants treated with CuO NPs had increased POD and PPO activity. Nair and Chung (2014) reported that soybean plants treated with CuO NPs had increased PPO and lignin levels. They also showed high nanoparticle concentrations increased enzyme activity. Sarkar *et al.* (2020) presented results showing that presence of CuO NPs mediated catalase activation, ascorbate peroxidase, PPO, and SOD enzymes in tobacco plants.

Phenolic compounds have important roles in the plant resistance against fungi and plant diseases through mechanisms including causing hypersensitive cell death and the lignification of cell walls (Rashad *et al*., 2020b). The present study demonstrated that CuO NPs gave the greatest phenol contents, which is similar to the results of Sarkar *et al.* (2020). They reported increased production of phenols and flavonoids after application of an optimum concentration of CuO NPs. Biswas *et al.* (2012) also discussed the role of phenols in the disease resistance. Additionally, nanoparticles of ZnO, TiO_2 and CuO have been shown to have uses in pathogen control programs by direct inhibition of disease-causing organisms or induction of systemic acquired resistance in host plants (Servin *et al.* 2015).

The present study field trials showed that the tested nanoparticles reduced root rot and wilt of chickpea, with CuO NPs) having the greatest disease reduction activity, comparable to that achieved with the fungicide thiophanate-methyl . The disease reductions from nanoparticle treatments was probably due to the large nanoparticle surface areas, facilitating robust adsorption to pathogen organisms, compromising cell permeability, and releasing vital components (Raffi *et al.,* 2010). These results are similar to those from other studies demonstrating elimination of black scurf of potato by CuO NPs (El-Shewy *et al.,* 2019) and efficacy of copper-based nanoparticles against several fungal pathogens (Giannousi *et al*., 2013; Elmer and White, 2016). Previous research has also shown the effectiveness of nanoparticles of zinc oxide titanium dioxide, and CuO for disease control by either directly inhibiting pathogens or enhancing systemic acquired resistance (Servin *et al.,* 2015).

Applying nanoparticles of CuO, SiO_2, or TiO_2 through seed soaking increased chickpea growth and

yield parameters. These enhancements were associated with reductions in disease incidence, indicating possible induction of disease resistance in chickpea. Treatment with CuO NPs had the greatest positive impact on chickpea growth and yield parameters. These results align with previous studies demonstrating the beneficial role of copper for promoting plant growth and productivity. Ngo *et al*. (2014) reported beneficial effects of copper on plant growth. Elmer and White (2016) found that CuO NPs outperformed other metallic oxide nanoparticles for enhancing growth parameters of tomato and eggplant cultivated in soil inoculated with specific pathogens. Immersing wheat plants in Cu NPs accelerated their growth (Yasmeen *et al*., 2015). Hafeez *et al.* (2015) demonstrated that wheat growth was increased in soils amended with Cu NPs at concentrations between 10 and 30 ppm. Baskar *et al.* (2018) showed that treating eggplants with CuO NP at 100 mg L^{-1} increased seedling root and shoot lengths. Badawy *et al.* (2021) found that CuO NPs (50 ppm) enhanced growth parameters of wheat plants. The present study gave similar results, showing that CuO NPs increased chickpea plant growth and yield parameters in the field trials. These increased seed yields indicate that use of CuO NPs is likely to be advantageous for augmenting profitability of chickpea farming.

This study assessed nanoparticles of CuO, SiO_2 and TiO_2 for antifungal effects against *R. solani* and *F. oxysporum* f. sp. *ciceris*. *In vitro* experiments demonstrated that increasing the concentrations of these nanoparticles increased inhibition rates of fungal growth and reduced the fungal populations. In the pot experiments, the tested nanoparticles, particularly at high concentrations, enhanced the resistance of chickpea plants against root rot and wilt. This was associated with increased levels of host defense compounds. TEM observations showed harmful alterations in cellular ultrastructures of *R. solani* and *F. oxysporum* f. sp. *ciceris* due to exposure to CuO NPs. These results indicate the potential for incorporating CuO NPs into management of Rhizoctonia rot and Fusarium wilt of chickpea plants. The field experiments further supported the effectiveness of these nanoparticles for reducing disease incidence and promoting chickpea growth and yield. CuO NP treatments are therefore recommended as potential alternative to potentially hazardous fungicides for managing chickpea root rot and wilt.

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LITERATURE CITED

Abou-Salem E., Ahmed A.R., Elbagory M., 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. https://doi.org/10.3390/su141912871

Al-Askar A.A., Abdulkhair W.M., Rashad Y.M., Hafez E.E., Ghoneem K.M., Baka Z.A., 2014. *Streptomyces griseorubens* E44G: A Potent Antagonist Isolated from Soil in Saudi Arabia. *Journal of Pure and Applied Microbiology* 8: 221–230.

Allahverdiyev A.M., Emrah S.A., Malahat B., Miriam R., 2011. Antimicrobial effects of TiO_2 and Ag_2O nanoparticles against drug-resistant bacteria and leishmania parasites. *Future Microbiology* 6: 933-940. https://doi.org/10.2217/fmb.11.78

Amin B.H., 2016. Isolation and characterization of antiprotozoal and antimicrobial metabolite from *Penicillium roqueforti*. *African Journal of Mycology and Biotechnology* 21(3): 13–26.

Amin B.H., Abou-Dobara M.I., Diab M.A., Gomaa E.A., El-Mogazy M.A., Salama H.M., 2020. Synthesis, characterization, and biological investigation of new mixed-ligand complexes. *Applied Organometallic Chemistry* 34(8): 1–18. https://doi.org/10.1002/aoc.5689

Amin B.H., Amer A., Azzam M., Abd El-Sattar N.E.A., Mahmoud D., Hozzein W.N., 2022. Antimicrobial and anticancer activities of *Periplaneta americana* tissue lysate: An *in vitro* study. *Journal of King Saud University*–Science 34: 102095. https://doi.org/10.1016/j.jksus.2022.102095

Badawy A.A., Nilly A.H.A., Salem S.S., Mohamed F.A., Amr F., 2021. Efficacy assessment of biosynthesized copper oxide nanoparticles (CuO-NPs) on stored grain insects and their impacts on morphological and physiological traits of wheat (*Triticum aestivum* L.) plant. *Biology* 10: 233. https://doi.org/10.3390/biology10030233

Baskar V., Nayeem S., Kuppuraj S.P., Muthu T., Ramalingam S., 2018. Assessment of the effects of metal oxide nanoparticles on the growth, physiology and metabolic responses *in vitro* grown eggplant (*Solanum melongena*). *3 Biotech* 8: 362. https://doi.org/10.1007/s13205-018-1386-9

Biswas S.K., Pandey N.K., Rajik M., 2012. Inductions of defense response in tomato against Fusarium wilt

through inorganic chemicals as inducers. *Journal of Plant Pathology & Microbiology* 3: 1–7. https://doi.org/10.4172/2157-7471.1000128

Bowler C., Montagu M.V., Inze D., 1992. Superoxide dismutase and stress tolerance. *Annual Review of Plant Biology* 43: 83–116.

Boxi S.S., Mukherjee K., Paria S., 2016. Ag doped hollow TiO_2 nanoparticles as an effective green fungicide against *Fusarum solani* and *Venturia inaequalis* phytopathogens. *Nanotechnology* 27: 085103. https://doi.org/10.1088/0957-4484/27/8/085103

Chakraborty M.R., Chatterjee N.C., 2007. Interaction of *Trichoderma harzianum* with *Fusarium solani* during its pathogenesis and the associated resistance of the host. *Asian Journal of Experimental Sciences* 21: 351–355.

Choudhary A.K., Kumar S., Patil B.S., Sharma M., Kemal S.,…Vijayakumar A.G., 2013. Narrowing yield gaps through genetic improvement for Fusarium wilt resistance in three pulse crops of the semi-arid tropics. SABRAO *Journal of Breeding and Genetics* 45: 341–370. http://oar.icrisat.org/id/eprint/1079

Cui H., Zhang P., Gu W., Jiang J., 2009. Application of anatasa TiO_2 sol derived from peroxotitannic acid in crop diseases control and growth regulation. *NSTI-Nanotech* 2: 286–289.

Elmer W.H., White J.C., 2016. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. *Environmental Science. Nano* 3: 1072–1079. https://doi.org/10.1039/C6EN00146G

Elshahawy I.E., Osman S.A., Abd-El-Kareem F., 2021. Protective effects of silicon and silicate salts against white rot disease of onion and garlic, caused by *Stromatinia cepivora*. *Journal of Plant Pathology* 103: 27–43. https://doi.org/10.1007/s42161-020-00685-1

El-Shewy E.S.A., Mohemed F.G., Abd-latif F.M., Hafez E.M., Mansour S.A., 2019. The efficacy of copper oxide, tri-calcium phosphate and silicon dioxide nanoparticles in controlling black scurf disease of potato. *Annals of Agricultural Science Moshtohor* 57: 129–138. https://doi.org/10.21608/assjm.2019.42223

Farias D.F., Souza T.M., Viana M.P., Soares B.M., Cunha A.P., … Carvalho A.F.U., 2013. Antibacterial, antioxidant, and anticholinesterase activities of plant seed extracts from Brazilian semiarid region. *BioMed Research International* 1: 510736. https://doi.org/10.1155/2013/510736

Fraternale D., Giamperi L., Ricci D., 2003. Chemical composition and antifungal activity of essential oil obtained from *in vitro* plants of *Thymus mastichina* L. *Journal of Essential Oil Research* 15: 278–281.

Giannousi K., Avramidis I., Dendrinou S.C., 2013. Synthesis, characterization and evaluation of copper-based nanoparticles as agrochemicals against *Phytophthora infestans*. *RSC Advances* 3: 21743–21752. https://doi.org/10.1039/C3RA42118J

Goffeau A., 2008. Drug resistance: The fight against fungi. *Nature* 452: 541-542. https://doi.org/10.1038/452541a

Gogos A., Knauer K., Bucheli T.D., 2012. Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *Journal of Agricultural and Food Chemistry* 60(39): 9781–9792 . https://doi.org/10.1021/jf302154y

Gohari G., Mohammadi A., Akbari A., Panahirad S., Dadpour M.R., Kimura S., 2020. Titanium dioxide nanoparticles (TiO_2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of Draco-cephalum moldavica. *Scientific Reports* 10(1): 1–14. https://doi.org/10.1038/s41598-020-57794-1

Gupta A.K., Gupta M., 2005. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials* 26: 3995–4021. https://doi.org/10.1016/j.biomaterials.2004.10.012

Hafeez A., Razzaq A., Mahmood T., Jhanzab H.M. 2015. Potential of copper nanoparticles to increase growth and yield of wheat. *Journal of Nanoscience with Advanced Technology* 1: 6–11. https://doi.org/10.24218/jnat.2015.02.

Hermida-Montero L.A., Nicolaza P.A.I, Mtz-Enriquez G.C., Paraguay-Delgado F., Greta R.S., 2019. Aqueous-phase synthesis of nanoparticles of copper/copper oxides and their antifungal effect against *Fusarium oxysporum*. *Journal of Hazardous Materials* 380: 120850. https://doi.org/10.1016/j.jhazmat.2019.120850

Ismail A.M., 2021. Efficacy of copper oxide and magnesium oxide nanoparticles on controlling black scurf disease on potato. *Egyptian Journal of Phytopathology* 49(2): 116-130 . http://dx.doi.org/10.21608/ejp.2021.109535.1050

Jukanti A.K., Gaur P.M., Gowda C.L.L., Chibbar R.N., 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *British Journal of Nutrition* 108: (S1) S11–S26. https://doi.org/10.1017/S0007114512000797

Kanhed P., Birla S., Gaikwad S., Gade A., Seabra A.B., Rai M., 2014. *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Materials Letters* 115: 13–17. https://doi.org/10.1016/j.matlet.2013.10.011

Kasana A., AliNiazee M.T., 1997. A thermal unit summation model for the phenology of *Rhagoietis completa* (Diptera: Tephritidae). *Journal of the Entomological Society of British Columbia* 94: 13–18.

Katsumiti A., Thorley A.J., Arostegui I., Reip P., Valsami-Jones E., Cajaraville M.P., 2018. Cytotoxicity and cellular mechanisms of toxicity of CuO NPs in mussel cells *in vitro* and comparative sensitivity with human cells. *Toxicology In Vitro* 48: 146–158. https://doi.org/10.1016/j.tiv.2018.01.013

Kaur P., Thakur R., Choudhary A., 2012. An *in vitro* study of the antifungal activity of silver/chitosan nanoformulations against important seed borne pathogens. *International Journal of Science and Technology Research* 1: 83–86.

Nair P.M.G., Chung I.M., 2014. Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignification, and molecular level changes. *Environmental Science and Pollution Research* 21: 12709–12722. https://doi.org/10.1007/s11356-014-3210-3

Naz S., Gul A., Zia M., 2020. Toxicity of copper oxide nanoparticles: a review study. *IET Nanobiotechnology* 14(1): 1-13. https://doi.org/10.1049/iet-nbt.2019.0176

Nel A.E., Xia T., Madler L., Li N., 2006. Toxic potential of materials at the nanolevel. *Science* 311: 622–627. https://doi.org/10.1126/science.1114397

Ngo B.Q., Dao T.T., Nguyen C.H., Tran X.T., Nguyen T.V., Huynh T.H., 2014. Effects of nanocrystalline powders (Fe, Co and Cu) on the germination, growth, crop yield and product quality of soybean (Vietnamese species DT-51). *Advances in Natural Sciences: Nanoscience and Nanotechnology* 5: 015016. https://doi.org/10.1088/2043-6262/5/1/015016

Oussou-Azo A.F., Nakama T., Nakamura M., Futagami T., Vestergaard M.C.M., 2020. Antifungal potential of nanostructured crystalline copper and its oxide forms. *Nanomaterials* 10: 1–13. https://doi.org/10.3390/nano10051003

Papavizas G.C., Davey C.B., 1962. Isolation and pathogenicity of Rhizoctonia saprophytically existing in soil. *Journal of Phytopathology* 52: 834–840.

Perez-de-Luque A., Rubiales, D., 2009. Nanotechnology for parasitic plant control. *Pest Management Science* 65: 540–545. https://doi.org/10.1002/ps.1732

Raffi M., Mehrwan S., Bhatti T.M., Akhter J.I., Yawar A.W., Masood ul Hasan, M., 2010. Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. *Annals of Microbiology* 60(1): 75–80. https://doi.org/10.1007/s13213-010-0015-6

Rashad Y.M., Aseel D.G., Hafez E.E., 2018. Antifungal potential and defense gene induction in maize against Rhizoctonia root rot by seed extract of *Ammi visnaga* (L.) Lam. *Phytopathologia Mediterranea* 57(1): 73–88. https://doi.org/10.14601/Phytopathol_Mediterr-21366

Rashad Y.M., Abbas M.A., Soliman H.M., Abdel-Fattah G.G., Abdel-Fattah G.A., 2020a. Synergy between endophytic *Bacillus amyloliquefaciens* GGA and arbuscular mycorrhizal fungi induces plant defense responses against white rot of garlic and improves host plant growth. *Phytopathologia Mediterranea* 59(1): 169–186. https://doi.org/10.36253/phyto-11019

Rashad Y.M., Aseel D.G., Hammad S.M., 2020b. Phenolic Compounds Against Fungal and Viral Plant Diseases. In: *Plant Phenolics in Sustainable Agriculture* (Lone R., Shuab R., Kamili A., ed.). Springer Nature Singapore Pte Ltd. pp. 201–219, from https://doi.org/10.1007/978-981-15-4890-1_9

Rashad Y.M., El-Sharkawy H.H., Belal B.E., Abdel Razik E.S., Galilah D.A., 2021. Silica nanoparticles as a probable anti-oomycete compound against downy mildew, and yield and quality enhancer in grapevine: field evaluation, molecular, physiological, ultrastructural, and toxicity investigations. *Frontiers in Plant Science* 12: 763365. https://doi.org/10.3389/fpls.2021.763365

Rashad Y.M., El-Sharkawy H.H., Elazab, N.T., 2022. *Ascophyllum nodosum* extract and mycorrhizal colonization synergistically trigger immune responses in pea plants against rhizoctonia root rot, and enhance plant growth and productivity. *Journal of Fungi* 8(3): 268. https://doi.org/10.3390/jof8030268

Rastogi A., Tripathi D.K., Yadav S., Chauhan D.K., Živčák M., Brestic M., 2019. Application of silicon nanoparticles in agriculture. *3 Biotech* 9(3): 90. https://doi.org/10.1007/s13205-019-1626-7

Regier N., Cosio C., Von Moos N., Slaveykova V.I., 2015. Effects of copper-oxide nanoparticles, dissolved copper and ultraviolet radiation on copper bioaccumulation, photosynthesis and oxidative stress in the aquatic macrophyte Elodea nuttallii. *Chemosphere* 128: 56–61. https://doi.org/10.1016/j.chemosphere.2014.12.078

Sadasivam S., Manickam A., 1996. *Biochemical Methods*. Second Ed. New Age Int. Pvt. Ltd. Pub. and T.N. Agril. Univ. Coimbatore, Tamil Nadu, India, pp. 108–110.

Sarkar J., Chakraborty N., Chatterjee A., Bhattacharjee A., Dasgupta D., Acharya K., 2020. Green synthesized copper oxide nanoparticles ameliorate defense and antioxidant enzymes in *Lens culinaris*. *Nanomaterials* 10(2): 312. https://doi.org/10.3390/nano10020312

Servin A., Elmer W., Mukherjee A., Torre-Roche R., Hamdi H., … Dimkpa C., 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *Journal of Nanoparticle Research* 17: 92. https://doi.org/10.1007/s11051-015-2907-7

Siddiqui M.H., Al-Whaibi M.H., 2014. Role of nano-SiO_2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.) *Saudi Journal of Biological Sciences* 21(1): 13–17. https://doi.org/10.1016/j.sjbs.2013.04.005

Singh A., Singh N.B., Hussain I., Singh H., 2017. Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants *Solanum lycopersicum* and *Brassica oleracea* var. *botrytis*. *Journal of Biotechnology* 262: 11–27. https://doi.org/10.1016/j.jbiotec.2017.09.016

Suriyaprabha R., Karunakaran G., Yuvakkumar R., Rajendran V., Kannan N., 2014. Foliar application of silica nanoparticles on the phytochemical responses of maize (*Zea mays* L.) and its toxicological behavior. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry* 44(8): 1128–1131. https://doi.org/10.1080/15533174.2013.799197

Wang X.P., Liu X.Q., Chen J.N., Han H.Y., Yuan Z.D., 2014. Evaluation and mechanism of antifungal effects of carbon nanomaterials in controlling plant fungal pathogen. *Carbon* 68: 798–806. https://doi.org/10.1016/j.carbon.2013.11.072

Worrall E.A., Hamid A., Mody K.T., Mitter N., Pappu H.R., 2018. Nanotechnology for plant disease management. *Agronomy* 8(12): 285. https://doi.org/10.3390/agronomy8120285

Xue X.J., Geng T.T., Liu H.F., Yang W., Zhong W.R., Zhang Z.L., 2021. Foliar application of silicon enhances resistance against *Phytophthora infestans* through the ET/JA- and NPR1-dependent signaling pathways in potato. *Frontiers in Plant Science* 12: 609870. https://doi.org/10.3389/fpls.2021.609870

Yadav R.K., Singh N.B., Singh A., Yadav B., Bano C., Niharika, 2020. Expanding the horizons of nanotechnology in agriculture: recent advances, challenges and future perspectives. *Vegetos* 33: 203–221. https://doi.org/10.1007/s42535-019-00090-9

Yasmeen F., Razzaq A., Iqbal M.N., Jhanzab H.M., 2015. Effect of silver, copper and iron nanoparticles on wheat germination. *International Journal of Biosciences* 6: 112–117. https://doi.org/10.12692/ijb/6.4.112-117

Zian A.H., El-Blasy S.A., El-Gendy H.M., El-Sayed S.A., 2023. Plant defense activation and down-regulation root rot and wilt in chickpea diseases by some abiotic substances. *E3S Web of Conferences* 462: 02014. https://doi.org/10.1051/e3sconf/202346202014

Zian A.H., El-Blasy S.A.S., Khalil M.S.M., El-Gammal Y.H., 2024. Improving the Efficiency of Bioagents Using Certain Chemical Inducers against Root Rot and Wilt Diseases of Soybean. *Journal of Plant Protection and Pathology* 15(10): 335–346. https://doi.org/10.21608/jppp.2024.320903.1266

Zilesin N., Ben-Zaken R., 1993. Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. *Plant Physiology and Biochemistry* 31: 333–339.