Antifungal Potent of Some Metallic Nanoparticles against *Sclerotinia Sclerotiorum* on Common Bean Plants: An Emphasis for Biochemical Alterations and Metal Accumulation

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Abstract

Antifungal efficacy for some oxides of metallic nanoparticles (NPs) e.g. magnesium (MgO), copper (CuO), silicon (SiO$_2$) and zinc (ZnO) was evaluated against fungi, *Sclerotinia sclerotioum* on bean plants under different conditions. The examined NPs exhibited significant effect on hyphal morphology and fungal linear growth under field trail in the following order: MgONPs> SiO$_2$NPs> ZnONPs> CuONPs compared with control group. However under storage condition, the disease severity along NPs-treated bean pods were in the order: MgONPs> SiO2NPs> ZnONPs> CuONPs compared with infected control which did not exceed 30.23% and non-infected (14.68%). Bean pods treated with NPs showed significantly increase in chlorophyll content, total phenols, and ascorbic acid compared with non-infected pods during storage period for 4 weeks. The examined NPs exhibited positive accumulation in pods tissues, except MgO was lower than non-infected group. The present findings may display the potential effect metal oxides in agricultural sector need more studies to achieve their adverse effects on consumers and environmental impacts.

Keywords: Metallic nanoparticles; Fungi; Biochemical alterations; Sclerotinia sclerotioum; Bean plants.

1. Introduction

The plants are frequently infected by different pathogenic fungal, bacterial and viral which result in significant loss to crops [1]. Widespread traditional methods have been applied to control of these pathogens. Today, lot of chemical compounds has been found to induce non-desirable effects to the humans and environment. Most fungal pathogens have generated resistance to many conventional fungicides [2]. In recent years, engineering nano-materials (ENMs) have been practically increased, attributed to their physico-chemical characteristics, which significantly differ than their bulk forms [3]. They range between 10 to 100 nm. In most cases; they have the potential to be directly applied on plant seeds, foliage, or roots against pest and pathogens. Metallic nanoparticle (NPs) *e.g.* silver (AgNPs), CuONPs, ZnONPs and titanium dioxide (TiO$_2$) have been intensively examined against bacterial and fungi [4-6]. It is important to achieve new antifungal agents instead of current control strategies. A new trend focuses on applying nano-technology in pest control. For example, some metallic NPs of SiO$_2$, MgO, and ZnO are able to destroy pathogenic fungi and inhibit their released toxins [7]. Another investigation demonstrated that, MgO was potential inhibitor to growth of fungi; *Fusarium* and *Aspergillus flavus* species [8]. Nanoparticles of ZnO affected on growth rate of fungi; *Botrytis cinerea* through cellular functions alteration, end to deformation in mycelia mats. Also, it had inhibitory effects on fungal growth for each conidiophores and conidia of *Penicillium expansum* arising death of fungal mats [9]. Different concentrations of ZnO and MgONPs induced remarkable inhibition of spore's germination of *A. alternate*, *F. oxysporum*, *R. stolonifer* and *Mucor plumbeus* [10]. Dimkpa, *et al.* [11], demonstrated the potential effect toxic of ZnONPs on wheat pathogen, *F. graminearum* in liquid or solid medium sand matrix. Nanomaterials, chitosan-based copper have been used as antifungal, antibacterial as well as plant growth promoting agents [12-15]. Common bean is considered as one of the greatest vital leguminous crops cultivated in Egypt, where the seeds and pods are rich in calcium, some vitamins, proteins, mineral salts, some amino acids, especially lysine. It is able to grow in the moderate regions [16]. Bean (*Phaseolus vulgaris* L.) is very sensitive to postharvest fungal infections. White mold disease in bean is caused by fungi, *Sclerotinia sclerotiorum*, resulting in significant decrease in produced crop [17-19]. Moreover, *S. sclerotiorum* causes devastating soft rot and white mold diseases for a large number of vegetable and non-gramineous field crops. Due to its wide host range and ability to survive many years as sclerotia, the control of this disease is particularly difficult [20]. Our study aims to 1) assess the antifungal potent of some metallic NPs against *S. sclerotiorum* on common bean plants under different conditions. 2) To assay the biochemical alterations and metal accumulation in edible part after storage condition.

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2. Experimental Design

2.1. Metallic Nanoparticles

Nanoparticles of MgO, CuO, SiO$_2$ and ZnO were supplied by Nano Lab., Dream land-6th October city, Egypt. They were employed to characterize on Scanning Electron Microscopy (SEM) (JOEL, JSM 5300) with high resolution at an accelerating voltage of 120 Kev. An aliquot of each powder was coated on a copper grid and scanned for its size and shape. On the other hand, they were suspended in 1% citric acid solution and subjected on Zeta sizer instrument (Malvern Ver. 6.20, Serial No. MAL 1054905, UK) at 25 $^{\circ}$C, count rate 202.1 and scan duration 70 sec.

2.2. Isolates of Fungi

An isolate of *S. sclerotiorum* was obtained from infected bean plants, where small pieces of infected samples (0.5 cm length) were washed by distilled water, dried on filter papers and sterilized by 2% sodium hypochlorite for 2 min. The selected pieces were then distributed on potato dextrose agar (PDA) media in Petri dishes (9 cm diameter). The treatments were incubated at 20±2 $^{\circ}$C for 7 days. The growing fungi were purified by hyphal tip transfer method, and identified according to the method of Gilman [21].

2.3. Evaluate Antifungal Activity

2.3.1. On Culture Media

Solutions of metallic NPs were mixed with PDA media at levels; 50,100, 200, 400, 600, 600, 800, 1000, 1200 and 1400 ppm as well as control without treatment. A disc with diameter (0.5 cm) for PDA culture (7 days-old) was placed at the center of each petri dish and incubated at 20±2 $^{\circ}$C (3 replicates for each). Fungal growth inhibition was calculated according to formula:

Inhibition (%) = (dc-dt)/dc x 100.

Where, dc is the average diameter of linear growth in control, and dt is the average diameter of linear growth in treatment. In addition, total number and dry weight of *sclerotia* per dish were evaluated.

2.3.2. Under Storage Conditions

Two concentrations for each metal oxide in triple number were used as follows: SiO$_2$ (400 and 600 ppm), MgO (100 and 200 ppm), ZnO and CuO (1200 and1400 ppm), respectively, to evaluate their antimicrobial efficacy during storage periods. Hundred g of Master bean was mixed with each concentration during 1 min, air dried at room temperature and placed in plastic boxes (60×40×30 cm), respectively. Then, they were inoculated with 5 ml of fungal spore suspension. The treatments were adjusted at 10±1 $^{\circ}$C and 90±5% relative humidity (RH). The rotten pods of bean were calculated and the disease severity was calculated using a disease index (5 degree) as follows: 0= no rot, 1=1-15% of rotted, 2=16-30% bean, 3=3-60% of and 4=61-100% of bean in rotted pods, respectively, according to method of Kobriger and Hagedorn [22].

Disease severity (%) =Sum (severity class × No. roots in class)/ (Total No. of roots × highest class No.)× 100

2.3.3. Pots Trail

The trials were conducted in the greenhouse using growing bean (Giza-3), plants cultivated in pots sterilized soil (25 cm diameter). The obtained isolates were grown on barley grain media in conical flasks for 10 days and separately as a source of inoculum. Inocula of tested isolates were applied at a rate of 5 % of the soil weight [23]. The treated soil was irrigated and allowed to 7 days before cultivation (Giza-3). Seeds were disinfested by dipping in NPs solutions as described above for 2 min, before cultivation. Four pots were used for each concentration and examined for basal stalk rot at 15, 20 and 25 days after cultivation.

2.4. Disease Assessment

Data of basal stalk rot severity were assessed at 15, 20 and 25 days of inoculation using an arbitrary 0-5, where: 0 = no visible symptoms, 1 = 1–25 %, 2 = 26 –50 %, 3 = 51 –75 %, 4 = 76–100 % of the basal stalk rot area, and 5 = dead plants, respectively. For each replicate rot severity was calculated according to Liu, *et al.* [24] as follows:

Basal stalk rot severity $= \frac{\Sigma dxnx}{dmax \; x \; n}$ x100

Where: d is the disease rating on each leaf or fruit, d$_{max}$ is maximum disease rating possible and n is the total number of leaves examined.

2.5. Postharvest Quality

2.5.1. % of Loss

The collected bean fruits were weighted after postharvest treatment and during storage periods; 7, 14, 21, and 28 days, respectively. The values were estimated as percentage of weight loss independent on the initial weight.

2.5.2. Pathogenicity Test

Surface sterilized snap bean pods (50 g for each) were separately placed in polyethylene container. Each one contains snap bean which inoculated with 3 discs of isolates 1 and 2 (5 mm diameter) from fungal culture on PDA of 5 days old [25].

$$severity \% = \frac{weight\ of\ diseased\ pods}{weight\ of\ the\ treatment} \times 100$$

2.6. Biochemical Quantifications

2.6.1. Phenolic Content

Level of phenol was determined as described by Singleton, *et al.* [26] with slight modifications. One hundred µl of bean extract was mixed with 6.0 ml of distilled water, followed by 0.5 ml of Folin-Ciocaltent reagent. The solutions were mixed and incubated at ambient temperature for 3 min. Then, 1.5 ml of 20% Na_2CO_3 was supplemented, the volume was remarked to 0 ml with distilled water and incubated at 25 $^\circ$C for 2 hr. The developed color was measured at 760 nm. Phenolic content was calculated using gallic acid as a standard. Phenol content was estimated as mg gallic acid per g mass.

2.6.2. Ascorbic Acid Assay

Bean pods were blended with a high speedy warring blender for 3 min. Five g of each sample was mixed with 45 ml of 0.4% oxalic acid and then filtered through filter paper. An aliquot (1 ml) of extract was mixed with 9 ml of reagent; 2, 6-dichlorophenolindo-phenol sodium salt, and the developed color was measured at 520 nm. Blank consisted of 1 ml filtrate and 9 ml of distilled water. The level of acid was estimated as mg per 100 g mass [27].

2.6.3. Total Chlorophyll Assay

Total chlorophyll content of bean was assayed by using spectrophotometric method [28]. One g of blended bean was homogenized with 10 ml chloroform: methanol and filtered on Whatman paper. Filtered samples were supplemented with chloroform: methanol to final volume (25 ml). Total chlorophyll was measured on UV-VIS spectrophotometer instrument at 663 and 645 nm against the blank. The pigment was estimated by the following formula.

Chlorophyll content $= 8.02 \times (A_{663}) + 202 \times (A_{645})$

2.7. Metal Accumulation in Pods

The treated pods were dried at 70 $^\circ$C until ash. Then, 1 g of each was digested in HNO_3 plus 5 drops of H_2O_2 under ultrasonic radiation at 40 $^\circ$C. The cleared solution was employed for cooling, dilution with deionized water and filtration to remarkable volume. The examined metals were quantified on Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES) instrument (Optima 7000 Perkin Elmer, USA). The samples were injected into the cyclonic spray chamber with mass flow-controlled laser nebulizer with gas flow at rate 0.65 L min^{-1}. The instrument was operated in a fast-sequential mode and featured to cooled CCD detector. Background and spectral interferences could be simply corrected and accurately using Agilent's MP Expert software.

2.8. Microscopic Investigation

Scanning Electron microscope (SEM) was used to examine morphological changes of NPs-treated fungi, *S. sclerotiorum* in comparing with control. Small parts of mycelia material cut from 7-day-old cultures were inoculated onto the PDA containing 400, 100, 1200 and 1200 of SiO_2NPs, MgONPs, ZnONPs and CuONPs, respectively, compared with control, and incubated for 7 days. Then, parts of mycelia were cut from the edge of the fungal cultures, and directly employed to electron microscopic observation. SEM images were taken at different magnification scales as required.

2.9. Data Analysis

The data were employed for the analysis by variance (ANOVA) and resented as mean±SE. The values were compared to significance by least significant difference (LSD) at the probability of 0.05 [29].

3. Results

3.1. Nanoparticles Characterization

Examined NPs exhibited characteristic spherical shape with size in ranges; 20-37, 20-40, 22-40 and 20-33 for MgO, ZnO, SiO_2 and CuO, respectively, as documented in SEM images (Figure 1a, b, c and d). Zeta sizer patterns for dispersion of particles in its solutions were achieved as plotting in Figures 1a, 1b, 1c, and 1d displaying the range of suspended particles; 10-40, 10-60, 10-12, and 10-50 nm, respectively, for the same types of NPs as described above.

Figure-1. Characterization of NPs, SEM image of NPs visualized at 35.000x for (a) MgO, (b) ZnO (c) SiO$_2$ and (dCuO, Zeta sizer patterns of (a1) Mg (b1) Zn (c1) SiO$_2$ and (d1) CuO nanoparticles respectively in prepared solutions
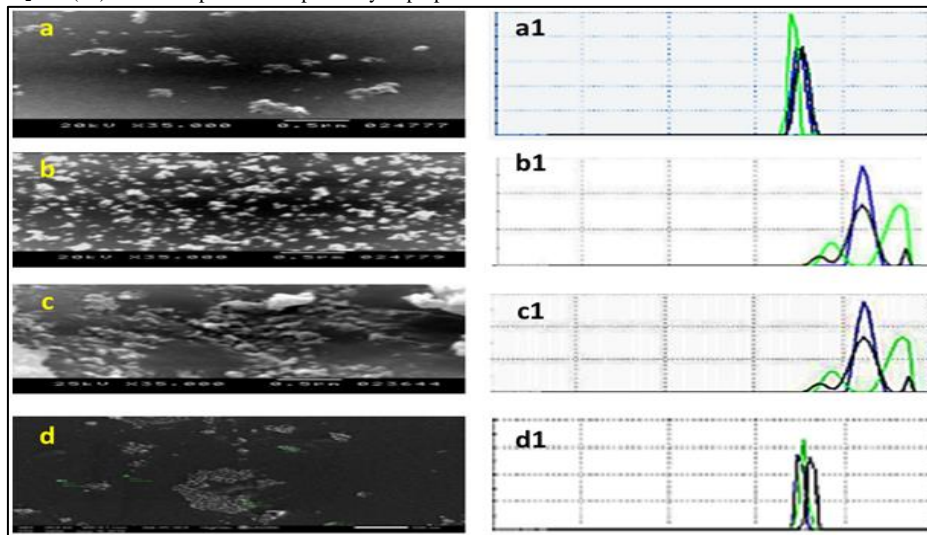
3.2. Pathogenicity Trails

Infectivity of *S. sclerotiorum* to bean pods cv. Paulista varied with different isolates (Table 1). All tested isolates were able to infect bean pods causing white mould under storage conditions. However, isolate I3 was the most pathogenic isolate expressed by the pods rotted which resulted from artificial inoculation, followed by isolates I2 and I1, respectively.

Table-1. Pathogenicity test of three different fungi isolates of *S. sclerotiorum* to bean pods cv. Paulista

| Number of isolates	Disease severity %				
	St$_1$	St$_2$	St$_3$	St$_4$	LSD5%
I1	10.00	15.00	25.00	36.00	5.38
I2	17.00	21.00	34.00	40.00	5.05
I3	20.00	25.00	37.00	45.00	5.31
LSD 5%	3.20	3.14	3.89	2.81	-

3.3. Alteration in Growth Rate

The potential effects of examined NPs on fungal linear growth percentage are illustrated in Figure 2. SiO$_2$NPs induced inhibitory effect at concentration 200 ppm (77.36 %), followed by 400 ppm (67.76 %). MgONPs exhibited inhibitory effect at concentration of 100 ppm (16.66%), followed by complete inhibition at concentration of 200 ppm (0.0 %). ZnONPs exhibited inhibitory effect at concentration of 1000 ppm (73.13%), followed by 1200 ppm (17.23%). Finally, CuONPs exhibited inhibitory effect at concentration 1000 of ppm (74.80%), followed by 1200 ppm (19.06%). The potential effects of examined NPs were in the order as follows: MgONPs> SiO$_2$NPs> ZnONPs> CuONPs.

Figure-2. Effects of NPs on fungal linear growth of S. sclerotiorum treated with (a) MgO, (b) SiO$_2$ (c) ZnO and (d) CuO nanoparticles respectively. Each value is the mean of three replicates. The letters indicate no Significant differences at 0.05
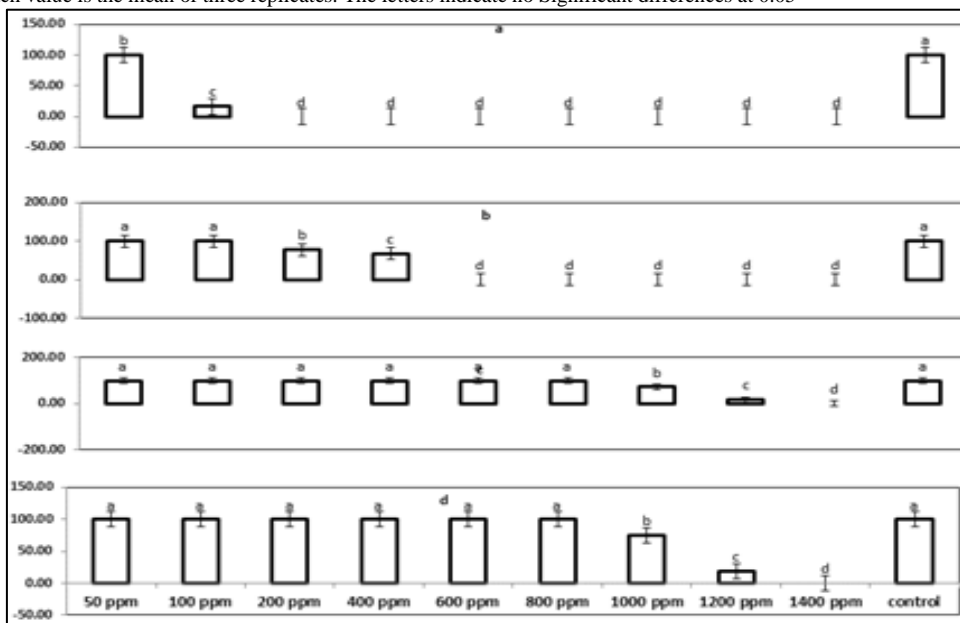
The effects of examined NPs on hyphal morphology were investigated by scanning electron microscope (SEM) after 24 hr of incubation (Figure 3). Interpretation of scanned images achieved that, control samples showed typical net structure and regular and smooth surface *S. sclerotiorum*. (Figure 3a) However, treatment with SiO_2 and CuONPs (Figure 3b, c) led to distracted hyphae with irregular and adsorbed shape. Some hyphae were wrinkly and depressed. MgONPs (Figure 3d) induced deformation and lysis of fungal hyphal, but some unusual on the surface of fungal hyphae were observed in case ZnONPs treatment (Figure 3e).

Figure-3. SEM images of hyphal morphological patterns of S. sclerotiorum after 24 hr incubation with examined NPs compared with (a) control, (b) SiO2, (c) CuO, (d) MgO, and (e) ZnO, respectively
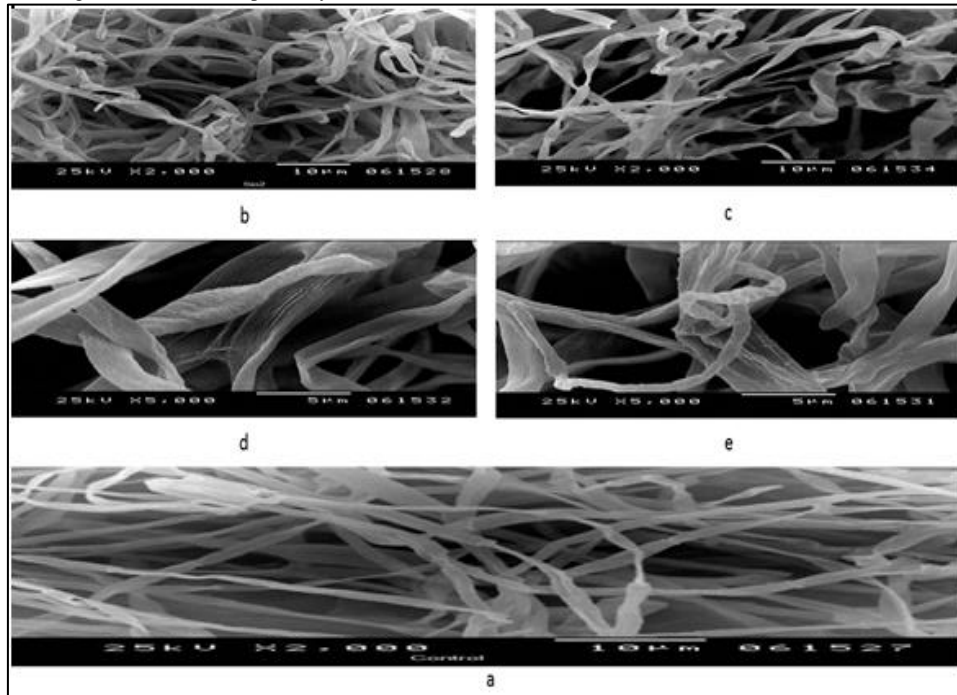
The effects of NPs on number and weight of sclerotia are illustrated in Figure 4. MgONPs exhibited the greatest potential effect on number of sclerotia plate^{-1} with mean values 16.30 and 2.66 at concentrations; 50 and 100 ppm, followed by complete inhibition for other concentrations. The lowest potential effect on number of sclerotia plate^{-1} was recorded for CuONPs with the following order: 21.33, 20.33, 18.66, 13.66, 7.33, 6.33, 5.33 and 2.66 at concentrations: 50, 100, 200, 400, 600, 800, 100, and 1200 ppm, respectively. The potential effects of examined NPs were in the following order: MgONPs> SiO_2NPs> ZnONPs> CuONPs.

Regarding weight of Sclerotia (g) plat^{-1}, ZnONPs exhibited the greatest effects; 0.17, 0.15, 0.13, 0.12, 0.10, and 0.03 g at concentrations; 50, 100, 200, 400, 600, and 800 ppm, respectively. CuONPs exhibited the least effects; 0.38, 0.31, 0.28, 0.17, 0.16, 0.13, 0.05, and 0.02 g at concentrations; 50, 100, 200, 400, 600, 800, 1000 and 1200 ppm, respectively. The potential effects of examined NPs were in the following order: ZnONPs> MgONPs> SiO_2NPs> CuONPs (Figure 5).

Figure-4. The effects of NPs on Number of sclerotia of fungi *S. sclerotiorum* during for (a) SiO2, (b)MgO (c) ZnO, (d) CuO, respectively Each value Is the mean$\pm$SE.The same letters indicate no significant different at 0.05 Levels
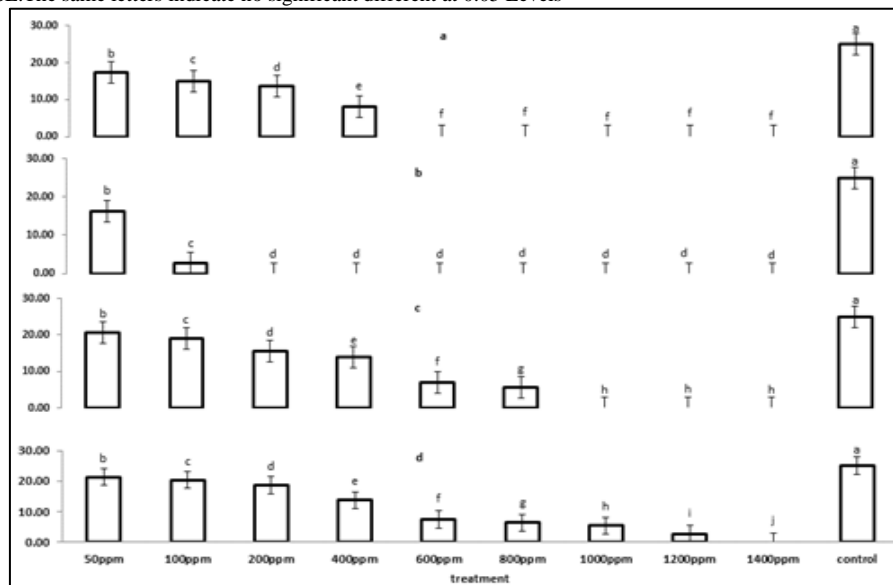
Figure-5. The effects of NPs on weight sclerotia [sclerotia (g) [plat^{-1}] in fungi, *S.Sclerotiroum* bean pods exposed to (a) SiO2, (b) MgO, (c) ZnO, and (d) CuO, respectively. Each value is the mean±SE. The same letters indicate no significant different at 0.05 levels
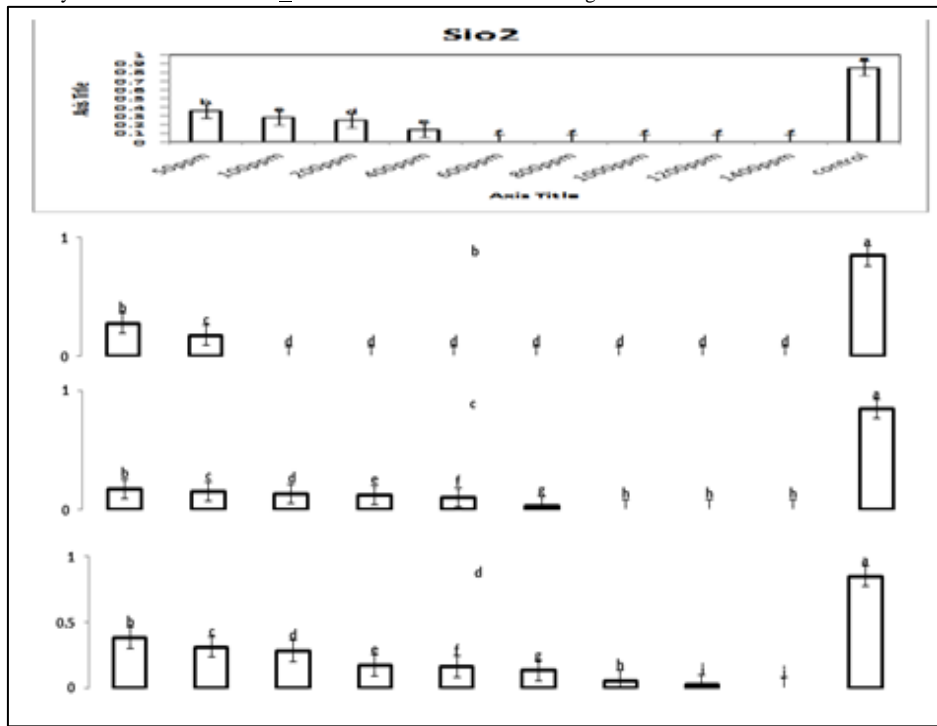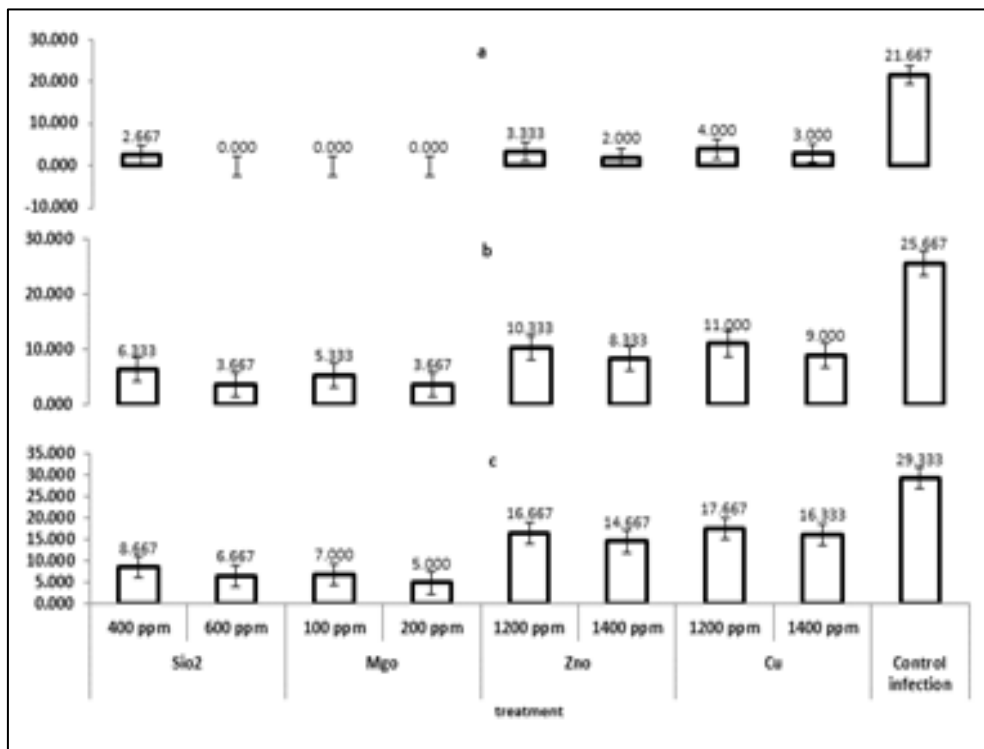

3.4. Disease Severity

The efficacies of examined NPs to reduced fungal growth or infection in treated pots are illustrated in Figure 6. MgONPs was the most potent to reduce disease severity as recorded for concentration 100 ppm; 0.00, 5.33, 7.00% and 200 ppm; 0.00, 3.67, 5.00%, after 15, 20 and 25 day, respectively. CuONPs was the least potent as recorded for 1200 ppm, 4.00, 11.00, 17.66% and140 ppm; 3.00, 9.00, 16.33% after the same periods. The potential effects of NPs to reduce disease severity were as follows: MgONPs> SiO$_2$NPs> ZnONPs> CuONPs compared with control which did not exceed 25.55%.

Figure-6. Disease severity of fungi, *S. sclerotiorum* on infected bean planted in pots after reatments withs NPs for (a) 15, (b) 20, and (c) 25 day, respectively

Regarding storage condition, the disease severities concern NPs treated bean pods are illustrated in Figure 7. MgONPs exhibited the greatest efficacy at storage 4 stage (2.37%) at concentration 100 ppm, followed by SiO$_2$NPs (4.37%) at the same storage period of concentration 400 ppm. CuONPs was the least potent to reduce disease severity with mean values 5.28 and 3.73% at concentrations; 1200 and 1400 ppm. The efficacies of NPs were in the

order: MgONPs> SiO₂NPs> ZnONPs> CuONPs compared with infected control which did not exceed 30.24% and non-infected was (14.68%).

Figure-7. Disease severity % concern NPs-treated bean pods under storage conditions
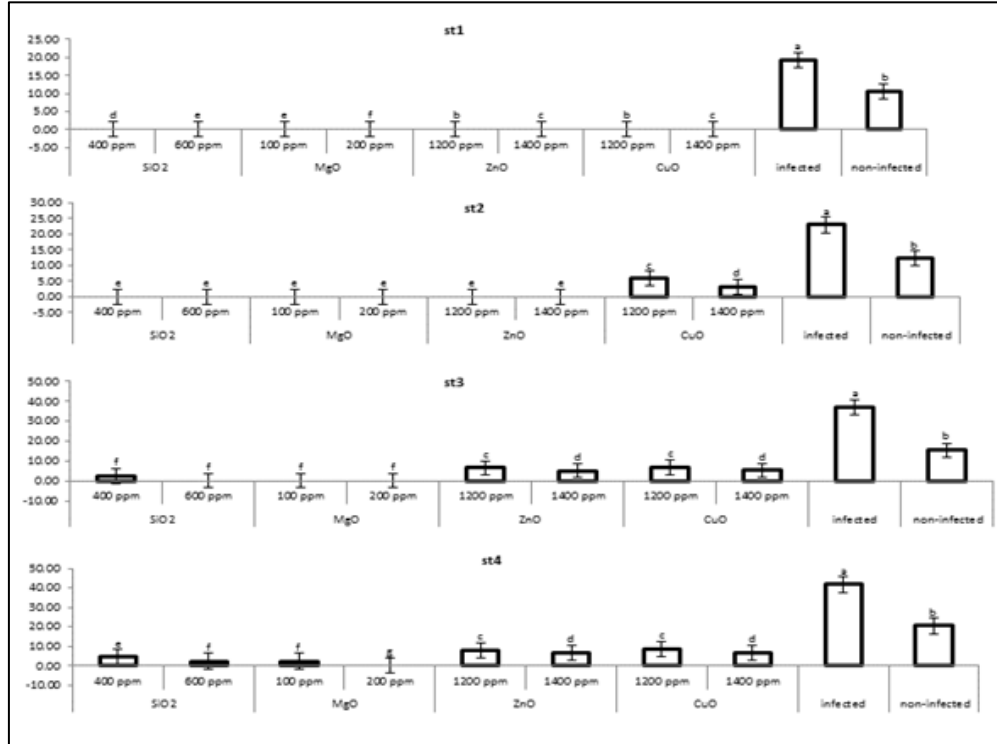
3.5. Pods Quality

The percentage loss of pods weight during storage for 4 weeks is illustrated in Figure 8. CuONPs was the most potent to increase % of loss with mean values 43.05 and 38.59% at concentrations; 1200 and 1400 ppm, followed by ZnONPs (39.77 and 35.93 %) for the same concentrations. MgONPs was the most potent to decrease % of loss of pods during storage as follows: 2.30, 11.81, 24.28, 33.31 % and 0.65, 8.70, 39.93, 31.00 % after 1, 2, 3, and 4 weeks, respectively, for 100 and 200 ppm. The % of loss was in the following order: MgONPs< SiO₂< ZnONPs> CuONPs compared with infected control which did not exceed 53.91%.

Figure-8. The effect of NPs on loss of weight after bean pods storage (a) after 1 week (b) after 2 weeks, (c) after 3 weeks and (d) after 4 weeks respectively. Each value is the mean±SE. The same letters indicate no significant different at 0.05 levels
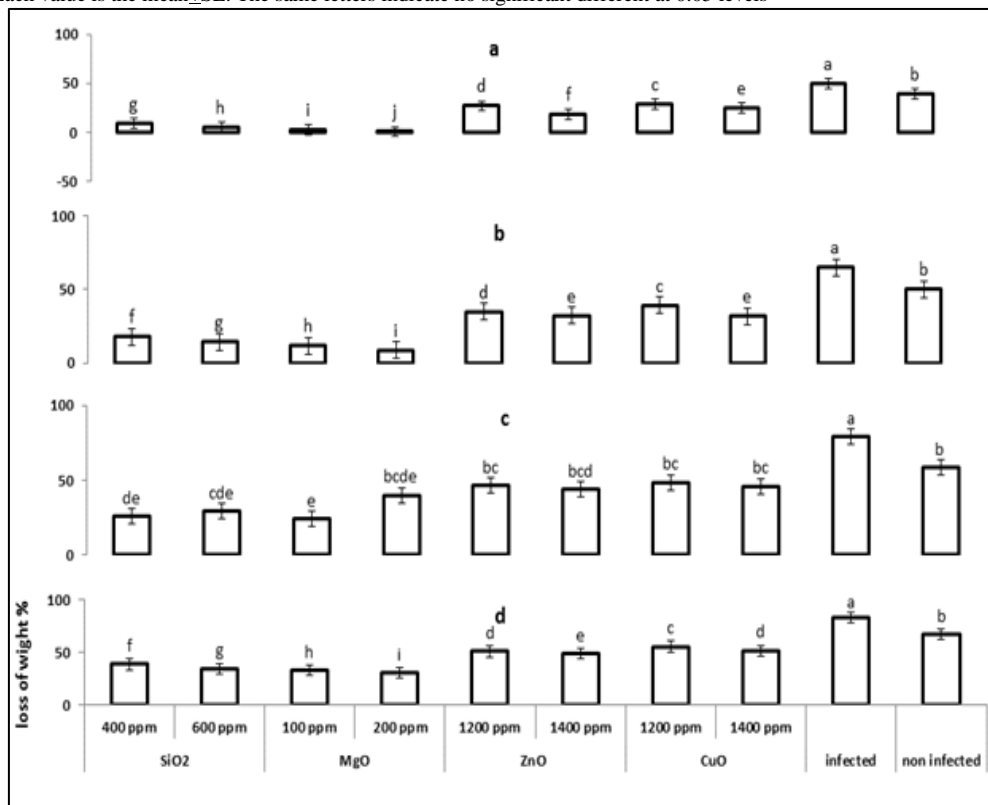
3.6. Biochemical Responses
3.6.1. Total Chlorophyll

Chlorophyll contents (mg g^{-1} mass) in treated pods are illustrated in Figure 9a. MgONPs exhibited the greatest increase in chlorophyll content; 8.88, 18.54, 8.31 and 8.11 mg g^{-1} mass during stages 1, 2, 3 and 4, respectively with mean value (8.46 mg g^{-1} mass; 100 ppm). At concentration (200 ppm), the values were 9.92, 8.82, 8.48 and 8.21 mg/g mass during the same periods. However, CuONPs exhibited the least increase in chlorophyll content; 3.65, 3.24, 2.66, and 2.45 mg g^{-1} mass with mean value (2.99 mg g^{-1} mass; 1200 ppm) at the same periods. The treatments displayed effects in the following order: MgONPs>SiO2NPs>ZnONPs> CuONPs> non- infected> infected with mean values; 8.66, 6.33, 4.59, 3.10, 1.92 and 0.93 mg g^{-1} mass, respectively.

3.6.2. Total Phenols Content

Phenols contents in pods were estimated as reflection to the effect of using different concentrations of NPs under infection by *S. sclerotiorum* during storage for 4 weeks (Figure 9b). MgONPs exhibited the greatest increasing 8.31, 2.50, 2.30 and 2.18 mg g^{-1} mass for periods1-4 weeks, respectively, with mean value (2.57 mg g^{-1} mass; 400 ppm). At concentration of 600 ppm, the values were 3.34, 2.91, 2.47 and 2.24 mg g^{-1} mass with mean value (2.74 mg g^{-1} mass; 600 ppm) during the same periods. However, CuONPs exhibited the last increase at concentrations, 1200 ppm (1.43, 1.38, 1.37 and1.35 mg g^{-1} mass) and 1400 ppm (1.51; 1.39, 1.38 and 1.36 mg g^{-1} mass) during the same periods. The treatments displayed increase of phenols content in the following order: MgONPs> SiO2NPs> ZnONPs> CuONPs> non-infected> infected with mean values; 5.02, 2.66, 1.80, 1.40, 1.27 and 1.06 mg g^{-1} mass, respectively.

3.6.3. Ascorbic Acid

All treatments exhibited increase in ascorbic acid levels (mg g^{-1} mass) compared with infected control (Figure 9c). MgONPs exhibited the greatest increase; 0.93, 0.87, 0.70 and 0.67 mg g^{-1} mass during storage periods 1-4 weeks, respectively, with mean value (0.79 mg g^{-1} mass). At concentration of 200 ppm, the values were 0.94, 0.91, 0.85 and 0.69 mg g^{-1} mass during the same periods with mean value (0.88 mg g^{-1} mass). However, CuONPs (1200 ppm) exhibited the least increase in acid content; 0.107, 0.107, 0.107 and 0.105 mg g^{-1} mass during the same period. The treatments induced increase in the following order: infected MgONPs> SiO2NPs> ZnONPs> CuONPs> non-infected> infected with mean values; 1.54, 0.82, 0.41, 0.17, 0.11 and 0.04 mg g^{-1} mass, respectively.

Figure-9a. Total Chlorophyll content (mg g^{-1} mass) in homogenate of stored bean pods treadted with different metallic nanoparticles during 4 weeks of four stages, Each value is mean of three replicates+SE. The same letters indicate no significant at 0.05 levels
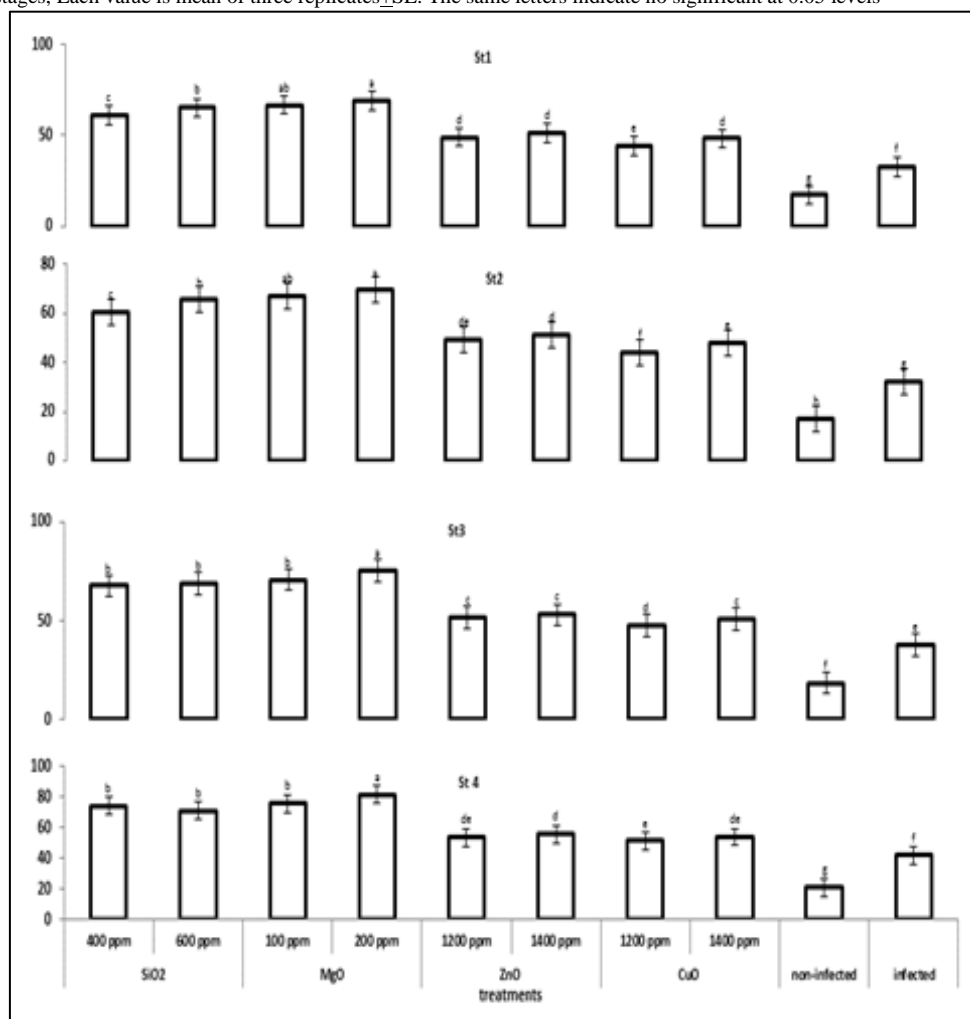
Figure-9b. Total phenol content (mg g⁻¹ mass) In homogenate of stored bean pods treadted with different metallic nanoparticles during 4 weeks of four stages. Each value is mean of three replicates+SE. The same letters indicate no significant differences at 0.05 levels

Figure-9c. Ascorbic acid level (mg 100 g⁻¹ mass) in homog of stored bean pods treated with different metallic nanoparticles during 4 weeks of four stages. Each value is mean of three replicates+SE. The same letters indicate no significant differences at 0.05 levels
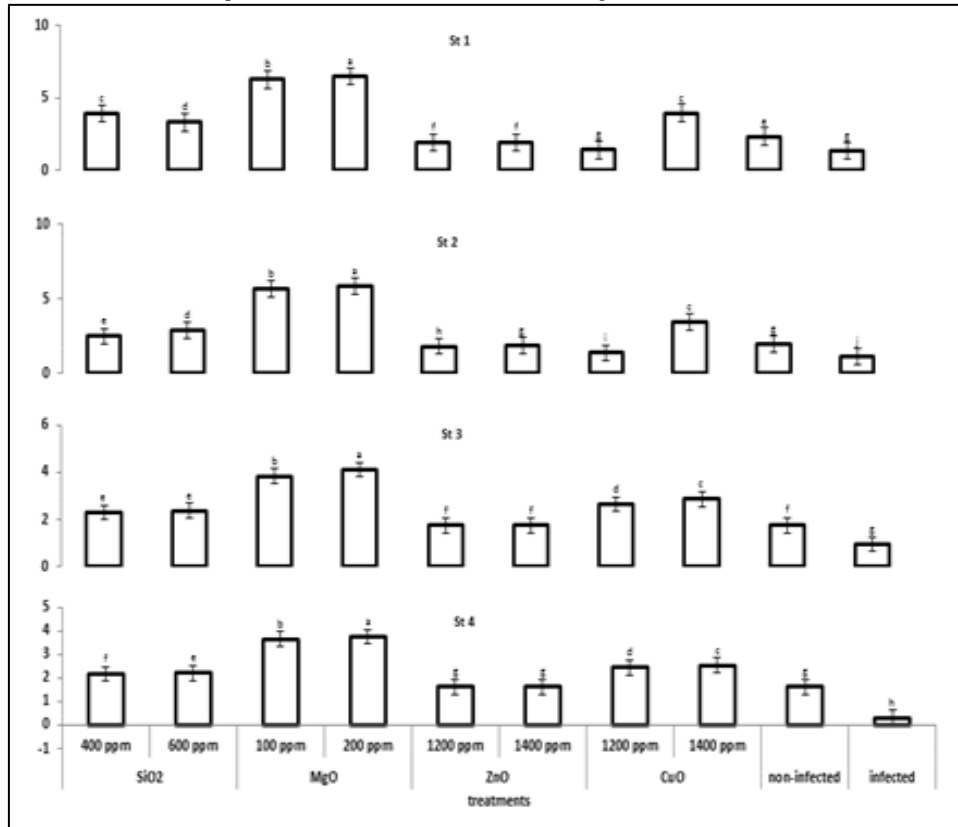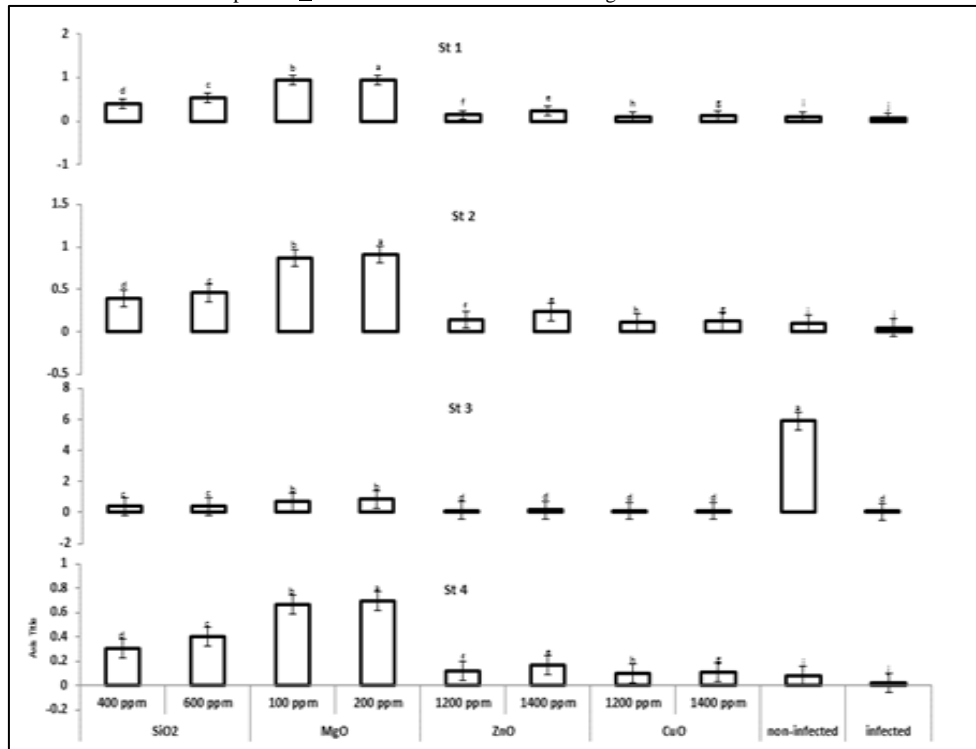

3.7. Metal Accumulation

The accumulation levels in stored pods are listed in Table 2. SiO$_2$NPs exhibited accumulation levels; 42.29 and 65.73 mg Kg⁻¹ dry w after treatments; 400 and 600 ppm, compared with non-infected control (15.50 mg Kg⁻¹ dry w). MgONPs (100 ppm) exhibited mean value (31.54 mg Kg⁻¹ mass) lower than control (883.35 mg/Kg dry w), but a concentration (600 ppm) exhibited accumulation value (907.29 mg Kg⁻¹ dry w). ZnONPs (1200 ppm) exhibited values, 128.50 mg/Kg mass compared with control (4.45 mg Kg⁻¹ dry w). Finally, CuONPs exhibited accumulation levels; 40.72 and 14.52 mg Kg⁻¹ dry w after treatment; 1200 and1400 ppm during storages, but non-infected group did not exceed 21.10 mg Kg⁻¹ dry w.

4. Discussion

The present findings may display the potential effect of some metallic NPs or fungi *S. sclerotoirum* on bean pods. The practices of these metal oxides in agricultural sector need more studies to achieve their adverse effects on consumers and environment impacts.

Table-2. The accumulation levels (mg Kg⁻¹ mass) of some metals in in homogenate of stored bean pods treated with different metallic nanoparticles during 4 weeks of four stages

Treatments (ppm)	Level (mg Kg⁻¹ mass)			
	S1	S2	S3	S4
SiO2(400)	78.07± 0.65 [ef]	74.17±0.59 [g]	16.92±0.22 [e]	0.00±0.00 [c]
SiO2 (600)	131.71±0.32 [de]	101.02±3.85 [f]	25.55±1.39 [e]	4.63±0.14 [c]
non-infected	11.24±0.82 [i]	7.16 ±1.73 [jk]	8.07±0.52 [e]	35.55±0.19 [c]
Infected	0.52±0.17 [i]	9.53±1.25 [jk]	0.62±0.21 [e]	13.24±0.11 [c]
MgO (100)	58.98±0.41 [fgh]	598.68±0.93 [a]	1148.22±17.42 [c]	720.27±197.70 [b]
MgO (200)	559.28±43.06 [a]	580.38±0.05 [b]	1216.79±0.98 [b]	1272.71±4.47 [a]
non-infected	68.66±0.47 [fg]	585.27±5.05 [b]	1414.50±26.76 [a]	1464.99±10.36 [a]
Infected	177.46±2.22 [fg]	563.31±5.71 [c]	700.78±7.98 [d]	1299.50±3.51 [a]
ZnO (1200)	223.44±0.27 [bc]	161.64±2.85 [e]	17.21±0.35 [e]	9.50±0.24 [c]
ZnO (1400)	253.07±0.84 [b]	240.61±2.06 [d]	12.83±0.15 [e]	7.48±0.67 [c]
non-infected	14.37±0.3 [hi]	1.82±0.24 [jk]	0.00±0.00 [e]	1.59±0.12 [c]
Infected	0.00±0.00 [i]	1.56±0.52 [k]	0.00±0.00 [e]	0.66±0.22 [c]
CuO (1200)	72.81±0.65 [f]	54.06±0.43 [h]	24.02±0.54 [e]	12.01±0.11 [c]
CuO (1400)	17.95±0.1 [ghi]	13.83±0.30 [j]	14.99±0.18 [e]	11.31±0.11 [c]
non-infected	3.91±0.88 [i]	39.13±0.33 [l]	26.88±0.14 [e]	14.49±0.07 [c]
Infected	2.04±0.68 [i]	5.27±0.09 [jk]	4.97±0.03 [e]	10.25±0.12 [c]

-The value is the mean of three replicates±SE. No significant differences are indicated for the same letters at 0.05 levels

The examined NPs displayed potential fungal effect against *S. sclerotoirum*. These findings are in accordance with that previously obtained [30-32], where NPs are in effective as nanocides against plant fungal pathogens as stated in species, mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*). Management of fungal diseases in plant nurseries is economically important and of environmental concern, to avoid the introduction and spread of diseases. Some diseases such as powdery mildew, needle casts and damping-off are among the most destructive foliar and soil-borne infections of forest tree seedlings [33]. It has been shown that nano-silver can cause significant reduction in seedling infection by *Fusarium culmorum*, an agent of damping-off [34]. However, an analysis of antioxidant enzyme activity characteristic of stress response indicated that, the toxicity of nano-silver treatment is comparable to damage caused by *Fusarium* treatment [35]**.** Qi*, et al.* [36]**,** demonstrated that, wood treated with copper-carbon core-shell nanoparticles is highly resistant to blue stain (*Ophiostoma minus*) and white rot (*Trametes versicolor*) fungi.

Many recent research studies have already demonstrated antimicrobial activities of various nanoparticles such as silver [37, 38], copper [39, 40], chitosan [41] and zinc oxide [42]. Copper oxide nanoparticles had a high activity against gram-positive bacteria, standard and clinical strains, including methicillin-resistant *Staphylococcus aureus*, comparable to silver nanoparticles and some antibiotics [43]. They also exhibited antifungal activity against *Candida* species. Also, the foliar applications of CuONPs limit growth of the oomycete *Phytopthora* [44]. Zinc oxide and oxide MgONPs at different concentrations brought about significant inhibition in the germination of spores of *A. alternata, F. oxysporum, R. stolonifera* and *Mucorplumbeus* [45]. In addition, it's have been proposed as an anti-microbial preservative for wood or food products [46-48]. ZnONPs toward plant pathogenic fungi including *Penicillium expansum* and *B. cinerea* was demonstrated by He*, et al.* [9]. Also, it was demonstrated against *Aspergillus* isolate [49]**.** Dimkpa*, et al.* [11]**,** reported that, ZnONPs are toxic to the wheat pathogen, *F. graminearum* both in medium and in a solid sand matrix. On the other hand, the combined copper-chitosan colloids were used as a new generation of copper-based bio-pesticides [50]. Chitosan-based copper nanomaterials have been used as antifungal, antibacterial as well as plant growth promoting agents [12-15].

Pilot studies showed that potential antibacterial effect of ZnONPs may be attributed to generated-free radicals on surfaces of NPs, and distractive of the lipids in cell membrane by these free radicals, which consequently lead to the leakage and breakdown of the cell membrane [51, 52]. However, the effect and mode of action of ZnONPs on the growth of fungi such as *F. oxysporum* and *P. expansum* have not been studied.

Studies in plants have demonstrated that, at least some NPs can be up-taken [53-56], transported [42, 57-59], and accumulated in specific subcellular locations such as cell vacuoles, nuclei and plasmodesmata [55, 60], and NPs can alter plant physiological processes, and influence plant growth and development [61-64]. The findings of this work show the impact of examined NPs on hyphal morphology. The alteration of hyphal cells was associated with the intake and behavior of NPs efficacy in fungal tissues after treatment. For example, ultra-small amounts TiO₂NPs have been shown to be able to enter into plant cells, accumulate in subcellular locations such as cell vacuoles and nuclei of root cells, and cause reorganization and elimination of microtubules, resulting in inhibition of root elongation in *Arabidopsis* [45, 55]. CuONPs have been shown to be able to transport in maize *via* xylem and phloem

[58]. Whereas, AgNPs and ZnONPs treatment lead to increase in contents of free radicals, including reactive oxygen and nitrogen species (ROS/RNS) and hydrogen peroxide (H_2O_2) in duckweed [65].

The cumulative use of metallic NPs highlights the need to study their toxicity and search for the potential plants that mitigate this environmental problem. It is known that, metallic NPs cause changes in cell metabolism, changing the intensity of biochemical reactions that have a huge effect on plant's resistance to various unfavorable conditions [66]. CuO and ZnONPs were shown to be among the most toxic metal nanoparticles and metal oxides [67]. It was noted that, the presence of NPs in aqueous solution may lead to generation of ROS, primarily hydroxyl radicals [68], which increase NPs toxicity.

The findings of this work illustrate significant effects of examined NPs on fungal infection. This concept is in accordance with that obtained by Shalaby, et al. [69], where the uptake of NPs on plants depends on many factors, such as the composition, concentration, size, the physical and chemical properties, and even the plant species under study. For example, a concentration of NPs above the optimal ranges of Zn, Cu, Ag, Ce, and Ti among others, produces stress and/or toxicity, generating ROS and resulting in the disruption of cellular metabolism. Under these conditions, plants produce antioxidant enzymes and non-enzymatic components that protect the cellular and subcellular system from ROS cytotoxic effects [70].

A significant induction of genes related to the responses to oxidative stress, sulfur assimilation, glutathione, and proline biosynthesis has also been shown under CuONPs stress [71], while the CuONPs (0–200 mg L^{-1}) applied to the leaves in cucumber plants significantly reduced the firmness of the fruit [72], The finding results obtain the increase of total phenol levels in pods. This concept is in agreement with that stated previously in literature. It also has been noted that, when applied to the substrate; CuONPs (0.006 mg L^{-1}) increased the total phenols and modified the concentration of the enzymatic and non-enzymatic compounds in tomato fruits [73]. Same findings were obtained in jalapeno peppers with CuONPs + Chitosan-polyvinyl alcohol (Cs-PVA) (0–10 mg L^{-1}) applied on the substrate [74]. In another finding concern the application of CuONPs, the production of antioxidant compounds [glutathione (GSH), vitamin C, and carotenoids] including antioxidant enzymes (ascorbate peroxidase; APx), superoxide dismutase (SOD), and catalase (CAT) are activated in plants to reduce oxidative stress caused by ROS [75].

5. Summary

The resent findings indicate the efficacy of metallic nanoparticles as antifungal agents against *S. sclerotoirum* under laboratory, storage and field conditions. Thus, they were able to destroy pathogenic fungi and inhibit their released toxins in pods during storage for 4 weeks and increased the intensity of some components i.g. chlorophyll content, total phenols and ascorbic acid. However, these agents need more studies to realize the undesirable changes and impacts on human and environment.

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