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Biosynthesis of Nano Zinc and Using of Some Nanoparticles in Reducing of Cercospora Leaf Spot Disease of Sugar Beet in The Field

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BIOSYNTHESIS of Zn nanoparticles(NPs) from 32 various plant samples were tested and subjected to 11 samples of ZnONPs from plant aqueous extracts, wherever, $ZnNo_3$(1mmole) was used as precursor to synthesizing of ZnONPs. The UV-Vis spectral analysis of *Morus nigra* and *Grevillea robusta* plant leaves mix extracts (reaction mixture) were confirmed and showed performance of Zn NPs and exhibited as new sources for clean production and could be explored in various fields. During two tested seasons 2015/2016 and 2016/2017 and field conditions showed that, NPs of Ti followed by Zn caused to reducing of cercospora leaf spot (CLS) disease severity percentage of sugar beet plants and enhancement of TSS and sucrose contents, especially under high disease severity stress in season 2015/2016 compared to protected plants by eminent fungicide and control. NPs led to activation and recorded high enzymes activity values of enzymes of peroxidase up to 6 min and polyphenoloxidase up to 4 min estimation periods compared to control, so exhibited as mechanism in defense against CLS disease. Unseasonable weather conditions of temperature degrees, relative humidity percentage, wind velocity, pan evaporation and rain played an essential role in changement of CLS disease severity and susceptibility under field conditions during two tested seasons. The results recorded high percentages with first date of planting in September month in the first season of 2015/2016 with seven sugar beet genotypes than the second ones of October month and season of 2016/2017.

Keywords: Biosynthesis ZnNPs, Cercospora leaf spot disease, Peroxidase, Weather conditions

Introduction

Cercospora leaf spot (CLS) disease caused by the polycyclic pathogen *C. beticola* is economically important in many beet growing regions and most serious foliar disease of sugar beet (*Beta vulgaris L.)* worldwide (Secor and Rivera 2010; Bolton et al. 2013) and can cause a reduction in gross sugar yield up to 42%. Losses of 30-48% in recoverable sucrose are common under uncontrolled moderate to heavy disease pressure and losses have been reported as high as 43% (Shane and Teng 1992; Khan and Smith 2005). Biosynthesis of green nanoparticles using plant extracts is interesting in the field nanotechnology, which has economic and eco-friendly benefits over chemical and physiological methods (Aniruddha and Bhalchandra 2013; Suzan et al. 2014). Several reports demonstrated the synthesis of ZnONPs from natural source like plants by green chemistry approaches (Babu and Prabu 2011; Salem et al. 2015; Manokari et al. 2016). Moreover, Kumar and Yadav (2009) indicated that, plant mediated NPs synthesis was performed as it was cost-effective, ecofriendly and safe for human.

In recent years, pathogenic fungi had grown increasingly resistant to commercially available antimicrobial agents. This prompted researchers to look for alternative means to combat microbial and fungal pathogens. Use of NPs in plant disease management is a novel approach that may prove very effective in the future with the progress of application aspects of nanotechnology. NPs may suppress the pathogen in a way comparable to chemical pesticides. Moreover, the NPs can be used as a carrier of some chemicals *viz*, pheromones, SAR inducing chemicals, polyamine synthesis inhibitors (Khan et al. 2014). TiO_2NPs have become one of the most

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important substances in nanotechnology which caused plant growth promoting to roots of oilseed rape and protected the plants against *Alternaria brassicae* infection and adhesive effects on bacteria (*Bacillus amyloliquefaciens*; Palmqvist et al. 2015). TiO_2NPs is useful as protective encapsulating agents that increase adhesive force in the interspecies relation between bacteria and plants (Chowdhury et al. 2012; Webster et al. 2008). Ruffolo et al. (2010) and Song et al. (2014) found that $ZnTiO_3$ and Zn hydroxide carbonate NPs showed higher growth inhibition efficiency of *A. niger* and fungal activity against cotton *Verticillium, Rhizopus, Mucorales* than ZnONPs, respectively. Abd–Elsalam (2013) and Mahendra et al. (2012) showed that application of NPs grower–friendly in agriculture need to be readily used for protecting crops and avoiding loss to plant pathogens diseases. Moreover, Singh et al. (2013) reported that, among 15 micronutrients nanoforms, $CuSO_4$ and $Na_2B_4O_7$ were found most effective in controlling rust disease of field peas. Also, Abd-El-Hai et al. (2009) added that, Mg and Zn NPs suppressed the damping off and charcoal rot disease in sunflower. Additionally, Graham et al. (2016) showed that, ZnNPs (zinkicide) spraying led to reducing of grapefruit canker lesion development and incidence than bacteriocide cuprous and Zn oxide also effective against fungal disease i.e. grapefruit scab and melanose. Khan and Rizvi (2014) added that, NPs have found suppressive to fungi. Zn plays a vital role for various metabolic pathways in plant system and plant diseases concerned to its deficiency, and improved growth and yield (Panwar et al. 2012). ZnONPs significantly inhibited the growth of many fungi *i.e. Botrytis cinerea* and *Penicillium expansum* (He et al. 2010 and Krishnaraj et al. 2012), A. flavus (Jayaseelan et al. 2012), *A. niger* and F. oxysporum had antifungal (fungicidal) effects due to induce intracellular the generation of reactive oxygen species (ROS), Lipovsky et al. (2011) and Patra et al. (2012), *Helmenthosporium oryzae* (Elamawi et al. 2016) and *F. verticilliods* (Farahat et al. 2017). Application of ZnNPs mediated cytotoxicity and increase of ROS which effective to control plant pathogens (Wani and Shah 2012), lipid peroxidation and reduced glutathione (Muthurman et al. 2014). Hamaza et al. (2013) added that, ZnNPs can be promising to control of late wilt disease in maize by increasing of PO enzyme with enhancement of yield.

As effect of weather conditions of development of CLS disease, Khan et al. (2008) reported that, wind was the major dispersal factor for *C. beticola* inoculum. Consequently, CLS was the most destructive in relatively warm and humid conditions worldwide (Kerr and Weiss 1990; Karaoglanidis et al. 2001) and responsible for significant reduction in root yield sucrose and sucrose, while increasing the concentration impurities and processing costs. Windels et al. (1998) showed that, infection by *C. beticola* depending on weather conditions after infection. Moreover, Khan et al. (2007) showed that, unseasonably low temperature was probably the main factor for low disease severity in average 15 and 16 °C compared to 22 °C. Jones and Windels (1991) pointed that, optimum temperature for spore germination is 24.4 ^oC, when relative humidity is 100%. Consequently, Wolf et al. (2001) and Wolf and Verreet (2005) showed that, meteorological variables were considered as potential reasons for variation in epidemic onset of *C. beticola*, temperature optimum at 20-25°C (disease development between 20-30 °C) and RH 75 – more 90% (free water) but strongly inhibition by temperature bellow10°C. Harveson (2013) reported that, epidemics of CLS depend on the presence of susceptible varieties, adequate inoculum and long periods of leaf wetness (RH above 90%, longer than 11 hours) accompanied by warm temperature in the crop canopy.

This work was aimed to provide data for biosynthesis of Zn nanoparticles using plant extracts, effect of zinc (Zn), magnesium (Mg) and titanium (Ti) nanoparticles of cercospora leaf spot disease under field and their relation to peroxidase and polyphenoloxidase enzymes activity, as well as effect of environmental conditions of cercospora leaf spot disease development under field conditions.

Materials and Methods

The present work was carried out at the experimental farm and Lab. of Sakha Agric. Res. Station, ARC, Egypt during 2015/2016 and 2016/2017 growing seasons.

1- Biosynthesis of Zn NPs

a- Plant materials and extracts preparation

Different plants as shown in Table 1 were used to make aqueous extracts. Healthy plant materials were collected from Kafr El-Sheikh location. Plant specimens were identified by Horticulture Sakha Research Station. Fresh leaves, stems and fruits were collected and washed with running

tap water, dried cut to small pieces and used to prepare the extracts as methods of Manokari et al. (2016). Five gm of chopped plant parts were boiled in 50 ml of distilled water for 5 min to prepare broth solution which filtrated and used as reducing agent.

b- Biosynthesis and spectral analysis of Zn NPs

Zinc nitrate hexahydrate [$Zn(No_3)_2.6H_2o$, Delta, Egypt Chemistries] 1millemole (mM) solution was used as precursor to synthesize ZnO NPs using plant extracts. Color changes observed with one hour by heating the reaction mixture at 60

TABLE 1. List and reaction of tested plant extracts to ZnNPs biosynthesis

Latin name	Used parts	Reaction
Melia azedarach	new leaves	+
M. azedarach	old leaves	++
M. azedarach	new stem	+
M. azedarach	fruits	+
Duranta erecta	leaves	+++
Cassia gloca	leaves	-
Ziziphus spina-christi	leaves	-
Ipomea palmate	leaves	-
Ipomea carnia	leaves	-
Ocimum basilicum	leaves	-
Ricinus commmunis	leaves	-
Bogainvillea spectabillis	leaves	-
Eucalyptus golobus	leaves	-
Rubus fruticocus	leaves	-
Conyza odorata	leaves	-
Lonicera japonica	leaves	-
Bohinia variegate	leaves	-
Gauva psidium	leaves	-
Morus nigra	leaves, T1	+++
M.nigra	leaves, T2	+++
M.nigra	leaves, T3	+
M. alba	leaves, T4	+++
M.nigra	leaves, T5	+++
Phragnites communist	leaves	-
Myoporum serratum	leaves	-
Thevetia nereifolia	leaves	-
Grevillea robusta	leaves	++
Impreta cylindrical	leaves	-
Nerium oleander	leaves	-
Pheonix dactylifera	leaves	-
Lantara camara	leave	-
L. camara	new stem	-

+: yellow ; ++: dark yellow ; +++: very dark yellow; -:no color

^{O}C for 15 min which turned to stable yellow color. The reduction of pure zinc ions and the synthesized ZnONPs were confirmed and characterized by using UV-visible spectrophotometer (Model Spectronic 21). The UV-Vis absorption spectra of Znic colloids from *Morus nigra* and *Grevillea robusta* plant extracts were confirmed by using wave length scan between 200 to 600 nm. Reaction mixture showed strong peak at 296 nm (absorbance peak) which was specific for the zinc NPs (Manokari et al. 2016).

c- Effect of boiling period of biosynthesis of Zn NPs

Three boiling periods, *i.e.* 1, 3 and 6 min were tested to preparing of extracts for ZnO NPs biosynthesis from two samples of *M. nigra* leaves *i.e.* T1, Sawalha location and T5, Sakha location. Extraction, synthesis procedure and spectral analysis of Zn NPs were performed as adopted by Manokari et al. (2016).

2-Effect of nanoparticles of cercospora leaf spot disease

Nanoparticles preparation

Zinc oxide (ZnO), magnesium oxide (MgO) and titanium dioxide (TiO_2) nanoparticles (NPS) were obtained from MKImpex Corp Mississauga, ON L5N 6X1, Canada. According to the manufacturing and observation on Transmission Electron Microscopy (TEM), the particles size distributions were ranged from 30 nm and nearly spherical to hexagonal shaped with ZnO NPs, 20 nm with TiO_2 NPs and 30 nm with MgO NPs, as shown in Fig. 1 (Farahat et al. 2017). For experiment, solutions were prepared freshly by dispersing nanoparticles in de-ionized water through ultra-sonication (300 W, 40 kHz) for 30 minutes. Different concentrations of nanoparticles of MgO NPs, ZnO NPs (25, 50 and 100 ppm) and TiO_2NPs (50,100 and 200 ppm) were used under field conditions.

The experiment was carried out in 6th October during 2015/2016 and 2016/2017 growing seasons. Randomized complete blocks design with three replicates was used. Every treatment applied in 3 rows, each with 6 m long at 15-20 cm distance between hills. Application of treatments and fungicide of eminent (recommended dose, 1 cm/L) as protected control were done after 90 days from sowing with three sprays 15 days intervals. All cultural practices were done at proper time. Sucrose and total soluble solids (TSS) were determined in fresh roots using sacarometer and refrectometer according to AOAC (1990) and McGinnis (1982), respectively as well as, disease severity percentage (DS) was recorded as modified scale of Shane and Teng (1992) and/or Stewart (2011) after 180 days from sowing.

3- Assay of peroxidase and polyphenoloxidase activities

Oxidative enzymes activity of peroxidase (PO) and poly-phenoloxidase (PPO) were estimated and expressed as changes of absorbance (optical density, OD and/or mg protein /gm fresh weight) after 10 days from the third spray application by TiO_2 NPs (200 ppm), ZnO NPs (100 ppm) and MgO NPs (100 ppm), compared with control treatment. Enzymes extraction and peroxidase were assayed as methods adopted by Anjum et al. (2012) and poly-phenoloxidase was assayed as methods of Matta and Dimond (1963) using spectrophotometer (Spectronic 21 D).

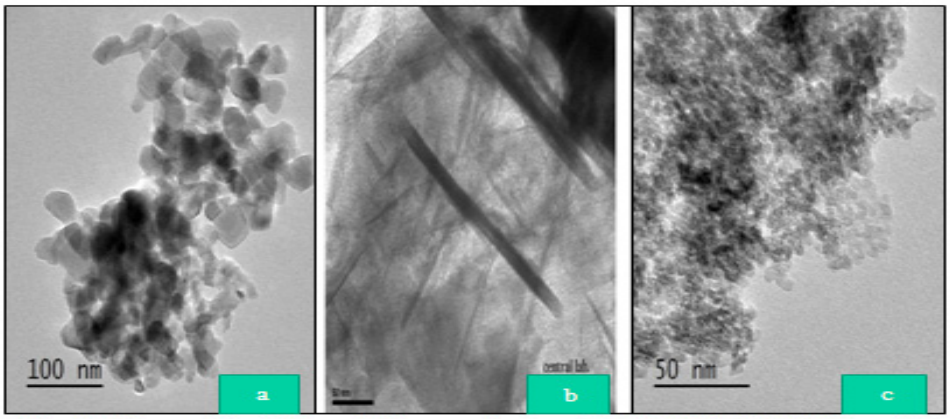

Fig. (1) : Transmission Electron Microscopy images of (a) ZnONP, (b) MgONP and (c) TiO_2NP.

4-Effect of environmental conditions of cercospora leaf spot disease in the field

Accurately determine the effect of weather conditions like air temperature degrees ◦C (max. and min.), relative humidity (RH % at 7:30 and 13:30 O'clock), pan evaporation (mm), rain (mm) and wind velocity (km / 24 h) of cercospora leaf spot disease development, meteor1ogical data of these parameters through the seasons 2015/2016 and 2016/2017 during of November and December (disease development period for first date cultivation) and during January to March (disease development period for second date cultivation) were obtained from Rice Res. And Training Center at Sakha Agric. Res. Station, Egypt and will be mentioned later. Nine sugar beet genotypes, *i.e*. Alouda, Maimouna, Beta 3980 and 8115, Nefirtitis clg, Cigogne, Toucan, Carnute and Oscar poly were cultivated at two dates, the first at 10th September and the second at 6th October during 2015/2016 and 2016/2017 growing seasons. Randomized complete block design with three replicates was used. Every treatment applied in 3 rows, each with 6 m long at 15-20 cm distance between hills. All cultural practices were done at proper time. DS were determined as mentioned above.

Statistical analysis

Data were analyzed statistically by WASP1 (WASP-Wep Agri Stat Package) developed by Ashok Kumar Jangam and Pranjali Thali at ICAR Research Complex for Goa, India program, the analysis of variance and the means were further tested using the least significant difference test (LSD).

Results and Discussion

1-Biosynthesis of Zn NPs:

From 32 plant samples were tested according to Manokari et al. (2016) protocol using leaves and stems extracts listed in Table 1, Zinc oxide NPs was synthesized by only 11samples after addition of aqueous zinc nitrate solution to extracts, color changes appeared within one hour by heating the reaction mixture at 60 °C for 15 min indicating the completing of the reaction. The intensity of colors steadily increased along the incubation period. In contrast, zinc nitrate solution without extracts and control (extract alone) showed negative reaction (no color changes). ZnNPs solutions exhibited dark yellow color, this due to reduction of aqueous extracts by Zn No3 (zinc ions) and formation of ZnNPs, Sangeetha et al. (2011). Four and five samples of both *Melia* and *Morus*, one sample of both *Duranta* and *Grevillea*, respectively, were designated to synthesis of ZnO NPS which yellow and / or dark yellow color was appeared, respectively. On the other hand, other tested samples showed no color change of reaction mixture and showed negative reaction. The UV-Vis spectral analysis of reaction mixtures of *M. nigra* and *G.robusta* confirmed the synthesis of ZnO NPs from leaves aqueous extracts by the using of wave length scan at 296nm (absorbance peak) and showed strong absorbance (Fig. 2 a and b). Yellow and/or dark yellow color were appeared as shown in Fig. 3 in chemical reaction due to reduction of aqueous extracts by Zn No3 (zinc ions) and formation of ZnO NPs from leaves aqueous extracts.

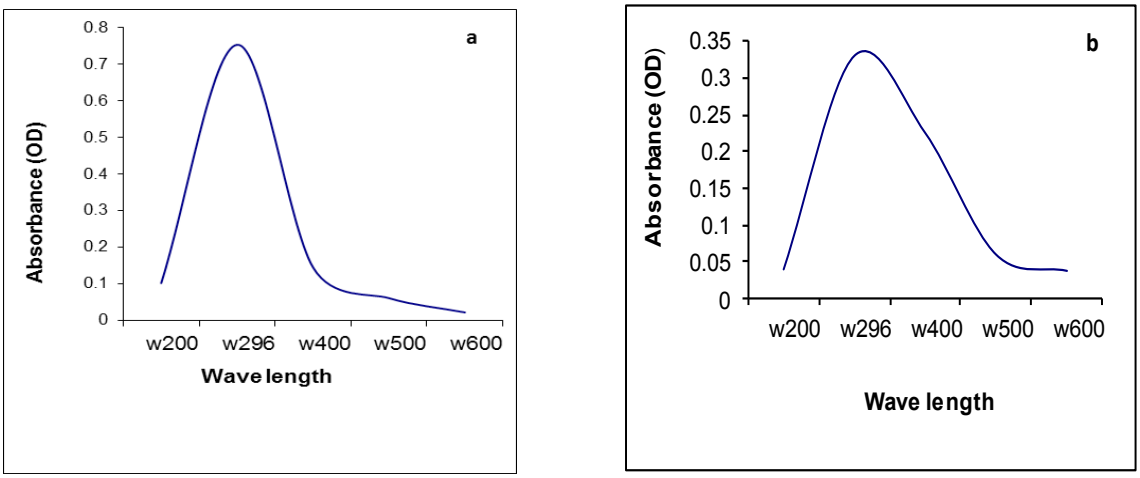

Fig. 2. UV-VIS spectra analysis of a) *Morus nigra* and b) *Grevillea robust*a mix extracts for Zn nanoparticles

Fig. 3. Dark stable yellow with reaction mixture of *Morus nigra* ,T5 (Zn+Ex) in the left, *Grevillea robusta* (Zn+S) in the right means confirmed of Zn NPS and Zn (1mM of Zn NO_3) in the middle .

The bio reducing of aqueous zinc ions by *M.nigra* and *G.robusta* plants had been demonstrated and could be anew and good source for synthesis of ZnO NPs eco-friendly method.

2- Effect of boiling period of biosynthesis of Zn NPs

Data in Table 2 showed that, 6 min boiling of leaves spices of two tested samples of *M. nigra i.e*. T1 and T5 was suitable for prepare broth solution which used as aqueous extraction for biosynthesis of ZnO NPs. Addition of zinc nitrate solution to aqueous extracts of this samples lead to appear dark yellow color. The intensity of colors increased along the incubation period and recorded the highest absorbance (OD) at wave length of 296 nm *i.e.* 0.753 and 0.473 compared to other boiling periods of 1 and 3 min.T5 (Sakha location) sample confirmed the synthesis of ZnO NPs from aqueous extract of leaves with three boiling period more than T1 (sawalha location) wherever dark yellow and very dark yellow color were appeared compared to yellow and dark yellow with T1extraxts.

The present study concluded that, aqueous extracts of leaves of *Melia, Duranta, Morus and Grevillea* were designated to synthesis of ZnO NPS which dark yellow color was appeared, this due to reduction of aqueous extracts by Zn NO_3 (zinc ions) and formation of ZnO NPs. UV-Vis spectral analysis of reaction mixtures of *M. nigra* and *G. robusta* confirmed the synthesis of ZnO NPs from leaves aqueous extracts by the using of wave length scan at 296 nm (absorbance peak) and showed strong absorbance (Manokari

TABLE 2. Effect boiling period of aqueous extraction of Zn NPS.

Plant extracts	Boiling Period /min	Absorbance (Optical Density, OD)				Color density
		200	296*	400	500	
M.nigra (T5)	1	0.040	0.523 c	0.125	0.015	++
	3	0.049	0.633 b	0.134	0.022	++
	6	0.100	0.753 a	0.145	0.061	+++
M.nigra (T1)	1	0.023	0.325 e	0.034	0.012	+
	3	0.035	0.329 e	0.050	0.013	+
	6	0.061	0.473 d	0.071	0.017	++

*296nm (absorbance peak) for Zinc NPs, Manokari *et al.* (2016). +: yellow ,++:dark yellow .+++: very dark yellow.

et al. 2016). Green synthesis of metallic NPs using plant extracts was proved to popular and safe including water and nature extracts as safe solvents (Sivakumar et al. 2011; Russell et al. 1997). Synthesis of ZnO NPs by *D. ericta* and *M. azedraech* leaves extracts were reported by Ravindran et al. (2016) and Manokari et al. (2016), respectively and supporting the present results. Radzimska and Jesionowski (2014) found that, synthesis of metallic NPs from plant derivatives were reported and more attention due to vast application in the fields, due to their physic –chemical properties. Consequently, colloidal solution of ZnO NPs were used as nanofertilizer, absorbed rapidly, save fertilizer by slow release, minimizing pollution, had remarkable optical, physiological and antimicrobial properties therefore had great potential to enhance growth and yield (Lin and Xing 2007; Selivanov and Zorin 2001; Batsmanova et al.2013).

3- Effect of nanoparticls of cercospora leaf spot disease

Certain Zn, Mg and Ti NPs were used to reduce and/or control of cercospora leaf spot disease under natural infection in the field in comparison of recommended fungicide Eminent and non sprayed control, results in Tables 3and 4 showed that Ti NPS was the most effective one in reducing of cercospora DS in the two tested seasons especially concentration of 200 ppm which recording 17.83 and 0.2% and efficiency against the disease *i.e.* 62.47 and 79.38% followed by 100 and 50 ppm *i.e.* DS ranged from 27.0 to 0.46 and efficiency ranged from 42.10 to 52.58% in comparison of 2.50 and 0.1DS and 94.74 and 98.96% efficiency of Eminent, whenever control recorded 47.51 and 0.97% DS in the two tested season, respectively. Zn NPs recorded positive effect against the disease *i.e*. DS recorded 37.53 -0.46% and efficiency 21.01- 52.58%, while Mg NPS showed the lowest effect in this respect especially in season of 2015/2016 and DS recorded 50.0-0.47% and efficiency 0.00-51.54 % in the two tested seasons. DS recorded very low percentages with all treatments and control in second seasons of study, so we will study effect of weather conditions of disease development later.

As to TSS and sucrose, TiNPs recorded the highest concentration especially in season of 2016/2017 with concentration 200 ppm *i.e.* 23.10 and 18.48 followed by 100 and 50 ppm *i.e.* 21.87 and 17.49; 21.73 and 17.39, respectively, as well

TABLE 3. Effect of nanoparticles spraying on cercospora leaf spot disease incidence, TSS and sucrose of sugar beet cv. oscarpoly, under field conditions in season 2015/2016

NPs-Cons(ppm)		Disease incidence %		TSS %	Sucrose %
		Severity %	Efficiency%		
Zn	25	37.50 cd	21.05	19.60 e	15.68 g
50		37.53 cd	21.01	19.93 de	15.95 fg
100		32.50 de	31.59	20.47 c	16.37 d
Mg	25	42.51 abc	10.52	21.02 b	16.80 be
50		50.00 a	-	21.67 a	17.33 a
100		40.12 bcd	15.55	20.60 c	16.59 cd
Ti	50	27.51 e	42.10	21.60 a	17.28 a
100		25.00 ef	47.38	20.62 c	16.33 de
200		17.83 f	62.47	20.40 c	16.32 de
Eminent		2.50 g	94.74	21.33 ab	17.07 ab
Control		47.51 ab	-	19.73 de	15.79 fg

In the same colum, means followed by a common letter are not significantly different at the 5% level by DMRT.

TABLE 4. Effect of nanoparticles spraying on cercospora leaf spot disease incidence, TSS and sucrose of sugar beet *cv.* oscarpoly, under field conditions in season 2016/2017.

NPs-	Cons(ppm)	Disease incidence, %		TSS (%)	Sucrose, %
		Severity %	Efficiency%		
Zn	25	0. 47 c	51.54	21.33 e	17.07 c
50		0.50 c	48.45	21.57 bc	17.10 c
100		0.46 c	52.58	23.00 a	18.40 a
Mg	25	0.47 c	51.54	20.93 d	16.75 d
50		0.63 b	35.05	20.03 e	16.03 e
100		0.63 b	35.05	20.13 e	16.11 e
Ti	50	0.46 c	52.58	21.73 b	17.39 b
100		0.47 c	51.54	21.87 b	17.49 b
200		0.20 d	79.38	23.10 a	18.48 a
Eminent		0.01 e	98.96	20.16 e	16.10 e
Control		0.97 a	-	20.00 e	16.01 e

In the same colum, means followed by a common letter are not significantly different at the 5% level by DMRT.

as,100 ppm of Zn NPs showed enhancement of TSS and sucrose in the two seasons *i.e*. 23.0 and 18.40 in 2016/2017 and 20.47; 16.37 in 2015/2016, while Mg do that with 25 ppm in the two seasons and 50 ppm in the first season only. The other treatments showed no significant effect of TSS and sucrose with exception of Eminent in the first season in comparison of control but uncharacterized effect was done in the second season which low DS was recorded. Data concluded that, NPs of Ti and Zn with used cons can reduce of cercospora leaf spot disease severity and enhancement of TSS and sucrose under heavy DS (first season) in highly susceptible sugar beet cv. Oscarpoly in the field. This results were supported by the findings of Palmqvist et al. (2015), they found that TiO_2NPs protected oilseed rape against *A. brassicae* infection and caused plant growth promoting of roots and increasing of adhesive force between *B. amyloliquefaciens* and plants by formation protective encapsulating interspecies (Chowdhury et al. 2012; Webster et al. 2008).

Moreover, Abd–Elsalam (2013) and Mahendra et al. (2012) added that NPs application needed to be readily used for protection against plant pathogens diseases. Also, NPs of Ti and Zn were promoted plant height, root length biomass yield and increase of chlorophyll, Raliya et al. (2015). In addition, TNPs lead to increase of nitrate reductase and enhance of absorbing water, stimulation the antioxidant effects and acceleration the germination and increased light absorption (Lu et al. 2002). Makhluf et al. (2005) and Stoimenov et al. (2002) mentioned that, NPs oxide of Zn, Ti, Mg are stable physically and optically; Zn and Ti (photocatalytic, increase light absorption), Baruah and Dutta (2009); Moreover, TiO_2 have been improved crop yield through nitrogen photo-reduction with beneficial physiological responses and to incorporated into fertilizer as a photocatalytic bacteriocid**,** Larue et al. (2012). Schiling et al. (2010) added that, TNPs are considered to be save up to 30% in products of food. The results in the same line of Abd El-Hai et al. (2009) and Hamaza et al. (2013), they reported that, Zn NPs suppressed damping off, charchol rot diseases in sunflower and control of late wilt disease of maize and enhancement of yield, respectively. Moreover**,** Singh et al. (2013) added that, NPs of $CuSO_4$ and $Na_2B_4O_7$ controlled rust disease of field peas, while Zn NPs spraying led to reducing of canker lesion and scab of grapefruit

and melanose (Graham et al. 2016). Panwar et al. (2012) added that, Zn plays a vital role for plant diseases concerned to its deficiency and improved growth and yield. Farahat et al. (2017) found that, Zn and Ti NPS reduced ears and kernels rot disease and enhanced the maize yield, as well as, had strongly fungicidal effect against many pathogens *i.e. A.niger* and *F. oxysprum* (Lipovsky et al. 2011; Patra et al. 2012), *A. flavus* (Jayaseelan et al. 2012), *Botrytis cinerea* and *Penicillium expansum* (He et al. 2010; Krishnaraj et al. 2012), *Helmenthosporium oryzae* (Elamawi et al. 2016), *F. verticillioids* (Farahat et al. 2017) and bacterial proliferation (Dimkapa et al. 2011) due to induce intercellular generation of ROS, Roy Choudhurry et al. (2011). Muthurman et al. (2014) added that, application of ZnNPs mediated cytotoxicity, increase of lipid peroxidation, ROS and had been effective to control plant pathogens by generation of ROS, Wani and Shah (2012). Lin et al. (2009) added that, inhibitory mechanisms of ZnNPs into bacteria and fungi may induce continuous release of membrane lipid and proteins which changes the permeability. Hernandez et al. (2013) reported that, Zn NPs did not accumulate in the grains and thus were safe to use as a nutrient.

4- Assay of peroxidase and polyphenoloxidase activities

Since enzymes of peroxidase and polyphenoloxidase played an important and essential role in plant defense. So, activity of these enzymes were estimated with NPs which recorded a positive effect against *C. beticola* and enhancement of control to cercospora leaf spot disease in the field. Results in Table 5 showed that, spraying of sugar beet plants by nanoparticles led to activate of PO enzyme expressed as optical density after 3 min period estimation and recorded significant increase and high values with 200 ppm of TiNPs and 100 ppm of ZnNPs *i.e.*1.463 and 1.326 (OD /3 min /gm fresh weight), respectively, compared to 0.976 of control. On the other hand, 100 ppm of MgNPs showed no significant deference with control treatment in this respect. Max. PO activity was recorded after 6 min, resulted in NPs application and the highest values of PO activity was recorded with TiNPs followed by ZnNPs while MgNPs recorded the lowest one *i.e.* 1.996, 1.801 and 1.691, respectively, compared to 1.651 of control treatment.

PPO enzyme activity continued up to 4 min. and the used NPs led to significant increase of PPO activity in all estimation activity periods. PPO activity was gradually increase up to end of estimation periods. Highest values of PPO activity were recoded after 1 min estimation period with ZnNPs *i.e.* 1.323 and 1.501 (OD / min /gm fresh weight) after 4 min with MgNPs compared to 0.943 and 1.096 of control with the same two periods of estimation. Also, TiNPs caused significant increase of PPO activity and ranged from 1.153 to 1.456 in the fourth estimation periods. So, this NPs treatments induce sugar beet plants to produce defense enzymes of PO and PPO against cecospora leaf spot disease in the field conditions and cause reduction by this mechanism as well as, PO was more active than PPO recording positive activity up to 6 min estimation period. Many authors supported these results like Lin et al. (2009), who found that, ROS stress induced fungicidal mode of action of ZnNPs. Hamza et al.(2013) added that, ZnNPs can be promising to control of maize late wilt disease by increasing of PO enzyme activity. In addition to, Raghu et

TABLE 5. Effect of nanoparticles spraying of peroxidase and polyphenoloxidase enzymes activity of sugar beet *cv.* oscarpoly

Concentration of nanoparticles (ppm)		PO/OD /3 min /g FW		PPO /OD / min /g FW			
		3	6	1	2	3	4
Zn	100	1.326 a	1.801 b	1.323 a	1.343 b	1.373 b	1.363 c
Mg	100	1.040 b	1.691 c	1.253 b	1.363 a	1.431 a	1.501 a
Ti	200	1.463 a	1.996 a	1.153 c	1.293 c	1.366 c	1.456 b
Control		0.976 b	1.651 d	0.943 d	1.010 d	1.056 d	1.096 d

In the same colum, means followed by a common letter are not significantly different at the 5% level by DMRT.

al.(2014) and Anusuya and Sathyabama (2015) reported that, NPs of selenium and B-d glucan were significantly alteration of ROS(PO,PPO, glucanase and protease inhibitors) and protected turmeric plants against rot disease. Moreover, Lu et al. (2002) added that, TiNPs increase of nitrate reductase and stimulation antioxidant effects. Dimkapa et al. (2011), Lipovsky et al. (2011) and Patra et al. (2012), showed that, ZnNPs bactericidal and fungicidal due to induce ROS generation intracellular. Ti and Zn NPs exhibited strongly activator of PO enzyme and may play an essential role in reducing ears and kernels rot disease symptoms, restricting development of causal pathogens (Farahat et al. 2017). Although, increase of ROS effective to control plant pathogens (Wani and Shah, 2012). Lower disease severity in different plants was in line of high activity of protective enzymes of PO and PPO in rice (Datnoff et al. 2007), wheat (Nanayakkara et al. (2008) and cucumber (Rezende et al.2009). These enzymes regulation production of antifungal compounds like phenols, lignin and phytoalexins which assigned role in disease resistance (Vidhyasekaran 1988).

5-Effect of environmental conditions of cercospora leaf spot disease

a- Responsibility of sugar beet cultivars to cercospora leaf spot disease

Data in Table 6 recorded high disease severity percentages in 2015/2016 season than other one 2016/2017, as well as, in the first date planting than the second one with most of tested sugar beet genotypes to cercospora leaf spot disease under field conditions. According to used scale of disease severity of **Shane and Teng (1992)**,

TABLE 6. Response of nine sugar beet cultivars to cercospora leaf spot disease in seasons 2015/2016and 2016/2017 with two dates planting.

Genotypes	Seasons	Disease severity %	
		First date (September)	Second date (October)
Alouda	2015/2016	30.00 de	31.66 c
	2016/2017	12.33 ghi	0.52 g
Maimouna	2015/2016	26.66 ef	17.33 e
	2016/2017	14.01 gh	1.01 g
Beta 3980	2015/2016	45.02 bc	36.66 bc
	2016/2017	14.33 g	0.53 g
Beta 8115	2015/2016	48.33 b	38.33 b
	2016/2017	41.66 c	1.33 g
Nefirtitis clg	2015/2016	9.00 ghi	12.33 ef
	2016/2017	8.33 hi	0.41 g
Cigogne	2015/2016	7.33 i	8.33 f
	2016/2017	8.33 ghi	0.17 g
Toucan	2015/2016	30.00 de	25.01 d
	2016/2017	21.66 f	0.21 g
Carnute	2015/2016	31.66 de	38.33 b
	2016/2017	31.67 de	0.93 g
Oscarpoly	2015/2016	55.00 a	45.08 a
	2016/2017	48.33 b	2.01 g

In the same colum , means followed by a common letter are not significantly different at the 5% level by DMRT

sugar beet genotypes of Alouda, Maimouna, Beta 3980 and 8115, Toucan, Carnute and Oscar poly expressed susceptible ones to the disease and recording disease severity ranged from 26.66 to 55.00% in season 2015/2016 with first planting date **(September)**, 17.33 to 45.08 with the second planting date **(October)** and 12.33to 48.33 and 0.21to 2.01% in season 2016/2017, respectively, while, Nefirtitis clg and Cigogne were resistant ones and recording disease severity ranged from 0.17 to 12.33 in the two tested seasons.The results showed significant differences in disease severity between two tested seasons with every tested susceptible genotype. Data showed also, dramatically decrease of disease severity in the second tested season (2016/2017) with the two planting dates wherever recorded slight disease severity (very low infection). So, effect of weather conditions must be studied to explain this point.

b-Cercospora leaf spot disease development and metrological parameters effects

Aforementioned studies of both NPs and sugar beet genotypes were recorded high disease severity of cercospora leaf spot disease in season 2015/2016 than season 2016/2017 which recorded slight infection, that reflects the favorable weather conditions to cause and development of cercospora leaf spot disease. Data in Table 7 introduce the explanation of this results, whereas, max air temperature degrees recorded low values of periods of disease development in Feb. and March in second season of 2016/2017 *ie* 19.7and 21.7 compared to 22.58 and 24.5 °C of these months in the first season 2015/2016 so, max temperature degrees were more suitable for doing infection and disease development in the first season than the second one. Air min temperature degrees in Nov. and March recorded high values in second season 2016/2017 *ie.* 17.90 and 17.91 compared to 14.42 and 11.6 in same months in first season 2015/2016, also, cool weather in Jan. in the second season, this may be retarding the disease and/ or cause slight infection and decreasing disease severity in this season (2016/2017). RH% at 13.30 PM recorded decreasing in months of Nov. and Dec. in season 2016/2017 (period of disease development of first date planting), as well as decrease of wind velocity in Nov., this led to decrease of disease severity in this date than the same one in season 2015/2016. Whenever, wind help to distribution and transport of cercospora inoculum from field, location and date planting to others. Pan evaporation (mm) recorded very high amount in 2015/2016 season with all months and safe suitable conditions to spores germination, success of infection and disease development in the mentioned experiments above but the reverse was true in the season 2016/2017, so slight disease severity was showed in this season. Season of 2015/2016 recorded high rain in the begging of disease development period (Nov.) and high total

TABLE 7. Grand mean of metrological parameters during the growing seasons 2015/2016 and 2016/2017 from November to March at Sakha Agriculture Research Station

Seasons	Month	Air Temperature °C		RH%		Wind	PE	Rain
		Max.	Min.	7:30 AM	13.30PM	Km/24hrs	(mm)	(mm)
2016/2015	Nov.	24.4	14.42	87.0	64.2	70.3	813.5	16.14
	Dec.	19.7	8.4	88.6	67.2	57.9	250.4	6.54
	Jan.	18.4	6.5	85.6	62.5	69.2	252.4	5.10
	Feb.	22.6	9.4	85.0	53.1	58.8	251.9	0.0
	Mar.	24.5	11.6	81.5	58.3	63.2	359.2	4.2
2017/2016	Nov.	24.9	17.9	77.9	56.8	56.0	198.1	0.0
	Dec.	19.3	10.8	85.4	65.1	64.7	156.4	7.1
	Jan.	18.2	5.2	87.3	62.9	51.9	136.2	4.8
	Feb.	19.7	10.2	85.8	60.1	58.3	214.4	4.8
	Mar.	21.7	17.9	84.9	60.4	83.8	295.4	0.0

Max. = Maximum,Min. = Minimum, PE = Pan evaporation, RH% = Relative humidity

rain amount *i.e.* 16.14 and 31.98 mm compared to 0.00 and 16.7 mm in season 2016/2017. High rain safe favorable RH to cause infection and disease development. So, grand mean of metrological factors were more suitable in season 2015/2016 than 2016/2017 for cercospora disease in sugar beet plants in Sakha location.Weather conditions were most effective elements of cercospora leaf spot disease infection and development and discussed by many researchers *i.e.* Kerr and Weiss (1990) found that, CLS was most destructive in warm humid conditions responsible for reducing of yield, sucrose and increasing of impurities and costs, Karaoglanidis et al. (2001). Windles et al. (1998) added that, *C. beticola* infection depending on weather conditions after infection. Consequently, Khan et al.(2007) reported that, unseasonably low temperature (15-16 compared to22^{o}C) was the main factor for low disease severity, since bellow 10^{o}Cwas strong inhibition for cercospora disease development, Shane and Teng (1984) but optimum ranged from 20-25^{o}C, disease was development between 20-30 ^{o}C and RH 75-90%, free water, Wolf et al. (2001) and Wolf and Verreet (2005). Moreover, Khan et al. (2008) showed that, wind was the major dispersal factor for *C. beticola* inoculum and optimum temperature for spore germination 24.4 ^{o}C when RH was100% (Jones and Windles 1991). Harveson (2013) concluded that, epidemics of CLS depending presence of susceptible varieties, adequate inoculum and RH above 90% longer 11 hours, accompanied by warm temperature in sugar beet canopy. Additionally, Salama (2017) reported that, adequate and suitable weather conditions for CLS disease were 16.66-26.9 ^{o}C, RH (83.36-95.86), wind velocity (35.0-91.88 km /24 hr and rain up to 16 ml). Ruppel (1986) added that CLS disease developed in favorable conditions *ie*. day temperature of 25 to 35 °C, night temperature of 16 °C, end prolong periods of RH of 90-95 % or free water on leaves.

Conclusion

This study has further revealed that, *M.nigra* and *G.robusta* confirmed the bio synthesis of ZnONPs from leaves mix extracts (reaction mixture), certain nanoparticles treatments can reduce the cercospora leaf spot disease of sugar beet under field conditions and enhancement of TSS, sucrose and yield by activation of peroxidase and polyphenol oxidase enzymes, as well as, metrological parameters effects of cercospora leaf spot disease development were subjected.

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