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Research Article
Efficacy of Some Nanoparticles to Control Damping-off and Root Rot of Sugar Beet in El-Behiera Governorate

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Abstract

Background: This study was conducted to examine the potential antifungal activity and protective effect of some nanoparticles in controlling root rot disease of sugar beet. Magnesium oxide, titanium dioxide and zinc oxide nanoparticles (MgO, TiO_2 and ZnO NPs) were investigated against three pathogenic fungi isolated from sugar beet roots, *Fusarium oxysporum* f. sp., *betae*, *Sclerotium rolfsii* and *Rhizoctonia solani in vitro* and under greenhouse conditions. **Materials and Methods:** Solutions of particles of MgO, TiO_2 and ZnO nanoparticles (NPs) were used in different concentrations *in vitro* to study the inhibition of fungal radial growth and by spraying fungal culture of the tested fungi to test the effect of nanoparticles on hyphal and spore formation and Scanning Electron Microscopy (SEM) was used to visualize the effect of this application. Greenhouse experiment was conducted and sugar beet seeds (cv., Kawamera) were treated with the nanoparticles concentrations as seed dressing then seeds were planted in infested soil with the tested fungi. Disease assessment was evaluated, also the effect of nanoparticles on the vegetative and the chemical characteristics of sugar beet were examined. **Results:** Obtained results showed that the three NPs tested with concentrations investigated (25, 50 and 100 ppm) increased the *in vitro* fungal growth inhibition by reducing the radial fungal growth with the best effect was recorded with the highest concentration. Meanwhile, TiO_2 NP (100 ppm) showed the highest effect in decreasing mean radial growth by 77.25%. Also, TiO_2 showed 100% inhibition on sclerotia formation of *Sclerotium rolfsii*, while MgO was most effective and decreased sporulation (number of conidia) of *Fusariun oxysporum* f. sp., *betae* by 69.23%. The greenhouse experiment showed that the tested all tested NPs significantly decreased the developed root rot severity and TiO_2 was most effective and decreased it to 1.39% compared to 28.2% for the untreated. On the other hand, treatments with the tested NPs significantly increased root fresh weight (biomass) of the plants and also the dry weight compared to the untreated infested control. This was accompanied with an increase in sucrose and Total Soluble Solids (TSS) and also the total phenol content and activity of the defense related enzyme, polyphenol oxidase. **Conclusion:** Based on the obtained results the use of magnesium oxide, titanium dioxide and zinc oxide nanoparticles could be a good and environmentally safe alternative of fungicides in controlling damping-off and root rot disease of sugar beet.

Key words: Nanoparticles, sugar beet, damping-off disease, root rot disease, biological control, magnesium oxide, titanium dioxide, zinc oxide

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) as an industrial crop is cultivated in 48 countries in the world for a total land of over 9 million hectares and the second highest source of sugar providing after sugar-cane. Around 30% of the total annual sugar production in the world is from sugar beets[1]. This plant is a good choice for crop rotation because it enriches soil with nutrients[2,3].

Sugar beet plants are often attacked by several pathogens such as fungi, bacteria and viruses which cause great losses in yield[4]. Root rot of sugar beet is considered the most effective disease that affects yield and quality as well as its sugar production. Several conventional methods have been used for the control of root rot of sugar beet. Some of these methods such the use of pesticides causes hazardous effects on the environment and human health. A great effort has been given to the development of safe non-traditional management methods that pose less danger to humans and animals[5,6]. Thus, use of nanoparticles has been suggested by Kumar and Yadav[7] as an alternative and effective approach which is eco-friendly and cost effective for the control of pathogenic microbes. In recent years, nanoparticle (NP) materials have received increasing attention due to their unique physical and chemical properties. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio enhance their interaction with the microbes to carry out a broad range of probable antimicrobial activities[8,9]. Some of the nanoparticles that have entered into the arena of controlling plant diseases are nano forms of silver[10-12], copper[13], titanium dioxide[14], zinc oxide[15] and carbon[16]. Meanwhile, nanoparticles play unique role by contributing direct uptake and accumulation of silica, which leads to leaf erectness and enhances defense response to fungal pathogens[17]. The ZnO NPs at concentrations greater than 3 mmol L^{-1} significantly inhibited the growth of *Botrytis cinerea* and *Penicillium expansum*[18]. Wani *et al*.[19] reported that ZnO and MgO nanoparticles brought about significant inhibition to the germination of *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* spores. Meanwhile, nanoparticles of ZnO and TiO_2 showed antifungal effect against *Macrophomina phaseolina*. Also, Min *et al*.[20] showed that silver nanoparticles strongly inhibited the fungal growth and sclerotial germination of *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor*. In the present study, nanoparticles of MgO, ZnO and TiO_2 were tested for antimycotic efficacy to control the root rot fungi affecting sugar beet in El-Behiera governorate and surrounding area as an alternative for the chemical fungicides.

MATERIALS AND METHODS

Tested sugar beet root rots fungi: Three good growing, highly virulent isolates of the sugar beet root rot fungi, *Fusarium oxysporum* f. sp., *betae*, *Sclerotium rolfsii* and *Rhizoctonia solani* were obtained from fungal collections of Plant Pathology Department, Faculty of Agriculture, Damnhour University. These isolates were previously isolated by the researcher from sugar beet fields, showed root rot symptoms in El-Behiera governorate during 2012-2013 growing season and the identification of the recovered fungi were done at the Department of Mycology and Plant Diseases Survey, Plant Pathology Research Institute, ARC, Giza. Confirmations were done to the identified fungi by comparison with those from the culture type collection of Maize and Sugar Crops Diseases Research Section of ARC, Giza.

Tested nanoparticle: Magnesium oxide (MgO), zinc oxid (ZnO) and titanium dioxide (TiO_2) nanoparticles (NPS) were obtained from MKnano, Canada and tested in the present study for their efficiency to control the root rot fungi affecting sugar beet cultivation in El-Behiera governorate. According to the source, the particles size ranged from 20-30±10 nm and spherical in shape. Size and morphology of MgO, ZnO and TiO_2 nanoparticles were confirmed by Scanning Electron Microscope (SEM) at Faculty of Science, Alexandria University (Fig. 1a-c). The elemental compositions of bio-transformed product containing nanoparticles in the solution were confirmed by Transmission Electron Microscopy (TEM) equipped with energy dispersive x-ray spectroscopy (EDX). The TEM–EDX (Fig. 1d-f) clearly show that the Zn nanoparticles show the maximum intensity at 8.467-8.807 keV, whereas, Mg and Ti showed maximum intensity at 1.148-1.347 and 4.6, 4.367-4.648 keV, respectively. Results clearly exhibited the purity of the metal nanoparticles.

***In vitro* effect of nanoparticles on growth of the sugar beet root rots fungi:** Three nanoparticle, MgO, ZnO and TiO_2 were tested in the present study. Different concentrations of nanoparticles (25, 50 and 100 ppm) were prepared by diluting the original stock solution (1000 ppm) using sterile deionized water. All solutions were stored at 4°C until use. Sterile deionized water was used as control.

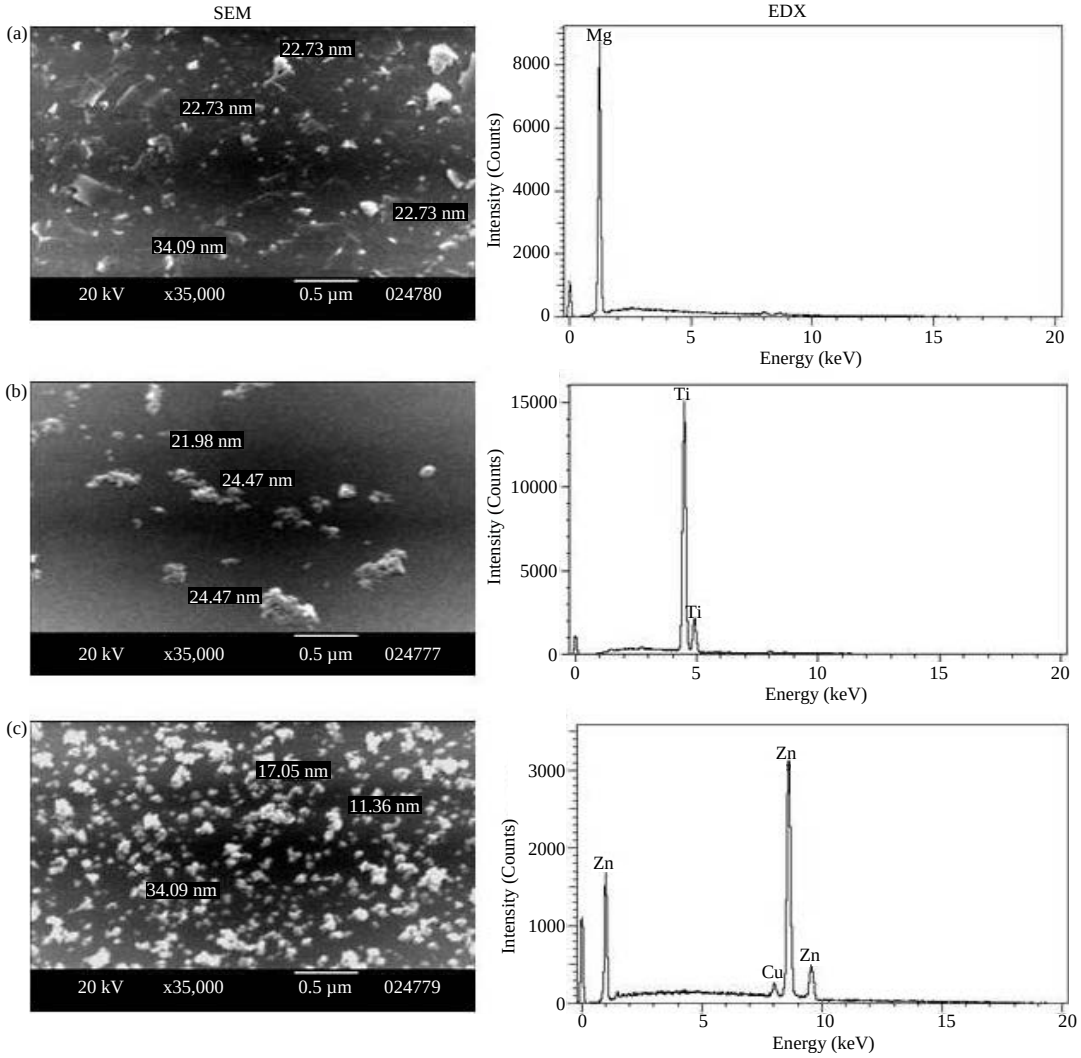

Fig. 1(a-c): Scanning Electron Microscopy (SEM) images and Energy Dispersive X-ray (EDX) of (a) Magnesium oxide (MgO), (b) Titanium dioxide (TiO_2) and (c) Zinc oxide (ZnO) nanoparticles use in the present study

Effect on radial growth: The *in vitro* assay was performed by the agar dilution method described by Fraternale *et al.*[21] with some modification. The autoclaved PDA media with concentrations of 25, 50 and 100 ppm for all nanoparticles tested, keeping one as control (PDA without nanoparticles) and NPs solution were poured into the petri dishes (9 cm diameter) before pouring the plates. Then, a disc of 0.5 cm diameter was taken from the edge of 7 day-old cultures of the tested fungi was placed in the center of each petri dish. The petri dish with the inoculum was then incubated at 25±2°C. Then, 7 days after incubation or when the fungal growth in the control completely colonized the plate, radial growth, sporulation and sclerotia formation of sugar beet root rot fungi were investigated. All tests were performed in four replicate plates.

Effect on fungal radial growth: Percentage of inhibition of mycelial radial growth was calculated according to Kaur *et al.*[22] using the equation:

$$\text{Inhibition (\%)} = \frac{\text{dc-dt}}{\text{dc}} \times 100$$

where, dc is a mycelial growth in control and dt is a mycelial growth in the treatment.

Effect on sporulation and sclerotia formation: Sporulation (conidia number) of the sugar beet root rot fungus *Fusarium oxysporum* f. sp., *betae* was calculated using a hemaecytometer. Also number of sclerotia of *Sclerotium rolfsii* was recorded using 40X magnifying lens.

Effect on hyphal and spore morphology: Petri dishes containing healthy 10 day-old cultures on PDA of the sugar beet root rot fungi were sprayed with 1 mL of 100 ppm MgO, ZnO and TiO_2 nanoparticle solutions. At the same time cultures were sprayed with sterile deionized water and served as control. Twenty four hours later, the cultures were investigated under a Hitachi S-3500N scanning electron microscope at Faculty of Science, Alexandria University according to Elamawi and El-Shafey[23] to reveal the effect of nanoparticles on hyphae and spore morphology.

***In vivo* effect of nanoparticles on root rot and damping-off on sugar beet:** Greenhouse experiments were carried out in 2015 to study the efficacy of ZnO and MgO and TiO_2 nanoparticles to control root rot and damping-off of sugar beet. The pathogens were grown on medium of sand and corn (2:3 w/w) in glass bottles for 2 weeks at 27°C. Plastic pots (25 cm diameter) were filled with soil sterilized with formalin (5%) as 3 kg soil pot^{-1} and left for 1 week until complete formalin evaporation. Pot soil (1 clay:1 sand v/v) was infested by mixing the inoculum of fungi at the rate of 2% of soil weight according to Papavizas and Devey[24] and Marwa *et al.*[25].

The infested soil was watered and left for a week before planting to stimulate the fungal growth and ensure its establishment in the soil. On the other hand, nanoparticles solution were prepared (100 ppm+0.2% tween 80) and used as seed coating before planting and vitavax 200 fungicide (3 g kg^{-1}) were used as a control treatment. The pots were then planted with the sugar beet treated seeds, cv., Kawamera, (10 seeds pot^{-1}), watered and fertilized as usual. Each treatment had three pots and other pots planted with seeds in sterilized soil and served as control.

Disease assessments: Pre and post emergence damping off were calculated after 15 and 45 days of planting, respectively, according to El-Shafey *et al.*[26] as follows:

$$\text{Pre-emergence damping-off (\%)} = \frac{\text{No. of non-emerged seed}}{\text{Total No. of seeds sown}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{No. of died plant}}{\text{Total No. of emerged plant}} \times 100$$

Root rot severity was scored 150 days after planting based on Rowe[27] and Liu *et al.*[28] with the following ratings: 0: No internal or external browning, 1: No internal browning, with superficial lesions ($\leq$25%) on tap root, 2: Slight internal browning with (<25 to $\leq$50%) surface covered with cankers, 3: Moderate internal browning with <50 to $\geq$75% cankers, 4: Severe internal and external (<75%) browning.

$$\text{Disease severity} = \frac{\text{Sum } (n\times r0)+(n\times r1)+....+ (n\times r4)}{4\ N} \times 100$$

where, n is No. of plants in each numerical rate (r0....r4) and N is the total No. of plants multiplied by the maximum numerical rate r4.

Effect of nanoparticles on the vegetative and the chemical characteristics of sugar beet: At the end of the pot experiment (150 days after planting), root yield was estimated as root fresh weight and dry weight, as well as the Total Soluble Solids (TSS), sucrose, total phenols and the defense related enzyme polyphenol oxidase in plant roots as follows.

Fresh and dry weight: After harvest, plant roots were thoroughly washed with tap water and dried at room temperature. Roots were then sliced and dried at 80°C for 72 h in air drying oven until constant weight was reached.

Total Soluble Solids (TSS) and sucrose content: The TSS was measured in juice of fresh roots by using hand refractometer (Hycle groupe lifasa bio 21320 Pouuilly by Auxxois-Fransa). Sucrose percent was determined by using polarimetric on lead acetate extract of fresh macerated roots according to the method of Carruthers and Oldfield[29] and Fatouh[30].

Total phenol: Total phenolic content of fresh root was estimated by Folin Ciocalteu method of Zieslin and Ben-Zaken[31] with some modification. One gram of sample was homogenized in 10 mL of 80% methanol and agitated for 15 min at 70°C, then 1 mL of methanolic extract was added to 5 mL of distilled water and 250 µL of Folin Ciocalteu reagent and incubated at 25°C, after 3 min, 1 mL of the saturated solution of sodium carbonate and 1 mL of distilled water were added and the reaction mixtures were incubated further for 1 h at 25°C. The absorption of the developed blue color was measured using spectrophotometer at 725 nm. The total soluble phenol content was calculated according to a standard curve obtained from a Folin Ciocalteu reagent with a phenol solution and expressed as catechol equivalent per gram of fresh tissue.

Polyphenol oxidase assay: Enzyme extraction was conducted according to the method described by Maxwell and Bateman[32] as follows. The root tissues were grounded in a mortar with 0.1 M sodium phosphate buffer at pH 7.1

(2 mL g^{-1} fresh root tissues). The triturated tissues were strained through layer of cheesecloth and filtrates were centrifuged at 3000 rpm for 20 min at 6°C and the supernatant fluids were used for enzyme assays. The activity of polyphenol oxidase was measured using spectrophotometer (Spectronic 601). The control cuvette contained the buffer solution plus distilled water. The reaction mixture contained 0.5 mL enzyme extract, 0.5 mL sodium phosphate buffer at pH 7 and 0.5 mL of catechol brought to a final volume of 3 mL with distilled water. The activity of polyphenol oxidase was expressed as the change in absorbance per minute in 1.0 g fresh weight at 495 nm. The polyphenol oxidase activity was measured after 150 days of inoculation. All the experiments were repeated thrice.

Statistical analysis: Data were analyzed using one-way analysis of variance (ANOVA) and the least significant difference test to estimate statistical differences between means at $p = 0.05$.

RESULTS

In vitro effect of nanoparticles on growth of the sugar beet root rot fungi

Effect on fungal radial growth: Data presented in Table 1 showed that all the tested nanoparticles, MgO, TiO_2 and ZnO at the different tested concentrations (25, 50 and 100 ppm) significantly inhibited the fungal radial growth of the tested sugar beet root rots fungi on PDA compared to the control (sterile deionized water). Meanwhile, TiO_2 at 100 and 50 ppm showed the highest mean inhibition effects being 77.52 and 69.72% for both concentrations, respectively. This was followed by 100 ppm MgO and 100 ppm ZnO, where mean inhibitions were 67.24 and 63.39% for both nanoparticles, respectively (Table 1). On the other hand, *F. oxysporum* f. sp., *betae* was best inhibited (55.71%) by 100 ppm MgO, while *R. solani* and *S. rolfsii* were best inhibited with the use of TiO_2 (100 ppm) being 78.8 and 100% of inhibition for both fungi, respectively.

Effect on number of conidia and sclerotia: Data illustrated in Fig. 2 showed that, all the tested nanoparticles (all at 100 ppm) significantly decreased the number of sclerotia of *S. rolfsii* and number of conidia of *F. oxysporum* f. sp., *betae* compared to control. The MgO NP was the most effective and posed 69.23% for the percentage of inhibition of number of *F. oxysporum* f. sp., *betae* conidia. This was followed by TiO_2 (61.45%) and ZnO (54.81%). On the other hand, TiO_2 posed 100% for the percentage of inhibition of number of sclerotia of *S. rolfsii* followed by MgO (55.1%) while, ZnO showed the lowest inhibition effect being 49.29%.

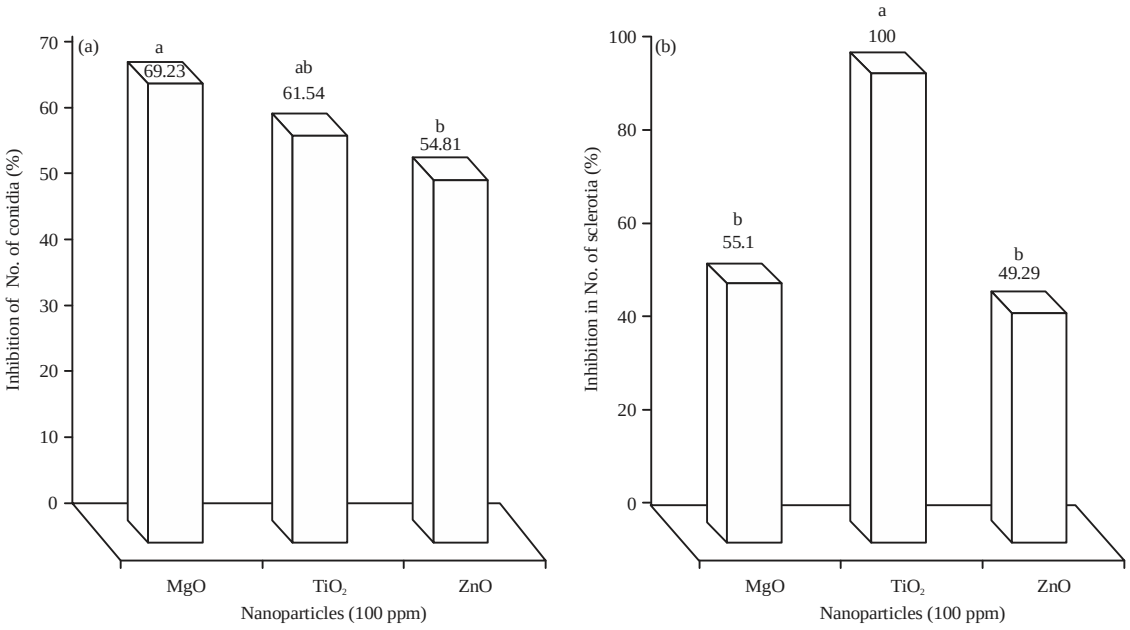

Fig. 2(a-b): *In vitro* percentage of inhibition of (a) No. of conidia of *Fusarium oxysporum* f. sp., *betae* and (b) No. of sclerotia of *Sclerotium rolfsii* on PDA amended with 100 ppm of the different nanoparticles

Table 1: *In vitro* percentage of inhibition of radial growth of sugar beet root rot pathogenic fungi on PDA by different nanoparticle concentrations

		Nanoparticles (ppm)								
		MgO			TiO_2			ZnO		
Fungi	Cont.	25	50	100	25	50	100	25	50	100
Fusarium oxysporum f. sp., *betae*	0.00	42.86	50.41	55.71	31.23	49.59	53.67	32.04	44.29	53.06
Sclerotium rolfsii	0.00	70.33	77.00	78.56	75.89	83.67	100.00	72.56	73.67	77.44
Rhizoctonia solani	0.00	52.22	62.22	67.44	51.11	75.89	78.89	49.22	50.00	59.67
Mean	0.00[d]	55.14[c]	63.21[b]	67.24[a]	52.74[c]	69.72[b]	77.52[a]	51.27[c]	55.99[b]	63.39[a]

Means followed by different letter(s) are significantly different at p = 0.05 of probability, inhibition effects were determined based on five replicates for each treatment, Cont.: Untreated control

Table 2: *In vivo* effect of nanoparticles on damping-off and root rot disease severity of sugar beet (cv., Kawamera) in pot experiments infested with the root rot fungi of sugar beet

	Treatments (%)														
	Pre-emergence					Post-emergence					Root rot severity				
Fungi	Cont.	Vitavax	MgO	TiO_2	ZnO	Cont.	Vitavax	MgO	TiO_2	ZnO	Cont.	Vitavax	MgO	TiO_2	ZnO
Fusarium oxysporum f. sp., *betae*	33.30	9.24	12.90	18.47	20.33	22.14	6.13	2.22	6.81	9.36	18.06	0.00	0.00	2.78	2.78
Sclerotium rolfsii	40.70	11.10	16.60	11.1	16.63	21.67	6.25	6.66	4.17	6.68	34.7	1.39	2.78	0.0	4.17
Rhizoctonia solani	42.57	18.50	22.20	20.3	24.03	22.73	6.66	7.14	6.96	7.32	31.92	4.17	4.17	1.39	2.78
Mean	38.86[a]	12.95[c]	17.23[b]	16.6[bc]	20.33[b]	22.18[b]	6.35[b]	5.34[b]	5.98[b]	7.79[b]	28.23[a]	1.85[b]	2.32[b]	1.39[b]	3.24[b]

Pre-emergence and post-emergence damping-off were recorded 15 and 45 days after planting, respectively, while root rot severity was recorded 150 days after planting, all nanoparticles were applied as 100 ppm, while vitavax was applied as 3 g kg^{-1}

Effect on hyphal and spore morphology: In order to reveal the effect of the tested nanoparticles on hyphal and spore morphology, healthy fungal cultures on PDA of the sugar beet root rot fungi were sprayed with 100 ppm MgO, TiO_2 and ZnO solutions and other plates were also sprayed with water as control and investigated by Scanning Electron Microscope (SEM). The microscopic observation showed the images of conidia and mycelia in the control for *F. oxysporum* f. sp., *betae* with typical net structure and smooth surface (Fig. 3) while, MgO, TiO_2 and ZnO nanoparticles clearly damaged the hyphae and conidia of *F. oxysporum* f. sp., *betae* as fungal mycelia and conidia were sunken, wrinkled and damaged after 24 h (Fig. 3). On the other hand, Fig. 4 showed the image of *R. solani* and *S. rolfsii* mycelia of the control with typical net structure and smooth surface. In contrast MgO NPs formed unusual bulges on the surface of fungal hyphae, while TiO_2 caused severe hyphal distortion. The ZnO NPs, however, caused deformation and lysis for fungal hyphae of both fungi (Fig. 4).

***In vivo* effect of nanoparticles on root rot and damping-off on sugar beet:** In pot experiment the effect of MgO, TiO_2 and ZnO NPs and vitavax on damping-off and root rot of sugar beet (cv., Kawamera) caused by *F. oxysporum* f. sp., *betae*, *S. rolfsii* and *R. solani* is shown in Table 2. Treatment with TiO_2 was the most effective and decreased mean percentage of pre-emergence damping-off to 16.60% compared to 38.86% for the untreated in tested control and this was not significantly different form the vitavax effect and also was not significantly different form MgO and ZnO NPs effect. On the other hand, all the tested NPs significantly decreased post emergence damping-off and MgO was most effective as decreased post emergence to 5.34% compared to 22.18% for the untreated control. Meanwhile, this effect was not significantly different form the vitavax fungicide effect and the other two NPs, TiO_2 and ZnO (Table 2). Concerning to the root rot severity, all NPs significantly deceased it where TiO_2 was the most effective and decreased disease severity to 1.39% compared to 28.2% for the untreated control. Meantime, TiO_2 effect was not significantly different vitavax and also the MgO and ZnO effect (Table 2).

Effect of nanoparticles on the vegetative and the chemical characteristics of sugar beet

Root fresh (biomass) and dry weight: Data in Table 3 showed the effect of MgO, TiO_2 and ZnO NPs on some agronomic parameters of sugar beet plants grown in soil infested with root rots fungi. Treatment with TiO_2 proved to be the best treatment as significantly increased the mean root fresh weight (biomass) to 193.4 g $plant^{-1}$ compared to 104.5 g for the untreated infected control. This effect was even significantly higher than vitavax which showed 175.5 g mean root fresh weight and was higher than MgO (167.5 g) and ZnO (151.5 g).

A similar trend was obtained for the dry weight of roots where TiO_2 showed the highest effect and increased dry

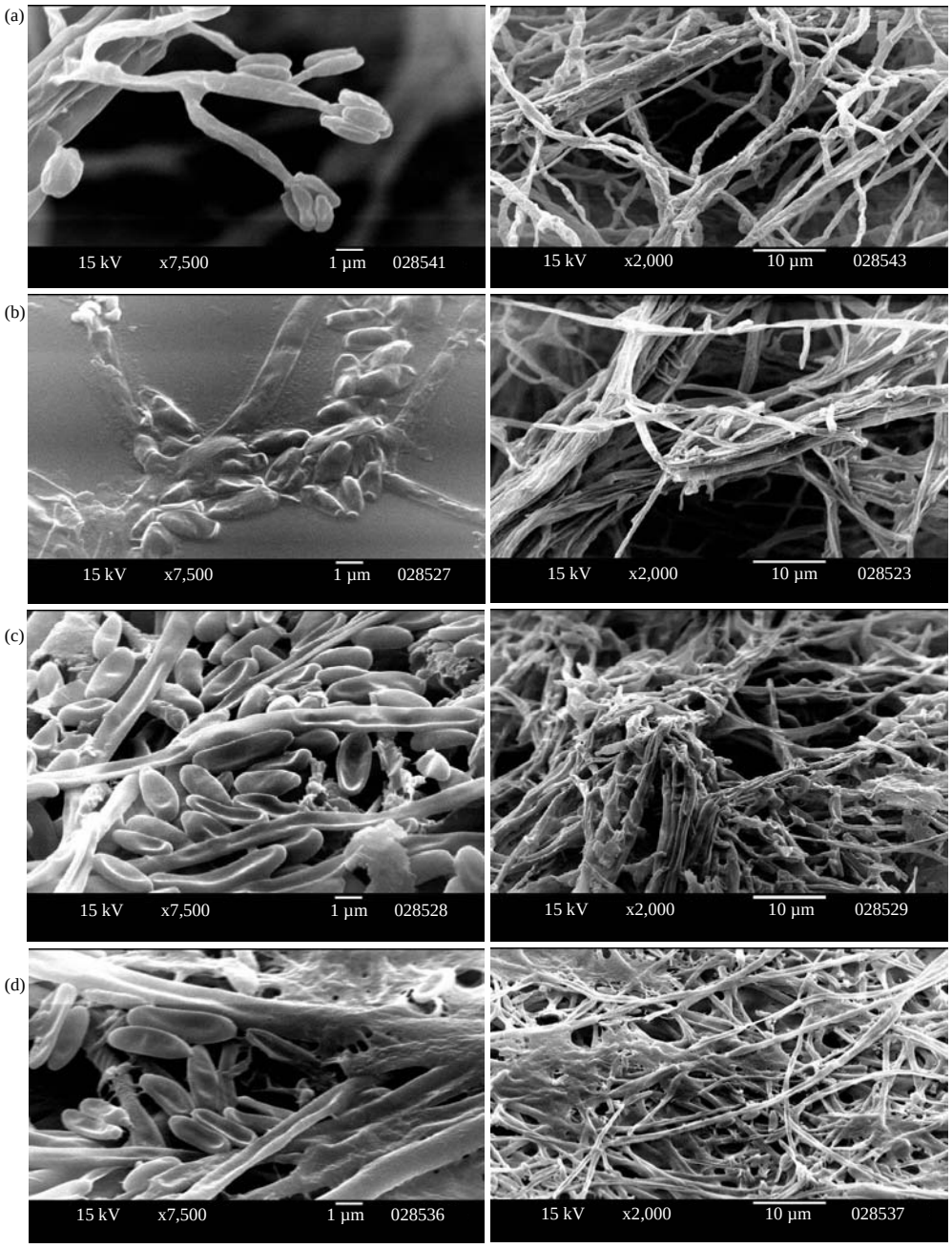

Fig. 3(a-d): Scanning Electron Microscopy (SEM) of conidia and hyphae of *F. oxysporum* f. sp., *betae* grown on PDA sprayed with either water as a (a) Control or 100 ppm of (b) MgO, (c) TiO_2 and (d) ZnO nanoparticle solutions, 24 h after treatment

Table 3: *In vivo* effect of nanoparticles on root fresh weight (biomass) and dry weight of sugar beet grown in soil infested with sugar beet root rot fungi in pot experiment, 150 days after planting

	Treatments (g plant^{-1})									
	Root fresh weight					Root dry weight				
Fungi	Cont.	Vitavax	MgO	TiO_2	ZnO	Cont.	Vitavax	MgO	TiO_2	ZnO
Fusarium oxysporum f. sp., *betae*	108.02	192.65	217.25	177.05	161.55	29.98	48.69	51.03	47.58	46.32
Sclerotium rolfsii	105.32	170.1	142.8	207.26	139.45	28.77	45.27	45.77	50.28	45.73
Rhizoctonia solani	100.22	163.95	143.28	195.85	153.7	27.5	46.07	44.87	48.82	45.81
Mean	104.52^{d}	175.57^{b}	167.78^{bc}	193.4^{a}	151.57^{c}	28.75^{d}	46.68^{abc}	47.22^{ab}	48.89^{a}	45.95^{abc}

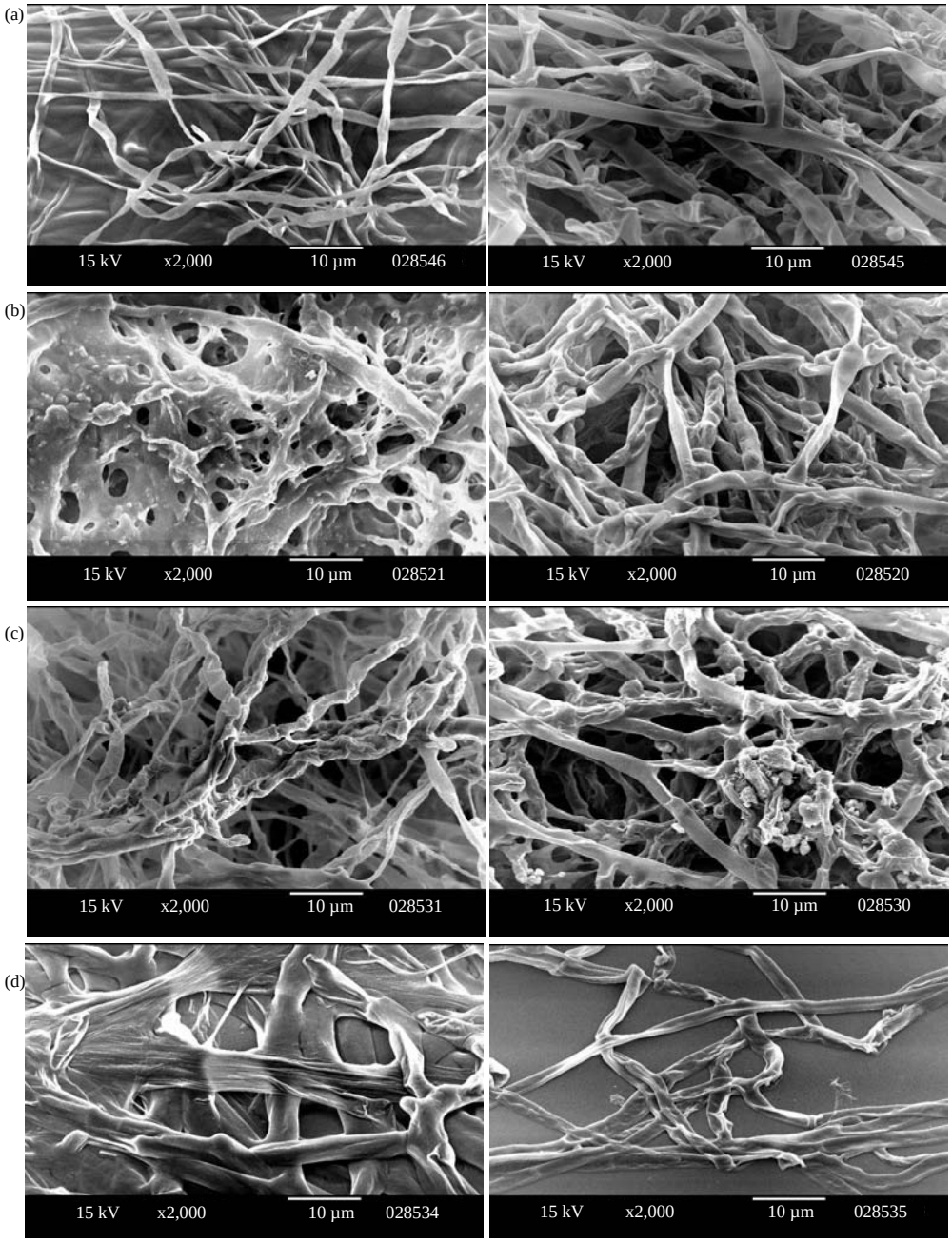

Fig. 4(a-d): Scanning Electron Microscopy (SEM) of *R. solani* and *S. rolfsii* hyphae grown on PDA sprayed with either water as a (a) Control or 100 ppm of (b) MgO, (c) TiO_2 and (d) ZnO nanoparticle solutions, 24 h after treatment

weight of roots to 48.8 g plant^{-1} compared to 28.7 g for the untreated infected control. Meanwhile, TiO_2 effect was not significantly different from the other NPs treatments and the vitavax (Table 3).

Total Soluble Solids (TSS) and sucrose: For TSS and sucrose content, data of Table 4 revealed a similar trend for both parameters. All the NPs tested and vitavax increased TSS (%) and sucrose (%) compared to the untreated infected control. However, TiO_2 showed the highest effect and increased TSS to 22.9% and sucrose to 18.3% compared to 16.7 and 13.8% for both parameter, respectively in the untreated control (Table 4).

Total phenols: Data of Table 5 revealed that all the nanoparticles tested increased the defensive reaction related to total phenols compared to the untreated infected control. However, TiO_2 was of the highest effect and increased mean

Table 4: *In vivo* effect of nanoparticles on TSS and sucrose content of sugar beet roots grown in soil infested with sugar beet root rot fungi in pot experiments, 150 days after planting

	Treatments (%)									
	TSS					Sucrose				
Fungi	Cont.	Vitavax	MgO	TiO_2	ZnO	Cont.	Vitavax	MgO	TiO_2	ZnO
Fusarium oxysporum f. sp., *betae*	17.17	24	24	23.67	20.33	13.97	18.78	18.63	18.56	17.25
Sclerotium rolfsii	16	22	21.67	23	21.67	13.43	17.52	17.91	18.48	17.51
Rhizoctonia solani	17	21	21.00	22	21.33	13.96	17.14	17.34	17.87	17.37
Mean	16.72^{c}	22.33^{a}	22.22^{ab}	22.89^{a}	21.1^{b}	13.79^{d}	17.8^{abc}	17.96^{ab}	18.31^{a}	17.38^{bc}

Table 5: Effect of MgO, TiO_2 and ZnO on total phenol content and activity of polyphenol oxidase of sugar beet roots grown in soil infested with sugar beet root rots fungi in pot experiments, 150 days after planting

	Treatments									
	Total phenols (mg g^{-1} fresh tissue)					Polyphenol oxidase activity (Absorbance min g^{-1} fresh tissue)				
Fungi	Cont.	Vitavax	MgO	TiO_2	ZnO	Cont.	Vitavax	MgO	TiO_2	ZnO
Fusarium oxysporum f. sp., *betae*	4.60	8.83	9.58	7.87	7.83	0.025	0.059	0.058	0.056	0.051
Sclerotium rolfsii	3.83	8.75	8.10	9.41	7.75	0.026	0.062	0.059	0.084	0.052
Rhizoctonia solani	4.73	8.49	7.45	9.26	8.68	0.030	0.059	0.057	0.112	0.073
Mean	4.39^{d}	8.69^{ab}	8.38^{bc}	8.85^{a}	8.09^{c}	0.027^{d}	0.060^{b}	0.058^{c}	0.084^{a}	0.059^{bc}

of the total phenols to 8.85 mg g^{-1} fresh weight compared to 4.39 in the untreated control (Table 5). Meanwhile, this effect was not significantly different from MgO nanoparticle and fungicide vitavax.

Polyphenol oxidase activity: Data of Table 5 revealed that all the nanoparticles tested increased of the defensive reaction related to enzyme activity (polyphenol oxidase) compared to the untreated infected control. The TiO_2 nanoparticles showed highest effect and increased mean of the polyphenol oxidase activity to 0.084 (absorbance min g^{-1} fresh tissue) compared to 0.027 in the untreated control (Table 5). The effect was significantly higher than vitavax (0.060), ZnO (0.059) and MgO (0.058).

DISCUSSION

Control of plant diseases is the most challenging aspect in crop production. In recent years, resistance to commercially available fungicides by phytopathogenic fungi has been increasing and has become a serious problem[33].

A greater effort has been given for the development of safe management methods that pose less danger to humans and animals. Thus, use of nanoparticles has been suggested as an alternative and effective approach with antifungal potential which is eco-friendly and cost effective for the control of pathogenic microbes[7,34-37].

In the present study, all the tested nanoparticles i.e., magnesium oxide (MgO), zinc oxid (ZnO) and titanium dioxide (TiO_2) at the tested concentrations (25, 50 and 100 ppm) significantly inhibited the *in vitro* fungal radial growth, sporulation and sclerotia formation of the tested damping-off and root rot fungi of sugar beet, *Fusarium oxysporum* f. sp., *betae*, *Sclerotium rolfsii* and *Rhizoctonia solani* on PDA compared to control (sterile deionized water). The TiO_2 at 100 ppm was of the highest effect as inhibited mean radial growth of the tested fungi by 77.52%, followed by MgO with 67.2% inhibition. However, MgO at 100 ppm was most effective and decreased number of conidia of *Fusarium oxysporum* f. sp., *betae* by 69.33% which was not significantly different than TiO_2 which decreased number of conidia by 61.54%. However, TiO_2 at 100 ppm was most effective to inhibit sclerotia formation of *Sclerotium rolfsii* by 100%, while MgO was the second in this respect being 55.1%. The ZnO, however, showed the least effect compared to the other two NPs but still significantly effective to decrease radial growth, sporulation and sclerotia formation compared to the control. These effects were accompanied with deformation and lysis of the fungal hyphae and spores. The results were in harmony with the findings of Sharma *et al.*[38] as reported that ZnO NPs shown antifungal activity against *Pythium debarynum* and *Sclerotium rolfsii*, causing a significant decrease in the fungal growth that corresponds to increase in the concentration of ZnO NPs. Besides, Kumar *et al.*[39] reported that Ag NPs treated plates containing *S. rolfsii* showed sclerotia formation either lacking or abnormal, if formed. There result were in contrast with the findings of Kasprowicz *et al.*[40] who reported that the number of *Fusarium culmorum* spores formed by mycelia increased in the culture after contact with silver nanoparticles (Ag NPs), especially on the nutrient-poor PDA medium but

in agreement with the findings of Al-Othman *et al.*[41] who reported that Ag NPs reduced *A. flavus* spores number. Also, Yehia and Ahmed[42] mentioned that ZnO NPs may disrupt and damage the conidia of *F. oxysporum* and deformate the structure of fungal hyphae and consequently the growth was deeply inhibited. The generation of active free hydroxyl radicals (-OH) by photoexcited TiO_2 particles is probably responsible for the antibacterial activity[43].

Meanwhile, the inhibitory effect of nanoparticles may be due to release of extracellular enzymes and metabolites[44]. Also, some studies proposed that ZnO NPs may cause structural changes of microbial cell membrane, causing cytoplasm leakage and eventually the death of bacterial cells[45,46].

The *in vivo* pot experiment conducted supported the *in vitro* results, treatment with TiO_2 was the most effective and decreased percentage of damping-off to 16.60% compared to 38.8% for the untreated control. This TiO_2 effect was not significantly different from the vitavax fungicide effect. For the post-emergence damping-off, MgO was most effective and reduced post-emergence to 5.3% compared to 22.1% for the untreated control. This effect was not significantly different from TiO_2 and the fungicide vitavax where post-emergence damping-off was 5.9 and 6.3%, respectively. All the tested NPs significantly decreased root rot severity and TiO_2 was the most effective and decreased root rot severity to 1.39% compared to 28.2% for the untreated control and was even not significantly different form the fungicide vitavax effect.

These findings were in agreement with Elamawi and Al-Harbi[47] who reported that *Fusarium* disease incidence on tomato was reduced by silver nanoparticles to 5% compared to 100% for the untreated control also, Jo *et al.*[48] found that silver nanoparticles, reduced the disease severity by *Bipolaris sorokiniana* which causes seedling blight, root rot, crown rot and leaf spot blotch on various gramineous species and *Magnaporthe grisea* which causes blast on rice. Servin *et al.*[49] showed that ZnO, TiO_2 and CuO nanoparticles may have significant use in pathogen control program by directly inhibiting disease causing organisms or by affecting the systemic acquired resistance.

On the other hand, the present study showed that MgO, TiO_2 and ZnO NPs significantly enhanced agronomic parameters of sugar beet plants grown in soil infested with root rot fungi of sugar beet. Treatment with TiO_2 proved to be the best treatment as increased the mean root fresh weight (biomass) to 193.4 g compared to 104.5 g for the untreated infected control. This effect was even significantly higher than the fungicide vitavax which showed 175.5 g mean root fresh weight and was higher than MgO (167.5 g) and ZnO (151.5 g). A similar trend was obtained for the dry weight of roots. Their findings were in harmony with that of Mahajan *et al.*[50] as mentioned that seedling roots of *Vigna radiate* and *Cicer arietinum* absorbed ZnO NPs and promoted the root and shoot length and root and shoot biomass. Also, De la Rosa *et al.*[51] applied different concentrations of ZnO NPs on cucumber and found that cucumber seed germination was enhanced. Mahmoodzadeh *et al.*[52] reported that TiO_2 NPs enhanced seed germination, promoted radicle and plumule growth of canola seedlings. Also, Jaberzadeh *et al.*[53] reported that TiO_2 augmented wheat plant growth and yielded components under water deficit stress condition.

Meanwhile, the present study also showed that the NPs tested increased TSS and sucrose (%) of the roots compared to the untreated infected control where TiO_2 was the most effective and increased TSS and sucrose to 22.9 and 18.3% compared to 16.7 and 13.7% for both parameters, respectively and this was not significantly different from the vitavax effect. Application of TiO_2 NPs was found to improve plant-photosynthesis efficacy, plant-enzyme activity and provide plants with more nitrogen nutrient by chemical fixation of nitrogen in the air[54-57]. Also, all the NPs tested in the present study and vitavax enhanced the plant defense and related the enzyme activity i.e., polyphenol oxidase and the total phenols compared to the untreated infected control. Phenol is one of the most stress-responsive plant compounds according to Rodrigues *et al.*[57]. In general, the toxicity of NPs is determined by their particle size, shape and biodegradability[58-60].

There are five theories, which have been proposed about the mechanisms of nano-metal toxicity: (1) Release of toxic ions (Cd^{2+}, Zn^{2+}, Ag^{+}) that can bind to sulphur-containing proteins, this accumulation prevents the proteins from properly functioning in the membrane and interfere in cell permeability, (2) They can be genotoxic _ ions that can destroy DNA which leads to cell death, (3) Interruption of electron transport, protein oxidation and membrane potential collapse due to its contact with CeO_2 or nC60, (4) Generation of Reactive Oxygen Species (ROS) mediated cellular damage and different metal-catalyzed oxidation reactions could underlie specific types of protein, membrane or DNA damage and (5) Interference with nutrient uptake. These mechanisms may not operate separately suggesting that more than one mechanism can occur simultaneously[61,62].

CONCLUSION

In vitro and in planta experiments clearly showed that the magnesium oxide, titanium dioxide and zinc oxide

nanoparticles (MgO, TiO_2 and ZnO NPs) increased the *in vitro* fungal growth inhibition. Meanwhile, TiO_2 NP (100 ppm) showed the highest inhibition effect of fungal radial growth and the formation of *Sclerotium rolfsii*, while MgO was most effective in decreasing the sporulation (number of conidia) of *Fusariun oxysporum* f. sp., *betae*. Application of all tested NPs significantly decreased the developed root rot severity and TiO_2 was most effective. On the other hand, treatments with the tested NPs significantly increased both root fresh weight (biomass) and dry weight of the treated plants. Also, the treated plants showed increased levels of sucrose, Total Soluble Solids (TSS), phenol content and activity of the defense related enzyme, polyphenol oxidase. The use of nanoparticles as an environmentally safe substitute of the traditional synthetic fungicide will provide a green method of controlling plant diseases. More attention should be given to develop strong delivery method of nanoparticles compounds in different agriculture systems.

SIGNIFICANCE STATEMENTS

Yearly average production of sugar beets in Egypt is about 10 million tons this makes it the world number 9 producer of sugar beet and sugar industry is almost relying on sugar beet plants. Our data presented in the study will help sugar beet growers to control the damping-off and root rot disease in a safe way that will reduce the risk of using harmful synthetic fungicides and to maintain more eco-friendly sugar beet cultivation system.

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