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The efficacy of Copper Oxide, Tri-calcium Phosphate and Silicon Dioxide Nanoparticles in Controlling Black Scurf Disease of Potato

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Abstract

Black scurf disease caused by *Rhizoctonia solani,* was a serious and common disease on potato worldwide. Effect of nanoparticles forms of Tri–calcium phosphate, copper oxide and silicon dioxide nanoparticle was evaluated at five concentrations to control black scurf disease in vitro and in vivo. Examination of treated *R. solani* with different tested nanoparticle was done using Transmission Electron Microscope (TEM). Activities of defense related enzyme were determined in artificially inoculated potato plants and tubers (cv. Spunta) with *R solani*. Physical characteristics of tested nanoparticles exhibit that all of them are spherical in shape and varied in their sizes. Also, all tested nanoparticles exhibited inhibitory effect of *R. solani*. Non-copper oxide was the most effective one (56.42 %) in suppressing the mycelial growth of *R. solani* at concentration 250 µl/L. The TEM examination of treated *R. solani* with different nanoparticles exhibit that instability they caused in cell wall thickness, abnormalities of nucleus and disappearance of nuclear membrane. All tested nanoparticles reduced of incidence and severity% of black scurf disease at concentrations 150 and 200 µl/L. during 2014 and 2015 seasons. Results cleared also that treating potato plants with different tested nanoparticles affected positively the activities of poly phenoloxidase, peroxidase, catalase and chitinase enzymes in leaves and tubers.

Key words: Potato, Black scurf disease, *Rhizoctonia solani*, Nanoparticles, Transmission Electron Microscopy (TEM), defense related enzymes.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth largest food crop worldwide after wheat, maize and rice (Kaguango *et al.* 2008; Haimdeldin and Hussien 2013). Potatoes play a significant role in human nutrition worldwide, where more than 320 million tons of potatoes are produced annually from 20 million hectares (Poczai *et al*. 2010; Sneyers 2010). Potatoes ranked after soybean for amount of protein/ha, with the major storage protein being patatin, one of the most known nutritionally balanced plant proteins (Liedl *et al.* 1987). About 150g of potato provides up to 45% of recommended daily allowance (RDA) for vitamin C, 10% vitamin B6, 8% niacin, 6% folate and other essential mineral nutrients (Patil *et al.* 2016). Egypt is one of 15 countries that export unprocessed raw potatoes with shipped dollar value, US$ 272.7 million during 2017 of (Workman, 2018).

Black scurf disease caused by *Rhizoctonia solani* (Telomorph*: Tanatephrus cucumeris*) *is one of the mostimportant fungal disease attacking potato* (Wilson *et al*. 2008; Rubayet *et al.* 2018). *Rhizoctonia solani* is wide spread fungus causing damage to many crops under wide range of temperatures from 10 - 24 ºC (Dorrance *et. al.* 2003). The plant pathogen *R. solani* is very difficult to control due to its persistent, long living sclerotial structures in soil (Zachow *et al.* 2011). Agriculture has been one of the most recent disciplines to join the nanotech race (Pérez-de-Luque & Hermosín, 2013). Several nanoparticles are being explored these days for their antimicrobial effects (Ren *et al*. 2009; Jia *et al*. 2012), which it can be beneficial or harmful, depending on the context. Nano-copper was reported to be highly effective in controlling bacterial diseases *i.e.* bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*) and leaf spot of mung bean (*X. campestris* pv. *phaseoli*) (Gogoi *et al.* 2009). Also, copper nanoparticles (CuNP) have been reported as effective antimicrobial in several studies (Cioffi *et al*. 2005; Ren *et al.* 2009; Jia *et al.* 2012). At low concentration, CuNPs promoted the growth of the plant pathogenic fungi; *Botrytis fabae*, *Fusarium oxysporum* f. sp. *ciceris*, *F. oxysporum* f.sp. *melonis*, *Alternaria alternata* and *Pseudomonas syringae*, and sporulation of *T. harzianum* (Banik and Perez-de-luque 2017).On the other hand, Nano sized silica-silver at concentration 10 µl/L revealed 100 % inhibition of mycelial growth of *Pythium ultimum, Magnaporthe grisea, Colletotrichum gloeosporioides, Botrytis cinere* and, *Rhizoctonia solani,* (Park *et al.* 2006). The Nano silica-treated plants showed higher expression of phenolic compounds and lower expression of stress-responsive enzymes against both fungi.

The present study aimed to evaluate the potential effect of Tri–calcium phosphate, copper oxide and silicion dioxide nanoparticles in controlling potato black scurf disease in vitro and in vivo. Also using the TEM as a good tool for clearing the effect of tested nanoparticles in controlling *R. solani* the causal of potato black scurf.

Materials and Methods

1- Isolation of potato black scurf Pathogen

Rhizoctonia solani was isolated on potato dextrose agar (PDA) medium from diseased potato tubers exhibiting typical symptoms of black scurf disease. Purification of the isolated fungi was done using the hyphal tip technique. The pathogen was identified as *R. solani* based on microscopic observation of morphological structures described by Sneh *et al.* (1991). *Rhizoctonia solani* was maintained on PDA in sterile disposable plastic Petri dishes at 25 ± 2 ˚C for further work.

2- Source of nanoparticles

Three materials i.e, Copper oxide, Tri-calcium phosphate and Silicon dioxide were obtained in form nanoparticles from NanoTech. Egypt for Photo-Electronics (Table .1).

Table 1. List of tested nanoparticles chemical structure and characteristics

Product name	Chemical structure	Solubility	Size average (nm)
Copper oxide	CuO NPs	Suspended in water	Less than 100
Tri-calcium phosphate	$Ca_3(PO_4)_2$ NPs	Dispersed in water	Less than 50
Silicon Dioxide	SiO_2 NPs	Dispersed in water or ethanol	Less than 50

3- Physical characteristics of tested nanoparticles

Images, crystal structure revelations and elemental analysis (qualitative and semi-quantitative analysis) were done of the three tested nanoparticales using High Resolution Transmission Electron Microscope (HR-TEM, Tecnai G20, FEI, Netherland). In this respect, Two different modes of imagine were employed; the bright field at electron accelerating voltage 200 kV using lanthanum hexaboride (LaB6) electron source gun and the diffraction pattern imaging. Eagle CCD camera with (4k*4k) image resolution was used to acquire and collect transmitted electron images. TEM Imaging & Analysis (TIA) software was used to spectrum acquisition and analysis of EDX peaks.

4- Effect of tested nanoparticles on growth of *R. solani* in vitro

Nanoparticles forms of Copper oxide, Tri-calcium phosphate and Silicon dioxide were evaluated in vitro against *R. solani* at five concentrations; 50, 100, 150, 200 and 250µl/L. The tested nanoparticles were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations then mixed gently. before solidification, the treated medium with nanoparticles were poured into 4 sterilized Petri dishes (9 cm diameter) per each treatment. After medium solidification, PDA plates were individually inoculated in the center with equal mycelial plugs (5-mm Ø) and incubated at 24 ± 2 ºC for 7 days. The inoculated PDA plates with only mycelial plugs served as control. The diameter of developed colonies was measured when fungus filled the control plate. The percentage of reduction in the colony diameter was calculated using the formula suggested by Sirirat *et al.* (2009) as follows:

$$\text{Reduction\%} = \frac{(de - di)}{de} \times 100$$

Where:

De = maximum linear growth in control set. Di = maximum linear growth in treatment set.

5- Examination of *R. solani* by Transmission Electron Microscopy (TEM)

Specimens of *R. solani* that treated by copper oxide, Tri-calcium phosphate and silicon dioxide were prepared for TEM examination using the protocol of Amin, (2013).

6- Efficacy of nanoparticles on black scurf disease under greenhouse conditions

The trials were carried out during the summer season of 2014 and 2015 on potato cv. Spunta at the Experimental Greenhouse of the Vegetable Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt.

6.1. Soil preparation and potato seeding:

Soil mixture of peat-moss and sand (2:1 w/w) was prepared and sterilized with formalin solution 5% concentration, then covered with polyethylene sheet for 2 weeks. Later on, the cover was removed and the mixture was exposed to air ventilation for 10 days to evaporate formalin residues.

Plastic pots (50 cm diameter) were sterilized by dipping in 5% formalin solution for 15 min. and then air-dried for 24 hrs. Pots were filled with sterilized soil mixture and six pots were used as replicates for each treatment. The filled pots were inoculated by *R. solani* isolates with rate of 20g/kg soil and watered for 4 days

before planting (Abd-El-Aziz *et al*. 2013; Mohamoud *et al*. 2013). Control pots were inoculated with only autoclaved sorghum-sand mixture at the same rate. The pots were then placed into greenhouse at 25 ± 3 ºC.

6.2. Inoculum preparation

For mass multiplication, fungal cultures were grown on sterilized sorghum grain in autoclavable glass bottles as following; one kilogram of sorghum grain were soaked in water for 12 h, soaked sorghum grain divided into five flasks 200 g/flask and autoclaved at 121º C for 20 min. All flasks were inoculated with mycelial plug (5mm) of 7 days old culture of *R. solani*. The flasks were then incubated at 25 ± 2 ˚C for 2 weeks.

6.3. Treatments

Copper oxide, Tri-calcium phosphate and silicon dioxide nanoparticles were evaluated at four concentrations against *R. solani* under greenhouse conditions. Application of nanoparticles materials was done twice. The first application was done by soaking potato tubers in tested nanoparticles at four concentrations (50,100,150 and 200µl/L) for 2 hr. pre-planting. The second application was done at 45 days post-planting as soil drench. After 90 days from planting, tubers were harvested and kept in a dry place at room temperature for two days. The tubers were then washed carefully to remove soil residues. The black scurf disease index was assessed and compared with the control of each plant. The ratio of tuber surface area covered with sclerotia was used as general method to estimate potato black scurf as the following scale (Hadi and Balali 2010 & Matny and Al-Jarrh 2014):

0: no sclerota present
1: less than 1% of tuber area covered
2: from 2-10% of tuber area covered
3: from 11-20% of tuber area covered
4: from 21-50% of tuber area covered
5: 51% or more of tuber area covered

Disease incidence% = No. of infected tubers / total No. of inspected tubers X 100

Disease severity%

$$= \frac{\sum (\text{No. of infected tubers} \times \text{No. of scale})}{(\text{Total No. of tubers} \times \text{highness No. of scale})} \times 100$$

7. Activities of defense related enzymes:

7.1. Enzymes extraction:

Artificially inoculated potato plant and tuber were used to determine the activities of the oxidative enzymes. In this respect, 50 g from representative plants and tubers of each treatment were blended with 100 mL phosphate buffer solution (7.1 pH) then centrifuged at 3000 rpm for 20 minutes. Clear supernatants were used as crude enzyme to determine enzyme activities

7.1.1. Peroxidase activity:

Peroxidase activity was determined according to the method described by Allan and Hollis (1972). Peroxidase was expressed as the change in the absorbance of the mixture every 0.5 minute for 3 minutes period at 425 nm by Spectrophotometer (Spectronic 601 Milton ROY).

7.1.1. Polyphenol oxidase activity:

The activity of polyphenol oxidase was measured as mentioned by Matta and Dimond (1963). Polyphenol oxidase was expressed as the change in the absorbance of the mixture every 0.5 minute for 3 minutes period at 495 nm by Spectrophotometer (Spectronic 601 Milton ROY).

7.1.3. Chitinase activity:

Chitinase activity was assayed according to Miller (1959). Briefly, the reaction mixture composed of 1mL of crude enzyme solution, plus 1mL of 0.5% colloidal chitin in 0.1 M citrate buffer (pH 7.0). The mixture was then incubated at 37˚C in a shaking water bath for 30 min. The reaction was then terminated by adding 2 mL DNS(Di nitro salicylic reagent) reagent. The color was developed in a boiling water bath for 5 min. The optical densities of samples were measured at 575 nm against a blank containing (1mL substrate-buffer solution 0.5 %, 1 mL buffer and 2mL DNS). N-acetyl glucosamine (GLcNAc) was used as a standard. The enzyme activity was expressed as µmoles GLc NAc g/mL.

Preparation of DNS reagent:

The following components; 1 g of dinitrosalicylic acid, 200 mg of crystalline phenol and 50 mg of sodium sulphite were dissolved simultaneously in 1% solution of NaOH. The reagent was stored in a stopper bottle at 4°C. The reagent deteriorates during storage due to atmospheric oxidation of the sulphite present.

7.1.4. Catalase enzyme:

Catalase enzyme activity was determined following the method of Kato and Shimizu (1987). In the sample cuvette; 0.1 ml of crude extract was mixed with 0.5 ml of 0.2 M sodium phosphate buffer at pH 7.6 and 0.3 ml of 0.5% H_2O_2 then the mixture brought to a final volume of 3 ml with distilled water. The breakdown of H_2O_2 was recorded by measuring the absorbance at 240 nm and the enzyme activity was calculated as the change in absorbance per minute.

Results and Discussion

1. Examination of nanoparticle materials

Tri–calcium phosphate, copper oxide and silicon dioxide nanoparticles were examined using Transmission Electron Microscopy (TEM) (Fig. 1A, B and C). These nanoparticles of Tri – calcium phosphate were spherical and displayed sizes, 85.2, 87.7 and 94.7

nm (Fig. 1A). The TEM micrographs revealed that copper oxide nanoparticles were spherical in shape with size between 28.6 and 38.6 nm (Fig. 1B). In addition, the TEM analysis concluded that silicon dioxide particles were also spherical between 26.4 and 32.2 nm in size (Fig. 1C). Our results revealed that the characteristics of calcium phosphate, copper oxide and silicon dioxide nanoparticles are similar with those previously reported (Caiab and Tang, 2008; Yuvakkumar *et al*., 2011; Suriyaprabha *et al*., 2014; Prachi *et al*., 2014) whot stated that nano-calcium had spherical shape between 25-100nm in diameter by using atomic force micrscopy (AFM) comfired by transmission electron silicon dioxide (TME) also silicon dioxide had spherical shape with dimater 20-40nm.

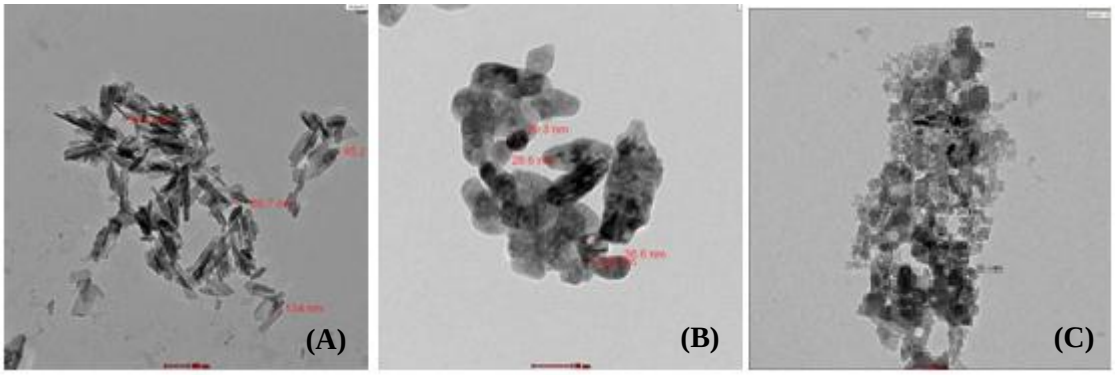

Figure. 1 TEM of nanoparticles materials used in this study; (A) nano- Tri - calcium phosphate, (B) nano - copper oxide (C) nano - silicon dioxide .

2. Effect of tested nanoparticles on the growth of *R. solani* in vitro.

Tri-calcium phosphate, copper oxide and silicon dioxide nanoparticles were evaluated at five concentrations for their inhibitory effect on *R. solani*. Results in Table (2) indicate that, all evaluated concentrations of nanoparticles suppressed the mycelial growth of *R. solani* in campning to control. However, copper oxide nanoparticles exhibited the highest reduction (56.42 %) of mycelial growth of *R. solani* at concentration 250 µl/L . Similarly, silicon dioxide exhibited a clear reduction in fungal growth at 250 µl/L with percentage 54.81%. On the other hand, silicon nanoparticles had the lowest effect on growth of *R. solani* at concentration 50 µl/L. The obtained results coincided with those obtained by Kim *et al.* (2011) who stated that the mycelial growth rate of *R. solani* was decreased typically by more than 90% at a 6 µg/ml concentration of Nano sized silica hybrid silver complex (NSS). Similar results were obtained by Ramyadevi *et al.* (2012) who found that copper nanoparticles showed inhibitory activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and fungi .*i.e. Aspergillus flavus* and *Aspergillus niger*, Moreover, Kanhed *et al.* (2014) reported that copper nanoparticles (CuNPs) showed remarkable activity against *Alternaria alternata*, *Fusariun oxysporum*, *Curvularia lunata* and *Phoma distructiva*. The enhanced antifungal activity of CuNPs was due to their large surface area to volume ratio. Bramhanwade *et al.* (2015) declared that copper nanoparticles exhibited significant and great activity against *F. equiseti.*

3. TEM of treated *R. solani* that with tested nanoparticles:

The effect of copper oxide nanoparticles on the ultrastructure of *R. solani* was shown in Figure 2 (C and D). There were clear morphological changes observed on the *R. solani* mycelium. These changes were; instability in cell wall thickens. Additionally, deformation of nucleus and disappearance nuclear membrane was also observed. This result takes the same direction obtained by Schrand *et al.* (2010) hypothesized that copper nanoparticles act as antibacterial agent against wide range of bacterial species due to interactions with SH-groups leading to protein denaturation. Moreover, Beveridge and Murray (1980) and Ren *et al.* (2009) indicated that copper nanoparticles exhibited an effect on cell membrane of *B. subtilis* due their affinity towards amines and carboxyl groups present on the cell surface. Furthermore, copper nanoparticles may bind with DNA molecules and disturb the helical structure by cross-linking within and between the nucleic acid strands. Gopalakrishnan *et al.* (2012) suggested the possible mechanism for mode of action of copper oxide nanoparticles against *E. coli* that nanoparticles adsorb on cell surface and interact with the cell wall causing damage to the cell membrane, increasing its permeability and leading to a decrease in

its viability. Furthermore, Chang *et al.* (2012) discussed three different mechanisms based on oxidative stress, coordination and no homeostasis effects that potentially explain why copper and zinc oxide nanoparticles display toxic effects on eukaryotic cells.

Table 2. Effect of three tested nanoparticles materials at different concentrations on the growth of *R. solani* in vitro.

Treatment	Concentrations µl/L	*R. solani*	
		Mycelial growth (mm)	Inhibition (%)
Copper oxide	**50**	70.20	22.00
	100	60.33	32.97
	150	50.20	44.22
	200	40.33	55.19
	250	39.23	56.41
Tri-calcium phosphate	**50**	70.25	21.94
	100	70.28	21.92
	150	57.95	35.61
	200	55.58	38.25
	250	50.53	43.86
Silicon dioxide	**50**	90.00	0.00
	100	70.60	21.56
	150	63.08	29.92
	200	50.63	43.75
	250	40.68	54.81
Control.		90.00	0.00
L.S.D at 5%		1.469	

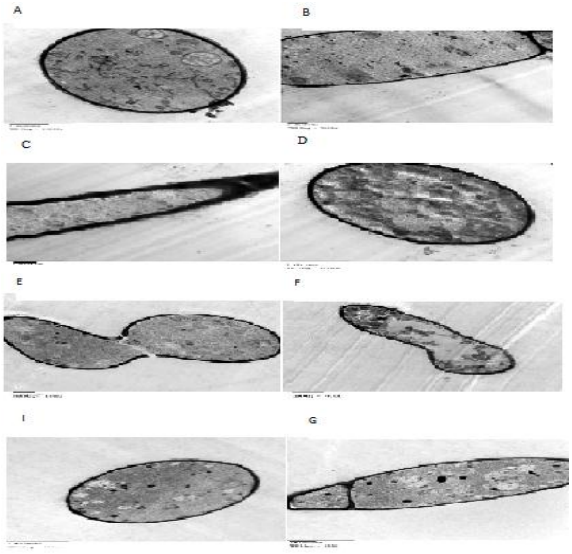

Figure. 2 TEM examination of *R. solani* hyphae; (A and B) untreated (C and D) *R. solani* treated with copper oxide nanoparticles; (E and F) *R. solani* treated with Tri-Calcium phosphate nanoparticles; (I and G) *R. solani* treated with Silicon dioxide nanoparticles

On the other hand, Tri-calcium phosphate nanoparticles had many effects on the ultrastructure of *R. solani*. These effects belonged that cell wall and membrane were thickened, cell wall and cell membrane were characterized by possessing thick weak, numerous vascular were observed, deformation of ultrastructure cytoplasmic organelles, deformed shape of nucleus and disappear of septum (Fig. 2E and F) compared with the control (Fig. 2A and B).

Also, silicon dioxide nanoparticles caused irregular and deformed shape of nucleus, abnormalities of nucleus and chromatin bodies (Fig. (2)I and G). There

are many theories explained the mechanism of silica nanoparticles against microorganisms. In this regard, the antifungal effect of silica nanoparticles may be achieved via breakdown of cell wall by forming hydrogen bonds between lipopolysaccharide-rides of the cell wall and surface hydroxyl groups present in silica nanoparticles (Capeletti *et al.* 2014). Also, the accumulation of silica nanoparticles in the membrane may induce cell lysis by preventing the trans-membrane energy cycle or form insoluble compounds in the fungal membrane that disrupt the electron transport chain (Gill *et al.* 2005). Other theory suggested that accumulation of silica nanoparticles induce oxidation of the cell membrane due to the positive charge of silica nanoparticles and the negative charge of cell membrane that produces the electromagnetic attraction between both resulting in immediate cell death (Rezaei-Zarchi *et al*. 2010).

4. Effect of nanoparticle materials on black scurf in vivo

Data in Table (3) indicate that all tested nanoparticle materials at all concentration reduced infection % and disease severity% of black scurf disease under greenhouse condition comparing with control. Also, all tested nanoparticles materials at concentration of 200 µl/L had reduced completely black scurf disease during seasons 2014 and 2015. Treatment with tri-calcium phosphate nanoparticles at 150 µl/L revealed a great reduction of diseases incidence and severity% nearly to Rizolex result with efficacy 80.91, 71.95 % respectively during season 2014 and with efficacy (DI) 81.82%, (DS) 69.94% of season 2015. Also, silicion dioxide and copper oxide nanoparticles revealed a significant reduction in DI% and DS% at 150 µl/L during the two seasons. All treatments with nanoparticles at concentration 150 µl/L displayed reduction in DI% and DS% and efficacy with values close to the Rizolex, being calcium phosphate nanoparticles is the closest. Also, it was clear that increasing the concentration of the tested nanoparticles materials from 50 to 200 µl/L , increased gradually their effect in reducing of DI% and DS% of black scurf disease.

These results are in agreement with those obtained by Brecht *et al.* (2004) who reported that silica nanoparticles increased resistance and stress resistance in plants. Also, Nano-copper was reported to be highly effective in controlling bacterial diseases such as bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*) and leaf spot of mung bean caused by *X. campestris* pv. *phaseoli* (Gogoi *et al*., 2009). Kanhed *et al.* (2014) declared also that synthesized CuNPs has antifungal activity against plant pathogenic fungi. This may open up new avenues in the field of plant disease control.

Table 3. Effect of three tested nanoparticles on black scurf disease in vivo.

Treatments	Con. µl/L	*R. solani*							
		Season 2014				Season 2015			
		DI	Ef.	DS	Ef.	DI	Ef.	DS	Ef.
Copper oxide	50	53.33	30.44	24.65	28.22	50.00	31.11	24.88	20.94
	100	35.00	51.35	19.97	42.38	33.33	54.54	19.97	36.54
	150	21.67	71.74	11.99	67.20	23.33	70.45	13.04	58.57
	200	0.00	100.00	0.00	100.00	0.00	0.00	0.00	100.00
Tri-calcium phosphate	50	50.00	34.26	24.04	20.79	50.00	31.81	24.71	21.48
	100	26.67	65.00	21.66	37.51	26.67	63.63	19.01	39.59
	150	15.00	80.91	9.55	71.95	13.33	81.82	10.09	69.94
	200	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Silicon dioxide	50	55.00	28.26	25.96	27.53	53.33	28.89	23.63	24.90
	100	31.67	43.48	20.85	42.15	31.11	57.58	18.63	36.29
	150	16.67	78.26	13.52	68.25	18.33	75.58	12.12	67.94
	200	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Rizolex		13.33	82.61	7.17	77.55	15.00	79.54	8.09	74.30
Control		76.67	0.00	34.66	0.00	73.33	0.00	31.47	0.00
LSD at 5%		12.78		2.16		14.89		2.23	

DI = %Disease incidence; DS =% Disease severity; Ef. = %Efficacy; Control = infected with *R. solani* and un-treated

6. Effect of tested nanoparticles on activities of defense related

Data in Table (4) illustrate that all tested nanoparticle materials displayed positive effect in increasing enzymes activity. The highest increase in the activity of polyphenol oxidase was observed in potato plants treated with tri-calcium phosphate in both leaves and tubers compering to control. In this respect, tri-calcium phosphate had strong effect in increasing peroxidase and catalase activity in potato leaves and tubers followed by silicon dioxide and copper oxide. while the highest activity of chitinase was obtained in

potato leaves treated with copper oxide and in potato tubers treated with silicon dioxide. Although, plants treated with Rizolex showed a high increase in polyphenol oxidase, catalase, chitinase activity, meanwhile peroxidase activity was higher in potato leaves treated with tested nanoparticles materials than plants treated with Rizolex. It has been reported that silicon stimulates the activity of these enzymes during plant–pathogen interactions (Fauteux *et al.*, 2005; Datnoff *et al*., 2007; Van *et al*., 2013). In this respect, several studies have reported the role of silicon in stimulating resistance by activating defense-related enzyme activities such as chitinase, peroxidase, polyphenol oxidase, b-1, 3glucanase, phenylalanine ammonia-lyase, uperoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, lipoxygenase, and glucanase. Phenylalanine ammonia-lyase involved in the synthesis of plant secondary antimicrobial substances and it is essential for plant disease resistance responses (Waewthongrak *et al*., 2015). The peroxidase, catalase, superoxide dismutase activities of *Lemna minor* increased with the increase in CuO NPs, bulk CuO, and 2× Cu2+ concentration released from CuO NPs (Song *et al*. 2016).

Table 4. Effect of some tested nanoparticls materials on activities of defense in potato plants and tubers cv. spunta

Treatment	Enzyme activity							
	polyphenol oxidase		Peroxidase		Catalase		Chitinase	
	leaves	Tubers	Leaves	Tubers	Leaves	tubers	leaves	Tubers
Copper oxide	0.33	0.23	2.88	1.98	1.76	1.47	6.22	4.21
Tri-calcium phosphate	0.49	0.43	3.85	3.27	1.88	1.63	4.65	3.60
Silicon dioxide	0.41	0.38	3.61	2.31	1.67	1.54	5.34	4.28
Rizolex	0.97	0.71	2.65	2.48	1.89	1.82	9.33	7.33
Control	0.190	0.189	0.5	0.7	1.04	1.12	3.78	2.54

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كفاءة أكسيد النحاس وفوسفات الكالسيوم الثلاثيه وفوق أكسيد السليكون بمركبات النانونيه في مكافحة مرض القشرة السوداء علي البطاطس

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تهدفت الدراسة إلي مقاومة مرض القشرة السوداء في البطاطس والناتج عن فطر *Rhizoctonia solani* والذي يعد من اخطر الامراض الفطريه التي تصيب البطاطس في انحاء العالم وذلك بإستخدام بعض المواد النانونية مثل أكسيد النحاس وفوسفات الكالسيوم وأكسيد السيليكون بتركيزات مختلفه بعد اختبار الخواص الفيزيائية لها وتأثيرهاعلي نمو الفطر في المعمل و كذلك علي نسبة وشدة المرض تحت ظروف الصوبه. أوضحت النتائج خفض نمو الفطر تحت ظروف المعمل وكانت افضل نسبه 56,42% تحققت بإستخدام أكسيد النحاس عند تركيز 250 ميكروليتر /لتر وأن كل المعاملات النانونية ادت الي تشوه في الخلايا الفطريه وتقليل سمك الجدر الخلويه وتشوه الانويه وألاغشيه النوويه عند دراستها تحت الميكرسكوب الالكتروني . كما اوضحت الدراسه أن المعاملات النانونية أدت إلي كبح مرض القشرة السوداء تماما عند تركيز 200 ميكروليتر/ لتر تحت ظروف الصوبه, كما أدت جميع المعاملات النانونية الي زياده في أنشطه الانزيمات المرتبطه بالدفاع النشط في النبات مما يزيد من أستخدمها في مكافحه المرض.