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ORIGINAL PAPER

Efficacy of Copper Oxide and Magnesium Oxide Nanoparticles on Controlling Black Scurf Disease on Potato

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ABSTRACT

Black scurf disease caused by *Rhizoctonia solani* is common on potato crop worldwide. The current study aimed to evaluate the antifungal activity of CuONPs and MgONPs on *R. solani* in laboratory, greenhouse and field trials. Field trials were conducted under naturally infected fields located in two Governorates, Behera and Menoufia. Potato tubers cv. Spunta were soaked in different concentrations (75, 150 and 200 mg/L) of the tested NPs for 2 hours pre-planting. Disease severity (DS) and disease incidence (DI) of black scurf symptoms on the harvested potato tubers were estimated. Ultrastructural changes in *R. solani* hyphae in response to the tested NPs were also detected using Transmission Electron Microscope (TEM). Physiological and biochemical activities were also determined in potato leaves after 20, 40 and 60 days of planting. *In vitro* results showed that MgONPs at 200 mg/L exhibited the greatest inhibitory effect on the mycelial growth of *R. solani*, with inhibition reached 73.47 %. Ultrastructural micrographs of TEM images of *R. solani* hyphae confirmed the damage induced by NPs. Greenhouse results exhibited that the great reduction in DI and DS% was achieved by MgONPs at concentration 200 mg/L, with efficacy reached 85.07 and 93.47 %, respectively. The same trend of greenhouse results was observed, of which MgONPs at concentration 200 mg/L, had significant ($p<0.05$) effect in reducing both DI and DS% in both Menoufia and Behera field trials. Also, MgONPs had a significant ($p<0.05$) effect in increasing the yield of potato tubers in both field trials. NPs had great impact in increasing enzyme activities, phenols and chlorophyll content when compared to untreated control plants. Energy Dispersive X-ray Spectrometry (EDX) analysis confirmed the presence of CuONPs in the tissues of treated harvested potato tubers compared with the control. The accumulation of CuONPs in edible plant tissues is a critical issue that could impact human health, and this should be taken in consideration when establishing control program for black scurf disease.

Key words: Potato, Black scurf, *Rhizoctonia solani*, chlorophyll, enzymes, nanoparticles, phenols.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is subjected to numerous fungal diseases such as powdery scab, powdery mildew, early blight, late blight, dry rot, wilt, silver scurf, stem canker and black scurf. Black scurf disease is caused by *Rhizoctonia solani* (Telomorph: *Thanatephorus cucumeris* (Frank) Donk). Damage caused by *R. solani* is common in potato crops worldwide, resulting in poor quality of tubers and reduction of yield (Jeger *et al*., 1996 and Kankam *et al*., 2021). The disease is very difficult to control due to persistent, long living sclerotial structures of *R. solani* in soil (Zachow *et al*., 2011). Thus, it is difficult to be entirely controlled, but severity may be limited by following a combination of crop protection strategies for successful disease management (Banyal, 2002). Crop rotation with non-susceptible crops for *R. solani* for 3–5 years is helpful to reduce both the incidence and severity of this disease. However, rotation is difficult to conduct in the main planting area of potato (Bakali and Martín, 2006). Therefore, alternative methods are needed for controlling this disease.

Nanotechnology is a new emerging and interesting field of sciences which currently applied in many areas. Nanoparticles (NPs) are commonly accepted as materials with two dimensions between 1-100 nm (Ball, 2002). Nanoparticles have potential prospects of use in plant disease management in different ways, such as application in soil, on seeds or foliage (Khandaker *et al.*, 2012). Due to their unique smaller size, larger surface area and better stability, NPs can serve as a preferable chemical (pesticide) delivery system and may also enhance crop yield by nutrient and water management (Rai *et al*., 2018), suggesting that nanotechnology is being a promising application approach for sustainable agriculture. An array of publications has been demonstrated the antifungal toxicity of CuONPs towards *Phytophthora infestans* (Giannousi *et al*., 2013)

and phytopathogenic fungi, such as *A. alternata* and *Botrytis cinerea* (Ouda, 2014), *A. alternata*, *Fusariun oxysporum*, *Curuvularia lunata* and *Phoma distructiva* (Kanhed *et al*., 2014), tomato Fusarium wilt and Verticillium wilt (Elmer and White, 2016), *R. solani* (El-Shewy *et al*., 2019) and powdery mildew of rose (Hao *et al*., 2019), Furthermore, MgONPs have enormous potential as antifungal agent against fungal diseases caused by *R. solani* (El-Argawy *et al*., 2017), *A. alternata*, *F. oxysporum*, *Rhizopus stolonifer*, and *Mucor plumbeus* (Wani and Shah, 2012), *F. oxysporum* f. sp. *lycopersici* (Parizi *et al*., 2014) and *P. nicotianae* and *Thielaviopsis basicola* (Chen *et al*., 2020).

The aims of the present study were to; (1) investigate the antifungal activity of CuONPs and MgONPs against *R. solani* in *in vitro* and *in vivo* trials; (2) detect the ultrastructural changes in *R. solani* hyphae in response to the tested NPs; and (3) determine of physiological and biochemical activities such as peroxidase and polyphenoloxidase enzymes, chlorophyll and phenol contents.

MATERIALS AND METHODS

Source of NPs and Potato Tubers:

CuONPs and MgONPs were obtained from Nanotechnology & Advanced Nano-Materials Laboratory (NANML), Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. The morphology and size of such NPs were confirmed using High Resolution Transmission Electron Microscope HR-TEM, with an accelerating voltage of 200 kV at National Research Centre (NRC), Dokki Giza, Egypt. Apparently healthy tubers cv. Spunta were obtained from Brown Rot Potato Project (ARC), Giza, Egypt.

Source of *R. solani*:

The isolate of *R. solani* used in this study was provided from Department of Vegetable Diseases Research, (PPRI), (ARC), Giza, Egypt. It was originally isolated from diseased potato tubers with typical black scurf symptoms. The identity of *R. solani* was confirmed by amplification and sequencing of Internal Transcribed Spacer (ITS) region containing; ITS1 and ITS2 regions and the 5.8S rRNA gene using ITS1 and ITS4 primers (White *et al.*, 1990). Genomic DNA extraction was performed using Genomic DNA Mini kit (Geneaid) according to manufacturer's protocol. PCR amplification was done in a reaction containing 1 µl of the fungal DNA extract, 2 mM $MgCl_2$, 2.5 of 10x PCR buffer,1.5 µL of 10 µM of each primer, 2.5 µl of 10 mM dNTPs, 0.3 µl of 5U Taq DNA Polymerase and the reaction was completed to 25 µL with Nuclease-free water. PCR was conducted in 2720 Thermal Cycler (Applied Biosystem), with initial denaturation at 95°C for 2 min, followed by 35 cycles of 95°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec, and the final cycle is a polymerization cycle performed at 72°C for 10 min. PCR products were sequenced through a commercial company (Macrogen Inc, South Korea). BLAST analysis was applied for sequences homology at NCBI (http://ncbi.nlm.nih. gov/BLAST).

Effect of NPs on the Mycelial Growth of *R. solani*:

Effect of CuONPs and MgONPs on the mycelial growth of *R. solani* was carried out on potato dextrose agar (PDA) medium using the poisoned media technique (Grover and Moore 1962). PDA medium was supplemented with a series of concentrations (75, 150 and 200 mg/L) of each NPs. Before solidification, PDA medium was poured into 4 petri plates for each treatment. Plates were then inoculated in the center with 5 mm fresh mycelial discs of 5 days old culture of *R. solani* and incubated at 24±2°C. Negative control plates contained only mycelial discs of *R. solani*. The diameter of the mycelium was measured, and inhibition ratio was calculated for each treatment.

Examination of *R. solani* Using TEM:

Ultrastructural and morphological changes in the hyphae of *R. solani* treated with CuONPs and MgONPs at concentration 200 mg/L were examined using Transmission Electron Microscope (TEM). The fungal hyphae were prepared following the procedure of Chen *et al*. (2020). Stained specimens were imaged using JEOL-JEM 1010 TEM at acceleration voltage 80 kV at The Regional Centre for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Greenhouse Trial:

Inoculum Preparation:

The inoculum of the tested *R. solani* was prepared in sterilized glass flasks (500 ml). Each flask contained a mixture of sorghum grains and sand (2:1 v/v), the ingredients were mixed well and wetted with tap water and then autoclaved for 30 min at 121°C. The flasks were inoculated with 6 mm mycelial disc of 5 days old culture of *R. solani*. The inoculated flasks were then incubated at 24±2°C for 15 days (Abd El-Aziz *et al.*, 2013).

Preparation of Soil and Pots:

Soil mixture of peat moss and sand (2:1 w/w) was prepared and sterilized with 5% diluted solution of commercial formalin. Plastic pots 30

cm in diameter were sterilized by dipping in 5% formalin solution for 15 min, then air dried for 24 hr. The pots were then filled in with 3 kg of the previously prepared sterilized soil mixture.

Treatment of Potato Tubers and Inoculation:

The tested NPs were applied by soaking apparently healthy potato tubers in a series of concentrations (75, 150 and 200 mg/L) of each for 2 hr. Each plastic pot was inoculated with *R. solani* inoculum at the rate of 2% and watered for 5 days before planting (Abd El-Aziz *et al.*, 2013). Potato tubers used for control were soaked in only water for 2 hr. Control pots were inoculated with the autoclaved sorghum-sand mixture without *R. solani*. Every two pots represented one replicate and three replicates were used for each treatment. The pots were kept under greenhouse conditions. Treated potato tubers were planted in the plastic pots (one tuber/pot) and they were watered and fertilized when needed.

Field Trials:

Field trials were conducted in January 2021. Two experiments were established in two different locations *i.e*., Kom-Hamada, El-Beheira Governorate, and El Khattatba, Menoufia Governorate. Each field of study has been cultivated with potato for several years and had a history of infestation with black scurf pathogen. Potato tubers were soaked for 2 hr. in a serious of concentrations (75, 150 and 200 mg/L) of each tested NPs. Potato tubers used for control were soaked in only tap water for 2 hr. Then, the treated tubers were planted in the field plots. Each plot comprised of 3 ridges, (3 meters long each), for each concentration, and each ridge represented one replicate. Both trials lasted 90 days then the tubers were harvested and kept in a dry place at room temperature for 2 days for dryness. After that, the harvested tubers were washed carefully to remove soil particles to show up the developed sclerotia of *R. solani*.

Disease Assessment:

In greenhouse trial, Disease severity (DS) and Disease incidence (DI) were measured on randomly collected 7 tubers for each replicate. In field trials, DS and DI were determined on randomly collected 25 tubers for each replicate. The DS was calculated using the scale of Rauf *et al*. (2007):

0= no sclerotia present

1= less than 1% of tuber area affected

2= 1-10% of tuber area affected

3= 11-20% of tuber area affected

4 =21-50% of tuber area affected

5= 51% or more of tuber area affected

$$\textbf{Disease Severity (DS)\%} = \frac{\sum \textbf{(No. of infected tubers} \times \textbf{No. of scale)}}{\textbf{Total No. of tubers X highest No. of scale}} \times 100$$

The disease incidence and efficacy were calculated as following:

$$\textbf{Disease Incidence (DI) \%} = \frac{\textbf{No. of infected tubers}}{\textbf{Total No. of tubers}} \times 100$$

$$\textbf{Efficacy\%= Control - Treatment/Control} \times 100$$

Assessment of Yield Parameters:

The yield parameters *i.e.*, tubers number/plant, potato tubers fresh weight (kg)/plant and dry weigh (g) were determined. Dry weight (g) was estimated in 100 (g) fresh weight of potato tubers following the method of Zelalem *et al*. (2009).

Assessment of Enzymatic Activity:

The activity of Polyphenoloxidase (PPO) enzyme was estimated in potato leaves after 20, 40 and 60 days of planting according to Matta and Dimond (1963). Polyphenoloxidase was expressed as change in absorbance at 495 nm. The activity of peroxidase (PO) was estimated as mentioned by Allam and Hollis (1972). Peroxidase was expressed as change in the absorbance at 422 nm every 10 sec for 1 minute period.

Determination of Phenols and Chlorophyll Contents:

Phenol content was colorimetrically estimated in potato leaves after 20, 40 and 60 days of planting date using the "Folin and Ciocaltei" reagent as described by Snell and Snell (1953). Chlorophyll A and B were estimated in potato leaves after 20, 40 and 60 days of planting according to Su *et al*. (2010). Chloroplast pigments were determined by measuring the optical density at 663 and 645 nm and calculated using the formula described by Arnon (1949). Measurements were done using Unico-2000 UV spectrophotometer (Unico Scientific, Hong Kong, China) at (NANML), Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt.

Detection of NPs in Potato Tubers:

The presence and accumulation of CuONPs and MgONPs in the tissues of harvested potato tubers was determined using Energy Dispersive X-ray Spectrometry (EDX) microanalysis (Scimeca *et al*., 2018) at National Research Centre (NRC), Giza, Egypt. Dry ashing of potato tuber tissues was done before EDX analysis following the method of Gichuhi *et al*. (2014).

Statistical analysis:

The obtained data were subjected to statistical analysis of variance according to Snedecor and Cochran (1980) and treatments means were separated by Fisher's protected least significant difference (LSD). Data were analyzed by software SPSS 8·0.

RESULTS

Identification of Potato Black Scurf Pathogen:

PCR amplification of the ITS1, 5.8S rRNA and ITS2 region resulted in a product of approximately 650 bp. BLAST search of the sequenced DNA product against a variety of nucleotide sequence in GenBank databases revealed 100 % similarity with *R. solani* strain CBS 280.36 (AG-3) (GenBank accession no. MH855798). The obtained sequence was deposited in GenBank database under accession no: OK275407. Sequence analysis of the ITS region confirmed that the *R. solani* isolate belongs to the AG-3 group.

Characterization of NPs Using TEM:

A close look at HR-TEM images at 200 nm confirmed the nanometer form with size ranges from 52.5 to 57.3 nm for MgONPs (Fig. 1A), and 23.1 to 27.3 nm for CuONPs (Fig. 1B). MgONPs were generally monodispersed, sometimes irregular and mostly have cubic-like shape (Fig. 1A). The nanoparticles of CuONPs were spherical and tended to agglomerate by forming aggregations, indicating unsatisfactory dispersibility (Fig.1B).

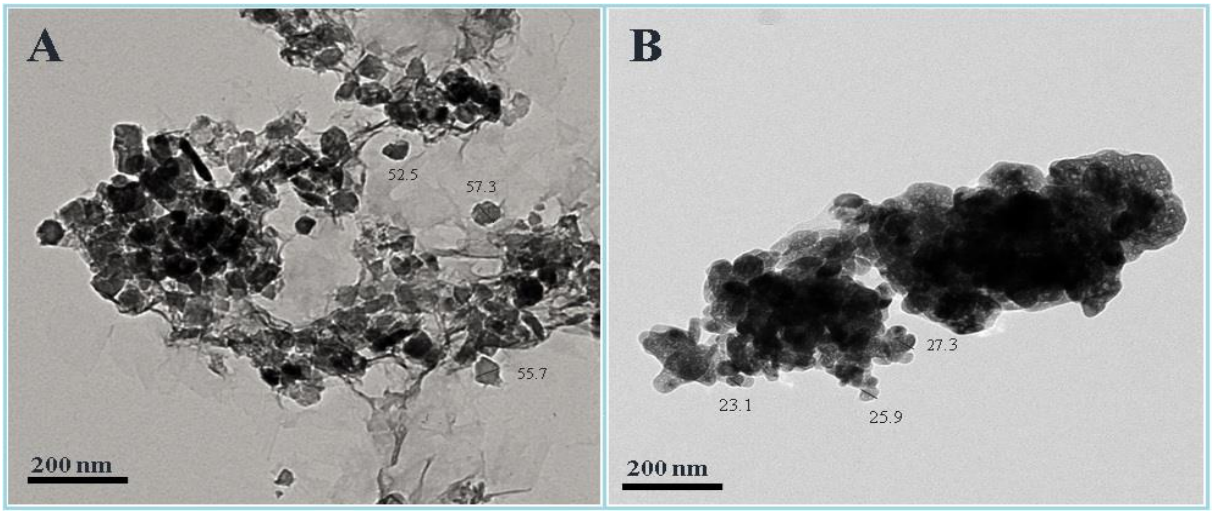

Figure (1): TEM micrograph images of MgONPs (A) and CuONPs (B) at 200 nm.

Effect of NPs on the mycelial growth of *R. solani*:

Results in Table (1) illustrate the antifungal effect of CuONPs and MgONPs on the mycelial growth of *R. solani in vitro*. All tested concentrations of the tested NPs reduced the mycelial growth of *R. solani* relative to control treatment. The treatment with MgONPs at 200 mg/L exhibited the greatest inhibitory effect in reducing the growth of *R. solani*, with inhibition reached 73.47 % followed by CuONPs at 200 mg/L, which recorded 62.02 % inhibition of the growth of *R. solani*. On the other hand, the low concentrations of CuONPs had weak effect on the growth of *R. solani*. Moreover, the inhibitory effect of CuONPs and MgONPs was decreased gradually with decreasing concentration. None of the tested NPs resulted in a complete inhibition of *R. solani* growth *in vitro*.

Table (1): Effect of CuONPs and MgONPs on the mycelial growth of *R. solani in vitro*

Treatment	Concentration (mg/L)	Mycelial growth (mm)	Inhibition %
CuONPs	75	60.03	33.30
	150	50.42	43.98
	200	34.18	62.02
MgONPs	75	44.93	50.08
	150	36.14	59.84
	200	23.88	73.47
Control	0	90.00	-
LSD at 0.05		0.84	

Examination of *R. solani* Using TEM:

Cross and longitudinal sections of *R. solani* mycelia were imaged through TEM (Fig. 2). Treatment with CuONPs revealed a malformation of fungal cell nucleus, degradation or deterioration of the fungal cell wall and coagulation of cytoplasm and most of cell organelles (Fig.2B, C) when compared with control treatment (Fig.2A). While treatment of MgONPs caused loss of matrix in vacuoles and obvious vacuolization, plasmolysis, cell wall thickening, conglomeration of cytoplasm and nucleus malformation relative to control treatment (Fig.2D, E).

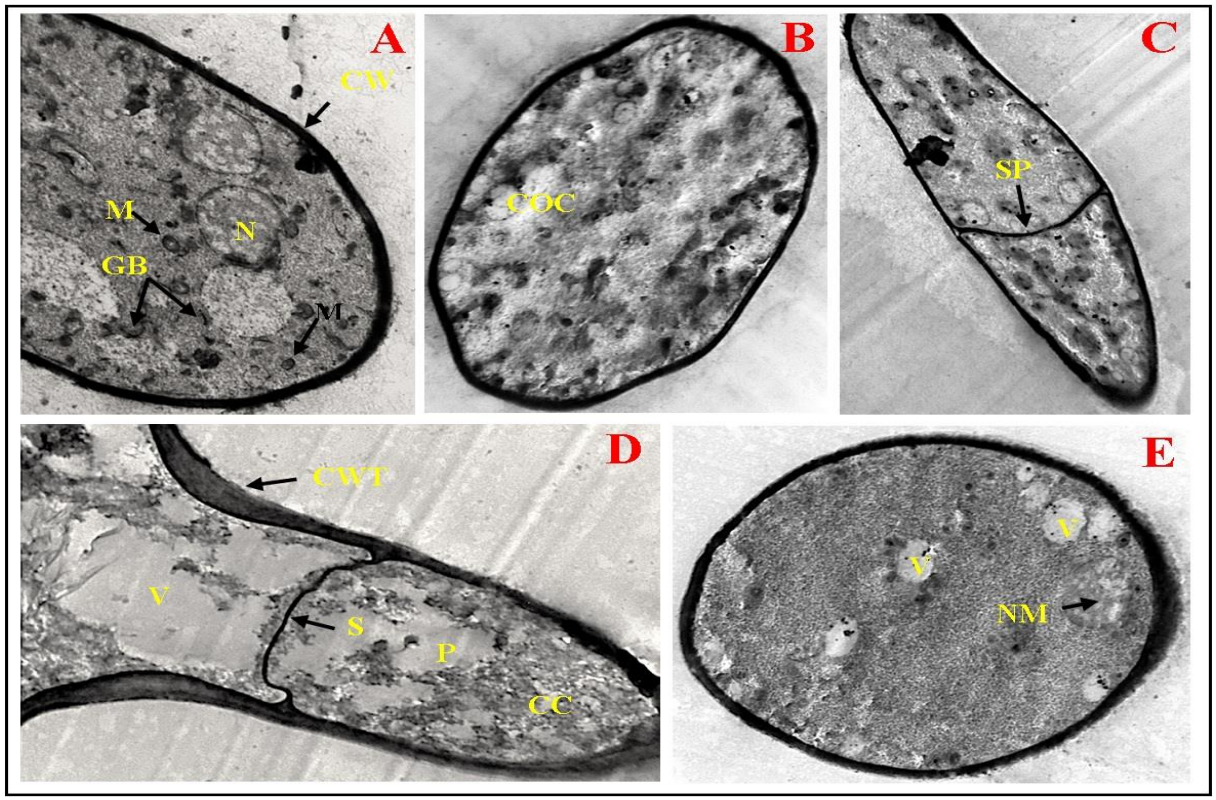

Figure (2): TEM of *R. solani* hyphae (A) transverse section of untreated hypha (control), mitochondria (M); cell wall (CW); Nucleus (N) and Golgi bodies (GB)). (B) transverse and (C) longitudinal sections of treated hyphae with CuONPs at 200 mg/L, showing cell organs coagulation (COC) and disappearance of septal pore (SP); (D) transverse and (E) longitudinal sections of treated hyphae with MgONPs at 200 mg/L, showing that cell walls become thick (CWT), loss of matrix in vacuoles and obvious vacuolization (V), plasmolysis (P), conglomeration of cytoplasm (CC) and nucleus malformation (NM).

Greenhouse Trial:

Data in Table (2) indicate that the tested CuONPs and MgONPs at all concentrations reduced DI and DS% of black scurf disease under greenhouse conditions over control. The greatest effect was obtained by MgONPs at 200 mg/L and 150 mg/L, which revealed a noticeable reduction in DI and DS%. Also, CuONPs revealed a significant reduction in DI% and DS% at 200 mg/L. It was clear that increasing the concentration of both the tested NPs from 75 to 200 mg/L increased gradually their effects in reducing DI% and DS% of black scurf disease on potato tubers.

Field Trials:

The evaluated CuONPs and MgONPs exhibited significant decrease in DI and DS of black scurf symptoms under field conditions of Behera and Menoufia trials (Tables 3, 4). In Behera trial, concentration 200 mg/L, MgONPs recorded significant decrease in DI of black scurf with efficacy reaching 76.67 % and DS with efficacy reaching 76.02 % (Table .3). However, CuONPs at the same concentration showed lower efficacy than MgONPs in such respect, but still significantly effective than the lower concentrations and control. In Menoufia trial, the same trend was obtained, of which the higher concentration (200 mg/L) of each of CuONPs and MgONPs had the greatest effect in decreasing DI and DS of black scurf symptoms (Table .4). Additionally, treatment of MgONPs at 150 mg/L resulted also in a remarkable decrease in DI and DS% of black scurf

symptoms when compared with the lower concentration and control in both trials. No phytotoxic effect was observed on potato leaves in response to treatments with CuONPs and MgONPs during the experimental studies.

Table (2): Evaluation of CuONPs and MgONPs against black scurf disease under greenhouse conditions

Treatment	Concentration (mg/L)	DI %	Efficacy %	DS %	Efficacy %
CuONPs	75	76.18	20.01	17.87	44.98
	150	57.14	40.00	11.65	64.13
	200	28.57	70.00	4.32	86.69
MgONPs	75	61.90	35.00	10.29	68.31
	150	19.10	79.94	3.14	90.33
	200	14.22	85.07	2.12	93.47
Control	0	95.24	-	32.48	-
LSD at 0.05		15.91		1.53	

Table (3): Evaluation of CuONPs and MgONPs against black scurf disease under field conditions in Behera Governorate

Treatment	Concentration (mg/L)	Behera Trial			
		DI %	Efficacy %	DS %	Efficacy %
CuONPs	75	30.67	23.32	18.55	23.59
	150	24.00	40.00	14.82	38.96
	200	13.33	66.67	7.02	71.08
MgONPs	75	26.67	33.32	15.75	35.13
	150	22.67	43.32	9.98	58.89
	200	9.33	76.67	5.82	76.02
Control	0	40.00	-	24.28	-
LSD at 0.05		5.69		1.89	-

Table (4): Evaluation of CuONPs and MgONPs against black scurf disease under field conditions in Menoufia Governorate

Treatment	Concentration (mg/L)	Menoufia Trial			
		DI %	Efficacy %	DS %	Efficacy %
CuONPs	75	25.33	29.63	11.99	27.81
	150	21.33	40.75	11.12	33.05
	200	10.67	70.36	5.65	65.98
MgONPs	75	25.33	29.63	10.41	37.32
	150	20.00	44.44	6.98	57.97
	200	8.00	77.77	4.59	72.36
Control	0	36.00	-	16.61	-
LSD at 0.05		6.40		2.13	

Effect of CuONPs and MgONPs on the Yield of Potato Plants:

Data presented in Tables (5, 6) show yield parameters of potato plants under field conditions. The tested NPs had significant effect in increasing yield of potato tubers when compared with control. Potato plants treated with MgONPs at concentration 200 mg/L in Behera trial showed significant increase in tuber number/plant with value reached 12.60 as well as fresh (2.67 kg) and dry weight (22.11g). At the same concentration, treatment of CuONPs exhibited also significant increase in tubers number/plant (10.73), fresh weight (2.64 kg) and dry weight (18.80 g) relative to control (Table .5). The same trend of the results was also observed in Menoufia trial, of which the highest concentration of MgONPs exhibited the highest values of tubers number/plant (11.13), fresh weight (2.92 kg) and dry weight (21.35 g) followed by CuONPs at the same concentration (Table .6).

Assessment of Enzymatic Activities, Total Phenols and Chlorophyll:

The activities of PO and PPO, total phenols and chlorophyll contents were estimated three times with 20-days interval after planting (Tables .7, 8). Based on the obtained results, the

highest activity of PO and PPO was recorded in leaves of potato plants treated with MgONPs at concentration 200 mg/L after 20 days of planting with values reaching 1.108 and 0.306 OD/ 10 sec/g FW, respectively (Table .7). The activity of PO and PPO was gradually decreased by time to reach 0.992 ΔA422/10 sec/g FW for PO and 0.272 ΔA495/10 sec/g FW for PPO after 60 days of planting. On the other hand, potato plants treated with CuONPs at concentration 200 mg/L exhibited also great activity of PO and PPO after 20 days of planting with values reached 0.791 ΔA422/10 sec/g FW and 0.282 ΔA495/10 sec/g FW, respectively. This activity was slightly decreased by time.

After 20 days of planting, potato plants treated with high concentrations of CuONPs and MgONPs revealed the highest content of total phenols with values reaching 7.66 and 8.25 mg/g FW (Table .8). Conversely, potato plants treated either with CuONPs or MgONPs at all concentrations displayed a gradual increase in chlorophyll contents started after 20 days from planting until the fortieth day (Table .8). After 40 days, phenols content in potato leaves was gradually decreased. The results elucidated in Table (8) show that the lowest concentrations 75 and 150 mg/L were also effective in increasing phenols and chlorophyll contents in potato plants when compared to control.

Table (5): Effect of CuONPs and MgONPs on the yield of potato plants cv. Spunta cultivated in Behera Governorate

Treatment	Concentration (mg/L)	Behera Trial		
		No. of tubers/Plant	Fresh weight/ Plant (kg)	Dry weight (g)/100 g of fresh weigh
CuONPs	75	9.60	1.94	16.95
	150	10.06	2.29	17.40
	200	10.73	2.64	18.80
MgONPs	75	10.00	2.48	19.19
	150	12.33	2.53	19.98
	200	12.60	2.67	22.11
Control	0	8.26	1.80	13.80
LSD at 0.05		2.11	0.39	1.5

Table (6): Effect of CuONPs and MgONPs on the yield of potato plants cv. Spunta cultivated in Menoufia Governorate

Treatment	Concentration (mg/L)	Menoufia Trial		
		No. of tubers/Plant	Fresh weight/ Plant (kg)	Dry weight (g)/100 g of fresh weigh
CuONPs	75	8.33	2.37	16.28
	150	9.00	2.46	16.37
	200	10.46	2.61	18.46
MgONPs	75	9.40	2.53	18.19
	150	9.66	2.86	19.32
	200	11.13	2.92	21.35
Control	0	6.60	1.64	12.63
LSD at 0.05		1.20	0.47	2.01

Table (7): Effect of CuONPs and MgONPs on oxidative enzyme activity of PO and PPO in potato plants cv. Spunta

Treatment	Concentration (mg/L)	PO (ΔA422/10 sec/g FW)			PPO (ΔA495/10 sec/g FW)		
		Days after planting			Days after planting		
		20	40	60	20	40	60
CuONPs	75	0.541	0.511	0.487	0.176	0.166	0.158
	150	0.587	0.547	0.521	0.224	0.214	0.203
	200	0.791	0.755	0.719	0.282	0.270	0.257
MgONPs	75	0.674	0.639	0.608	0.220	0.208	0.198
	150	0.930	0.883	0.841	0.259	0.247	0.235
	200	1.108	1.041	0.992	0.306	0.285	0.272
Control	0	0.505	0.482	0.459	0.147	0.140	0.133

OD= Optical density; FW= fresh weight; PO= peroxidase; PPO= polyphenoloxidase

Table (8): Effect of CuONPs and MgONPs on total phenols and chlorophyll in potato plants cv. Spunta

Treatment	Concentration (mg/L)	Total phenols (mg/g FW)			Total chlorophyll (mg/g FW)		
		Days after planting			Days after planting		
		20	40	60	20	40	60
CuONPs	75	4.67	4.26	4.03	33.40	38.14	36.67
	150	5.32	4.79	4.56	33.97	40.73	38.21
	200	7.66	7.12	6.72	35.42	46.29	42.54
MgONPs	75	6.58	6.08	5.94	39.22	44.23	40.21
	150	7.46	7.11	6.83	40.23	47.33	44.12
	200	8.25	7.49	7.32	46.54	48.75	45.32
Control	0	3.70	2.97	2.46	31.12	34.69	31.54

Detection of CuONPs and MgONPs in Potato Tubers:

Energy dispersive X-ray (EDX) spectrum analysis confirmed the presence of CuONPs and MgONPs in the tissues of harvested potato tubers (Fig. 3A, B) comparing to control (C). Other elements were also determined, and their weight percentages are presented in the incorporated Tables in Figure 3.

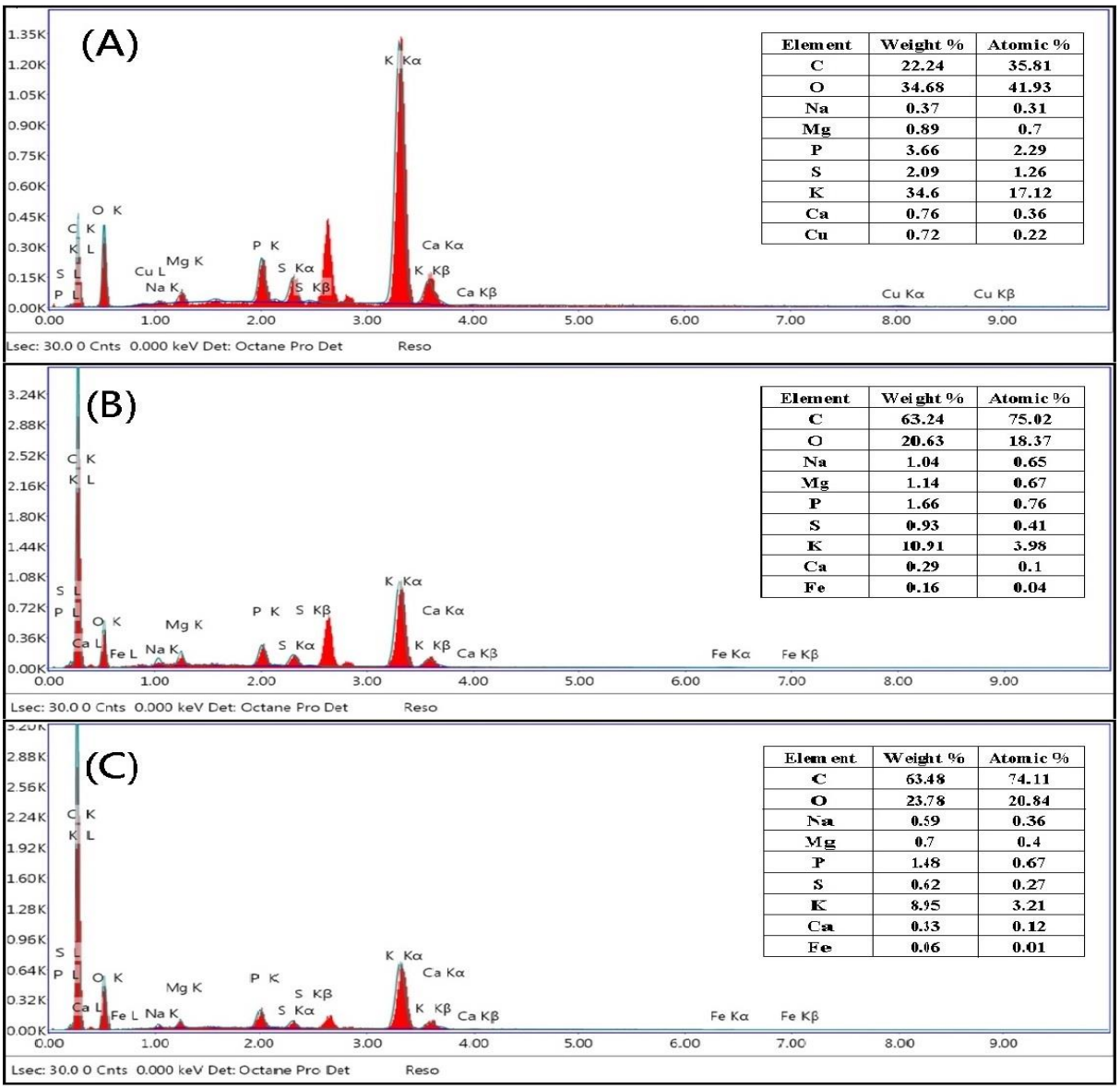

Figure (3): Energy Dispersive X-ray Spectrometry (EDX) profiles of harvested potato tubers treated with CuONPs (A), MgONPs (B) and untreated control (C) showing the weight percentages of NPs and other elements.

DISCUSSION

The group of multinucleate *R. solani* Kühn (teleomorph: *Thanatephorus cucumeris*) is the most extensively studied and widely recognized species. Hyphal anastomosis has been used for AG allocation of *Rhizoctonia* isolates, however it is not sufficient to delineate them at the subgroup level (Fang *et al*., 2013). According to Sharon *et al*. (2008), sequencing the ITS-5.8S rDNA region is considered to be the appropriate method for delineation of multinucleate and binucleate *Rhizoctonia* isolates into AGs and subgroups. Therefore, the results of amplification and sequencing of ITS-5.8S rDNA region confirmed that the tested isolate of *R. solani* belongs to AG3 group.

In vitro results of the current study elucidated the antifungal capacity of CuONPs and MgONPs at all tested concentrations to inhibit the mycelial growth of *R. solani*, when compared with control. The efficacy was high at concentration 200 mg/L and decreased with decreasing the concentration. Similar results were given by El-Shewy *et al*. (2019) who reported that CuONPs inhibited the growth of *R. solani* by 55.19 % at concentration 200 µl/L. The recent study of Oussou-Azo *et al*. (2020) demonstrated that the mycelial growth of *Colletotrichum gloeoesporioides* was inhibited by 76.8% with Cu-NP at concentrations of 200 mg/ml. Moreover, Kanhed *et al*. (2014) reported that CuONPs showed remarkable activity against *A. alternata*, *F. oxysporum*, *Curvularia lunata* and *Phoma distructiva*.

Based on the literature search, there are no reports on the use of MgONPs against potato black scurf caused by *R. solani*. However, the recent study of Ahmed *et al.* (2021) indicated that the CS-Mg nanocomposite had a remarkable antimicrobial activity against *A. oryzae* and *R. solani* on rice. Our results revealed that MgONPs at concentration 200 mg/L had the greatest inhibitory effect against *R. solani* with an efficacy reached 73.47 %. Similar to our results, El-Argawy *et al*. (2017) stated that MgONPs at concentration 100 ppm inhibited the mycelial growth of *R. solani* with efficacy value of 67.44 %. Previous *in vitro* studies demonstrated also the inhibitory effect of MgONPs on the germination of spores of *A. alternata*, *F. oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* (Wani and Shah, 2012), mycelial growth of *F. oxysporum* f. sp. *lycopersici* (Parizi *et al*., 2014), and *Phytophthora nicotianae* and *Thielaviopsis basicola* (Chen *et al*., 2020). Our results revealed that the antifungal activity of CuONPs and MgONPs restrained the mycelial growth of *R. solani* under all tested concentrations, in a dose-dependent manner, which is consistent with other metal nanoparticles (Rodriguez-Gonzalez *et al*., 2016 and Sun *et al*., 2018). From these perspectives, our study indicates the benefit of using MgONPs as a non-phytotoxic fungicide to control black scurf disease.

Ultrastructural micrographs TEM images of *R. solani* hyphae confirmed the damage induced by treatment with CuONPs and MgONPs. The treatment with CuONPs revealed that malformation and irregular disposition of cell organelles were clearly visible. The same trend of our results was also reported by El-Shewy *et al*. (2019). Moreover, Xi *et al*. (2018) stated that exposure of *R. solani* hyphae to 1,2,4,9-tetrahydro-3-thia-9-aza-fluorene caused disappearance of mitochondrial intermembrane space and obvious vacuolization. Our results revealed that exposer of *R. solani* hyphae to MgONPs resulted in cell wall thickening, loss of matrix in vacuoles and obvious vacuolization autolysis, depletion of hyphal cytoplasm and organelles. Likewise, Chen *et al*. (2020) reported the same effect of MgONPs at concentration 500 µg/ml against hyphae of *T. basicola* and *P. nicotianae*.

Considering the greenhouse and field experimental results, there are reasons to believe that CuONPs and MgONPs might be very suitable alternatives to fungicides for black scurf disease control. CuONPs displayed satisfied results at concentration 200 mg/L against *R. solani* in both greenhouse and field trials when compared with control. Almost, the same trend of our results was recorded by El-Shewy *et al*. (2019) who stated that CuONPs at 200 µl/L caused a 100 % reduction in DI and DS of black scurf in the field trials. Copper-based NPs displayed effectiveness in controlling tomato late blight caused by *Phytophthora infestans* (Giannousi *et al*., 2013) as well as several fungal diseases including, *A. alternata* and *Botrytis cinerea* (Ouda, 2014), tomato Fusarium wilt, and Verticillium wilt (Elmer and White, 2016), powdery mildew of rose (Hao *et al*., 2019), and avocado fruit rot (Hassan *et al*., 2021).

MgONPs exhibited the superior efficacy in reducing DI and DS under greenhouse and field conditions, being the higher concentration 200 mg/L was the greatest. Similarly, throughout greenhouse trial, El-Argawy *et al*. (2017) demonstrated that MgONPs at 100 ppm was successfully able to reduce pre-emergence, post-

emergence damping-off and root rot disease severity of sugar beet caused by *R. solani*. Other studies exhibited the antifungal activity of MgONPs against foliar and soilborne phytopathogens. For example, Chen *et al*. (2020) stated that the application of MgONPs at 500 µg/mL was highly effective against black shank and black root rot pathogens *P. nicotianae* and *Thielaviopsis basicola***.** Furthermore, Liao *et al*. (2019) reported that the application of MgONPs at 200 µg/mL significantly reduced disease severity of bacterial leaf spot on tomato under field conditions. None of the tested nanomaterials exhibited phytotoxicity on potato plants during the experiment progress. However, the phytotoxicity and biocompatibility of NPs toward potato plants need to be investigated in consequent studies.

The comprehensive mechanisms of NPs against phytopathogens *in vitro* and *in vivo* are not clarified in this study. These mechanisms could be discussed in the light of the previous studies. The biocidal activity of CuNPs could be attributed to the effect of the CuNPs and/or the copper ions discharged from CuNPs. Because of the great surface area of the NPs, it could be strongly adsorbed onto the surface of the microbial cells resulting in disruption of cell permeability and release of integral components (Raffi *et al*., 2010). It has been reported that Cu interacts with microorganisms in different ways including cell membrane permeabilization, membrane lipid peroxidation, protein alteration, and denaturation of nucleic acids, ultimately leading to cell death (Oussou-Azo *et al*., 2020). The antifungal behavior of MgONPs includes: injuring the cell plasmalemma originating from the direct physical interaction and affecting energy metabolism and cell membrane potential (Cai *et al*., 2018a), vacuolation and disorganized enterocytes (Chen *et al*., 2020). In addition, Mg is an important element of structural tissues, such as lignin, suberin, and the middle lamella, together with Ca, which makes some pectic substances more resistant to degradation by pectolytic enzymes of various bacterial and fungal pathogens (Huber and Jones, 2013). The different inhibitory mechanisms of MgONPs are discussed in details in the very recent study of Saied *et al*. (2021).

The results of field trials revealed that under treatments with CuONPs and MgONPs, all concentrations significantly enhanced potato yield compared to control. These results are supported by previous studies, which reported that Cu plays an important role in plant growth and development, as well as productivity (Xue *et al*., 2014 and Ngo *et al*., 2014). Interestingly, Elmer and White (2016) reported that CuONPs was the best among other six metallic oxide nanoparticles (ZnO, TiO, AlO, FeO, NiO, or MnO) in their efficiency to improve the growth of tomato and eggplants grown in soil infested with *Verticillium dahliae* and *F. oxysporum* f.sp. *lycopersici*, respectively. Conversely, Sarkar *et al*. (2020) reported that CuONPs at higher concentration (0.05 mg/mL^{-1}) caused a significant reduction of seeds vigor of *Lens culinaris* compared to control, indicating its toxic effects to the plants. Additionally, Nair and Chung (2014) stated that the higher concentration of CuONPs reduced the shoot growth, weight, and total chlorophyll content in soybean. On the other hand, Chen *et al*. (2020) demonstrated that there was a significant improvement in wheat root growth and grain yield after foliar MgONPs application under greenhouse conditions. Moreover, Cai *et al*. (2018b) found that MgONPs enhanced tobacco growth under greenhouse conditions. Additionally, Raliya *et al*. (2014) demonstrated that biosynthesized MgONPs improved shoot–root growth (18.2 to 49.2%) and chlorophyll photosynthetic pigment (76.1%) in clusterbean (*Cyamopsis tetragonoloba*). Based on the previous published results, it was not surprising that MgONPs and CuONPs displayed a positive effect on the growth of potato indicated by number and biomass of potato tuber in both field trials.

Enzyme activity of PO and PPO was estimated in potato leaves in response to treatments with CuONPs and MgONPs. All tested NPs exhibited a noticeable tendency of an increase in PO and PPO after 20 days of planting, but to a limited extent. The trend of our results was supported by previous studies, which reported that applied copper-based NPs compounds increased the antioxidant systems, including superoxide dismutase and PO activities and total antioxidant levels (Regier *et al*., 2015 and Singh *et al*., 2017). A similar kind of results was observed by El-Shewy *et al*. (2019) where potato plants treated with CuONPs exhibited a noticeable increase in PO and PPO enzymes. Furthermore, Nair and Chung (2014) reported that the CuONPs increased the PPO and lignin contents in soybean plants. This increment was in a concentration-dependent manner, being the higher concentrations induced higher enzyme activity. A similar kind of increase in the defense related enzymes was observed by Sarkar *et al*. (2020) who demonstrated that catalase, ascorbate

peroxidase, PPO and superoxide dismutase activities have also steadily increased in tobacco plants by increasing concentration of CuONPs. Contrarywise, Yang *et al*. (2020) under hydroponic conditions found out that activity of both catalase and superoxide dismutase was decreased in rice leaves treated with CuONPs at the concentration of 250 mg/L. Our results showed that MgONPs displayed a trend similar to that observed for CuONPs treatment, which was concentration dependent. Furthermore, the higher concentration 200 mg/L of MgONPs induced higher activity in both PO and PPO enzymes. Likewise, Cai *et al*. (2018b) found out that the higher concentration 250 µg/mL of MgONPs enhanced the activity of the superoxide dismutase and PO enzymes. While, the low concentrations of 50 and 150 µg/mL revealed lower activity. By contrast, Ramadan *et al*. (2020) stated that a higher concentration 40 mg L^{-1} of MgONPs, exhibited low activity of PO in soybean plants. El-Argawy *et al*. (2017) reported also that MgONPs had a positive effect on the activity of PPO in sugar beet plants. The increased production of the antioxidant molecule under the influence of NPs confirms the regulation of antioxidant system as a response to NPs interaction with plant (Da Costa and Sharma, 2016).

Chlorophylls have an important role in photosynthesis system, which highly correlate with plant biomass and recover and productivity (Karamanos *et al*., 2004 and Regier *et al*., 2015). In this regard, chlorophyll and phenol contents were estimated in potato plants in responses to treatments with CuONPs and MgONPs. Only MgONPs, when compared to CuONPs and control treatments that resulted in a distinct increase in total chlorophyll in potato plants after 20 and 40 days of planting. Consistent with these results, Jhansi *et al*. (2017) indicated that soaking of peanut seeds in a 500 µg/mL MgONP for 12 h enhanced the chlorophyll content in plant leaves. Moreover, after 30 days of treatment, Cai *et al*. (2018b) demonstrated that MgONPs increased chlorophyll a and b contents in tobacco plants. Additionally, Raliya *et al*. (2014) demonstrated that MgONPs improved shoot–root growth with values reached 18.2 to 49.2% and chlorophyll photosynthetic pigment (76.1%) in clusterbean (*Cyamopsis tetragonoloba*). Treatment with CuONPs at all concentrations exhibited also positive impact on the chlorophyll content when compared to untreated control. However, Yang *et al*. (2020) demonstrated the contrary, in which chlorophyll *a* and chlorophyll *b* and carotenoid content in rice leaves were decreased after 7 days of exposure to CuONP. Also, Pontes *et al*. (2020) indicated that CuONPs negatively affected the chlorophyll *a* content in duckweed leaves. Moreover, the results of Santos *et al*. (2021) confirmed the negative impact of CuONPs on the maximum emission of chlorophyll a, which was concentration-dependent and inhibited root growth of *Sesbania virgata* plants.

According to Biswas *et al*. (2012) phenols are involved in disease resistance in many ways such as lignification of cell walls, hypersensitive cell death, etc. In this concern, highest phenol content was recorded with treatment of MgONPs at 200 mg/L, which showed decrease trend with decreasing concentration. However, Ramadan *et al*. (2020) documented that the highest phenol content was obtained by lower concentration 20 ppm of MgONPs. Abdel-Wahab *et al*. (2019) demonstrated that lower concentrations 50, 100 and 150 mg/L of CuONPs increased phenolic compounds in callus cells of *Solanum nigrum*, while the higher concentration of 200 mg/L significantly decreased it. A similar kind of findings was observed by Sarkar *et al*. (2020) who indicated that the optimum concentration of CuONPs was found, 0.025 mg/mL^{-1}, which showed higher production of phenol and flavonoid. Contrarily, our results indicated that the concentration of 200 mg/L of CuONPs increased the phenol content in potato leaves when compared to lower concentrations and control.

Energy Dispersive X-ray (EDX) could be necessary for a positive detection of the nanomaterials in tissues and cells (De Jong *et al*., 2010). This study indicated the translocation of NPs from treated mother tubers to aerial parts after plantation and accumulated in daughter tubers. Our finding is supported by previous work of Rico *et al*. (2011) and Tripathi *et al*. (2017) who reported that NPs could transfer through roots to plant shoot and seeds. The accumulation of NPs in edible plant tissues is a critical issue that could impact human health via the food chain. In this regard, the study of Barakat *et al*. (2019) revealed that CuONPs caused DNA damage and histopathological alteration in the brain and the lungs of adult male albino rats. Previous studies have reported also that TiO_2 NPs and FeNPs caused lung damage and cytotoxic and genotoxic effects in rats (Gonzalez *et al*., 2008; Fraser *et al*., 2011 and Mohamed and Hussien, 2016). Furthermore, TiO_2 NPs and fullerene have been shown to cause brain damage in fish and dogs (Shaw and

Handy, 2011). Therefore, the application of NPs requires further interdisciplinary research work by involving scientists, medical researchers, toxicologists and environmental engineers.

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