J. Plant Prot. and Path., Mansoura Univ., Vol.7 (3): 171– 181, 2016

EFFECT OF ZINC OXIDE NANOPARTICLES ON BROWN SPOT DISEASE AND RICE PRODUCTIVITY UNDER SALINE SOIL

Elamawi, R. M. [1*], S.M. Bassiouni[2], W. M. Elkhoby[2] and B.A. Zayed [2]

[1]Rice Pathology Dept., Plant Pathol. Res. Inst., Agric. Res. Center, Sakha, 33717, Kafr El-Sheikh, Egypt.

[2] Rice Research Department, Field Crops Research Institute, Agricultural Research Center, Egypt.

*** Corresponding author: rabab.elamawi@yahoo.com**

ABSTRACT

The present study investigates the effect of zinc oxide nanoparticles (ZnO-NPs) on rice plants growth and their role in management of brown spot disease caused by the causal agent *Helmenthosporium oryza*. The antifungal activity of ZnO-NPs (20 to 35 nm particle size) was evaluated at different concentrations. Spores germination percentage, colony formation and sporulation of *H. oryza* were reduced at concentrations 25 and 50 ppm, *in vitro.* The greenhouse results showed that seed soaking treatment and foliar spray 5 day before inoculation (DBI) of ZnO-NPs leaded to reduce infection percentage of brown spot without significant difference between ZnO-NPs concentrations. Foliar spray ٢ days post-inoculation (DPI) with the lower concentrations 10 and 25 ppm of ZnO-NPs were able to reduce infection percentage of brown spot. Under field conditions, During 2013 and 2014 seasons at El-sirw Agriculture Research Station, Dammietta, Egypt, rice varieties Giza 177, Giza 178 and Giza 179 under ZnO-NPs level (0, 10, 20 and 30 ppm) as foliar spray twice at mid tillering and panicle initiation stages were evaluated. Application of ZnO-NPs at level 20 ppm effectively reduced brown spot disease severity and discolored grains of all tested varieties. The studied rice varieties were varied in their growth, yield attributes, grain yield and brown spot severity whereas Giza 178 and Giza 179 had good performance under ZnO-NPs treatments. Therefore both Giza 178 and Giza 179 had showed significant salt tolerance at 20 ppm of ZnO-NPs. The performance of Giza 177 as a salinity-sensitive variety was improved at 20 ppm ZnO-NPs. The all ZnO-NPs treatment positively improved rice growth, yield attributes, rice grain yield and brown spot severity over control treatment. Finally, ZnO-NPs can be used as future "nanofertilizers".

Keywords: Nanofertilizer, Zinc Oxide, *Helmenthosporium oryza*, *Oryza sativa*, L.

INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple food crop for half of the world's population. In Egypt, rice is the second staple food after wheat, and is important for local consumption and export. In Egypt, rice is annually grown in more than one million feddans, mostly in the Northern part of the Nile Delta. The cultivated area in 2015 season was 1.1 million feddans that produced about 4.2 million tons of paddy rice (RRTC, 2015). However, rice diseases can reduce yield production by about 5 % in normal or mild disease outbreaks, but during epidemic seasons the yield losses may reach as high as 30-50 % (Sehly *et al.* 2002). In Egypt, Brown spot disease is caused by *Helminthosporium oryzae* (Breda de Haan) Shoemaker (Ou 1985), it comes in the second rank after blast disease, especially under specific conditions as nutritionally deficient and unfavorable soils. However, these nutritional disorder promote the disease outbreak (El-wahsh 1997). The disease can occur at all crop development stages, it causes seedling blight and damages of the foliage and panicles of rice, causing seed discoloration (Elwahsh el al., 2008).

Micronutrients are very important for health of plant and have a great concern with the yield. Zinc deficiency is one of the most important micronutrient problems in submerged soil, globally (Quijano-Guerta *et al.* 2002). So, Zinc becomes unavailable to the plant because of their precipitation in the form of carbonate, phosphate and ZnS due to the reduced conditions (Ponnampperuma 1984 and Sims 1986). In Egypt, under high pH and saline sodic soil as well as using high yielding rice varieties and non-crop rotation, the rice varieties were varied in their growth, yield attributes and grain yield under that condition (El-wahsh *et al.* 2005). So, zinc fertilizer was become necessary to improve rice growth and obtaining high grain yield (Shehata *et al.* 2009 and Amira, 2011). Zinc is essential for biochemical processes in rice crop such as Nucleotide and cytochrome synthesis, metabolism of auxin, production of chlorophyll, enzymes activity and membrane integrity (Kirk and Bajita 1995).

Nanotechnology has the potential to play a critical role in global food production, food security, and food safety. Applications of nanotechnology in agriculture include fertilizers to increase plant growth and yield, pesticides for pest and disease management, and sensors for monitoring soil quality and plant health (Selivanov and Zorin 2001, Raikova *et al.* 2006, Batsmanova *et al.* 2013 and Servin *et al.* 2015). The potential benefits of nanotechnology have been widely reported but fate of nanomaterials on agriculture or environment is not well studied. The impact of nanomaterials on agriculture and the environment, in general were discussed by Phogat *et al.* (2016). Zinc oxide (ZnO) was one of the nanomaterials which selected for testing in the OECD (Organization for Economic Co-operation and Development (OECD) Sponsorship Programme OECD (2010). Zinc oxide nanoparticle (Zn-ONPs) is a plant nutrient without the harmful factors of chemical fertilizer. As far as their usage is concerned nanoparticles play a significant role in agriculture, where ZnO-NPs is used in nanofertilizers (Milani *et al.* 2015). Application of ZnO-NPs to crops increases their growth and yield. Nanopowders can be successfully used in very small amounts with several crops as fertilizers and pesticides as well (Selivanov and Zorin 2001, Raikova *et al.* 2006 and Batsmanova *et al.*

2013). The adsorbed nanoparticles are gradually penetrated into the plant tissues, and can enter in the plants through the shoot and root, like, cuticle, epidermis, stomata, hydathodes, stigma, root tips, rhizodermis, cortex lateral plants, root junctions, bark and other several surfaces of plants (Eichert *et al.* (2008) and Dietz and Herth, 2011).

Antifungal activity ZnO-NPs against fungi was evaluated. The effect of ZnO nanoparticles against the fungus *Penicillium expansum* and *Aspergillus niger* were reported by He *et al.* (2011) and Chitra *et al.* (2013), respectively. High inhibition rate in the germination of fungal spores of *Alternaria alternate, F. oxysporum, Rhizopus stolonifer,* and *Mucor plumbeu* was reported by Wani and Shah (2012). The inhibition effect of Silver nanoparticles was examine for two plant-pathogenic fungi on rice plants, *Bipolaris sorokiniana* (Young *et al.* 2009) and *Magnaporthe grisea* (Young *et al.* 2009 and Elamawi and El-shafey 2013). Biosynthesized silver NPs improved the seed germination percentage, vigour index and, reduced the disease incidence caused by *Fusarium oxysporium* on faba bean, tomato and barley (Elamawi and Al-Harbi 2014). Studies reveal that the enhanced bioactivity of ZnO-NPs as smaller particles is attributed to the higher surface area to volume ratio. Active oxygen species generated by ZnO-NPs could be the main mechanism of their antibacterial activity.

The objectives of this study were to determine the inhibitory property of zinc oxide nanoparticles on spore germination ,colony formation and sporulation of plant-pathogenic fungus *Helminthosporium oryzae In vitro* and to evaluate the efficacy of the ZnO nanoparticles for rice brown spot disease control under green house and field saline conditions. In addition, influence of ZnO nanoparticles feeding on the growth and development of rice plants under saline soil.

MATERIALS AND METHODS

Materials preparation: Nano-ZnO was prepared ZnO nanopowder (MKImpex Corp Mississauga, Canada). For each experiment, Nano-ZnO solution was prepared freshly by dispersing nanoparticles in de-ionized water through ultrasonication (300 W, 40 kHz) for 30 minutes. According to the manufacturer and the TEM (Table 1), the particle sizes were ranged from 20 to 35 nm and were nearly spherical to hexagonal shaped.

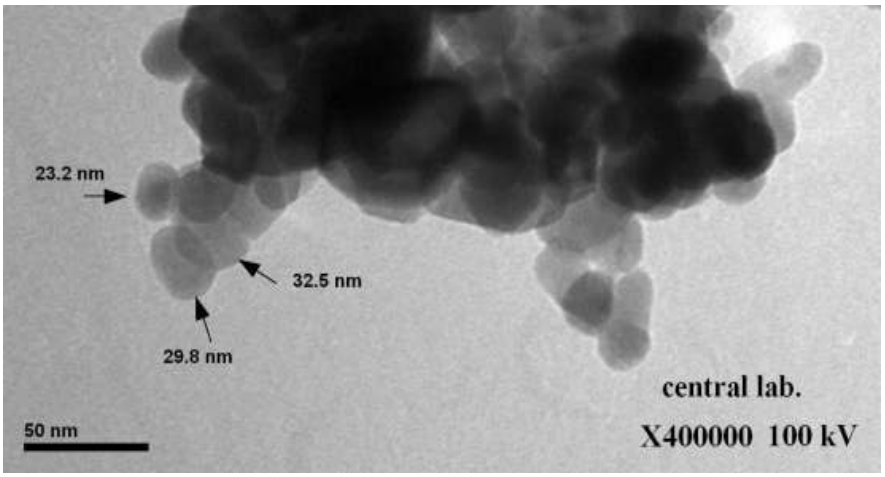

Fig. 1: TEM picture of Zinc Oxide nanoparticles

Laboratory Experiments: A pure culture of *H. oryzae* was grown on Potato dextrose agar (PDA) medium at 28 ^{o}C. ZnO-NPs concentrations of 10, 25, 50, 100, 200 and 400 ppm were evaluated for their effect on the spore germination, colony formation and sporulation capacity of *H. oryzae.* Spore suspension of isolate was prepared from 7 days old fungal culture. One drop about 0.1 ml of spore suspension at concentration $1x10^5$ spores/ml was put in a cavity glass slides containing a drop (about 0.1ml) of different concentration of nanoparticles and were incubated at 28°C for 48 hours. Each treatment was replicated five times. The percentage of germinated spores was recorded. The colony formation was prepared according to Elamawi and El-Shafey 2013. Whereas, spore suspension at concentration of $1x10^6$ spore/ml were mixed with serial concentrations of ZnO-NPs or sterile deionized water as control. Volume of 50 µl aliquot of each treatment was spread on PDA and incubated at 28^{o} C. The number of formed-colonies on plates was counted after 2, 4 and 5 days. The Sporulation capacity was estimated by adding 10 ml of distilled water to each dish, and then the spores were harvested by spatula. The suspension was filtered. The number of spores/ ml was counted using the hemocytometer.

Greenhouse assay: ZnO-NPs at concentrations 10, 25, 50, 100 and 200 were used against brown spot disease. ZnO-NPs were applied as follow: seed soaking, 5 days before inoculation (DBI), 2 days post inoculation (DPI). ZnO-NPs solutions were sprayed on rice seedlings at 21-days old. Inoculation with spore suspensions was at concentration $1x10^5$/ml. Control was applied as spray with water and inoculated. The inoculated seedlings were held in a moist chamber with at least 90%

R.H. and 25-28 °C for 24 hr. and then moved to the greenhouse. Disease assessment for brown spot infection was assessed as a percentage by counting the number of infected leaves of 10 randomly selected leaves per pot.

Field experiments: Under saline soil at the research farm of El-Sirw Agricultural Research Station, Dammitta, Egypt. During 2013 and 2014 rice growing seasons, three rice varieties Giza 177, Giza 178 and Giza 179 had been selected according to their different performance under saline soil. Different concentrations of ZnO-NPs; 10, 20 and 30 ppm were applied as foliar spray at mid tilling and panicle initiation stages. $ZnSo_4$ 2% as seed soaking for 48h and untreated control were applied as a comparison check. Representative soil samples were taken from 0-30 cm depth and subjected to chemical and physical analysis according to Piper (1950) and Black (1983) and listed in table 1 .

Table 1: Chemical analysis of the experimental sites at El-Sirw Station.

Season	pH	E.C.	Cat ion meqL^{-1}				Anion MeqL^{-1}			$CaCo_3$	Available (ppm)		
		dSm^{-1}	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	So_4	Cl^{-}	HCO^{-}_3		P	K	Zn
2013	8.2	7.7	7.3	13.5	56	0.46	21.8	45.9	10.5	0.43	18	297.2	1.09
2014	8.3	8.0	9.8	15.5	59	0.75	18	52.4	11	0.73	17	296.1	99.6

The experiment was designed in split plot arrangement with completely randomized block design with four replicates. The varieties were arranged in main plots. The zinc treatments were allocated in sub plot. The size of each plot was 10m^2 (5 x 2 m). The plants aged 30 days were transplanted in the space of 20x20 cm apart by 4 seedlings hill. Phosphorous fertilizer (21kg P_2O_5/fed) and Potassium sulphate (24 kg K_2O/fed) were applied basally at tillage stage. Nitrogen fertilizer in the form of Urea at the rate of 69 kg N/ fed was applied in three equal doses at; 15 days after transplanting (DAT), 30 DAT and 45 DAT. The rest of management issues were typically followed according to Rice Research and Training Center recommendations.

Pathological Measurements: samples of rice leaves were taken at maximum tillering and panicles stage. Total number of brown spot lesions was counted from randomly collected hundred leaves and recorded as severity of infection (El-Wahsh 1997). Samples of one hundred grains were taken for estimating the discolored grains as disease percentage after harvest.

Agronomic Measurements: For measuring leaf area index, dry matter production and chlorophyll content, 5 hills were randomly taken at heading and transferred to lab. Leaf area was measured by leaf area meter while total chlorophyll content was measured by SPAD value meter model502 according to Yoshida *et al.* (1976). Prior to harvest, five main panicles were randomly collected from each plot to determine the main yield attributes; panicle length, panicle weight, filled grains./panicle, unfilled grains/panicle, 1000-grain weight. The tiller and panicles of five hills were counted to determine tillers and panicles number m^2. The plants of the sex inner rows of each plot were harvested, dried and threshed to determined rice grain yield and straw yield. The grain yield was adjusted based on the moisture content of 14%.

Statistical analysis: The collected data were analyzed for analysis of variances according to Gomez and Gomez (1984). Multiple mean comparison analysis for treatment combinations of variety and stress treatment was performed using least significant difference at α = 0.05 level when F-test was significant.

RESULTS AND DISCUSSION

Laboratory Results: Effect of ZnO nanoparticles on spore germination, of *H. oryzae* was tested. Microscopic observation appears that different concentrations of ZnO-NPs inhibited the spore germination after 48h post treatment. The maximum inhibition was found at concentration 50 and 100 ppm without significant differences as compared to untreated control whom showed least inhibition (Table 2). In addition, ZnO-NPs caused reduction in germ tube growth as shown in Fig 2.

Different antimicrobial efficiency of the ZnO-NPs was observed on *H. oryzae* colonies numbers. The formed-colonies were counted after 2, 4 and 5 days. The results showed that ZnO-NPs treatments were retarded up to 4 days compared to those developed with the zero concentration (control with water), that appeared after 2 days and strongly increased with time. The growth inhibition with ZnO-NPs was not dose-dependent where formed colonies were increased at 10 and 25 ppm of ZnO-NPs concentrations more than zero concentration after 3 days of inoculation on PDA (Table 2**)**. After 5 to 7 days post inoculation, the characteristic olive color with white sterile heads for *H. oryzae* on cultural media were appeared with different concentrations and the control (water), except the concentration 25 and 50 ppm (Fig 3). In parallel, the both concentration 25 and 50 ppm were produced lower number of spores /ml.

Nanoparticle ZnO is recently shown to provide effective pathogen growth control. With lower toxicity and secondary benefits on soil fertility, ZnO-NP has clear advantages over Ag for fungal pathogen control efforts (Dimkpa *et al.* 2013). He *et al.* 2011 showed that ZnO-NPs (3-12 mmol) significantly inhibited *B. cinerea* (63–80 %) and *P. expansum* (61–91 %) growth in a plating assay, systemic disruption of cellular function within both pathogens was observed, thereby resulting in hyphal malformation and fungal death (He *et al.* 2011). Wani and Shah (2012) reported a high inhibition rate in the germination of fungal spores of *Alternaria alternate, F. oxysporum, Rhizopus stolonifer,* and *Mucor plumbeus* upon exposure to ZnO-NP at concentrations as low as 100 mg/L. There are several

mechanisms which have been proposed to explain the antimicrobial activity of ZnO nanoparticles. The generation of hydrogen peroxide from the surface of ZnO-NP is considered as an effective mean for the inhibition of fungal growth (He *et al.* 2011). It is presumed that with decreasing particle size, the number of ZnO powder particles per unit volume of powder slurry increases resulting in huge surface area and increased production of hydrogen peroxide. Another possible mechanism for ZnO-NP as antifungal activity is the release of Zn^2+ ions which can damage the cell membrane and interact with intracellular contents (Sirelkhatim, 2015).

Greenhouse Results: It was necessary to investigate if the inhibitory effects of different concentrations of ZnO-NPs on symptom expression in rice plants might have resulted from their direct toxic action on the pathogen. So, ZnO-NPs at concentrations 10, 25, 50, 100 and 200 ppm were used as seed soaking, foliar spray 5 DBI and 2 DPI on Giza 177 as brown spot susceptible variety. The results showed that seed soaking treatment and foliar spray 5 DBI leaded to reduce infection percentage of brown spot without significant difference between ZnO-NPs concentrations. In contrary, the treatment of ZnO-NPs two days post inoculation was failed to reduce infection percentage of brown spot at higher ZnO-NPs concentrations. But lower concentrations 10 and 25 ppm of ZnO-NPs were able to reduce infection percentage of brown spot. In addition, there is no noticeable phytotoxicity was observed on different varieties in comparison with the control. Contrary results were observed by Lin and Xing 2008 which, phytotoxicity of commercially available ZnO nanoparticle to rye grass was reported. Moreira *et al.* 2013 showed that high foliar concentration of Zn was associated with high concentrations of Zn in leaf tissues consequently increasing rice susceptibility to brown spot (Moreira *et al.* 2013). According to Duffy (2007), the intensity of several diseases can be reduced or increased by supplying Zn to plants. The mycelial growth of several fungi species in vitro can be stimulated by Zn deficiency due to an increase in nitrogen uptake and the production of cytotoxic secondary metabolites (Duffy 2007).

Fig 2: Micrograph of germ tube growth of *H. oryzae* after 28 h of ZnO nanoparticles treatment. A: non-germinated spore, B: unipolar germination, C: D: dipoler germination, E; unipolar germination with long mycelium. Scale bar =10 µm

Table 2: Effect of ZnO-NP on spore germination, Colony formation and sporulation of *H. oryzae, In vivo*.

Concentration of ZnO-NPs ppm	Spores germination %	Number of formed-Colonies(cm^2)	*No. of spores /ml
Control	42.00e	42.33b	٣٨.٧c
ZnO-NP10	27.60abc	54.00a	٢٨.١ba
ZnO-NP 25	23.75ab	42.50b	٢٣.٨a
ZnO-NP 50	17.80a	36.33b	٢٠.٥a
ZnO-NP 100	18.50a	28.33c	٢٢.٧a
ZnO-NP 200	33.10bcd	55.00a	٣١.٠ab
ZnO-NP 400	39.45cd	54.33a	39.4c

***No. of spores×10^4**

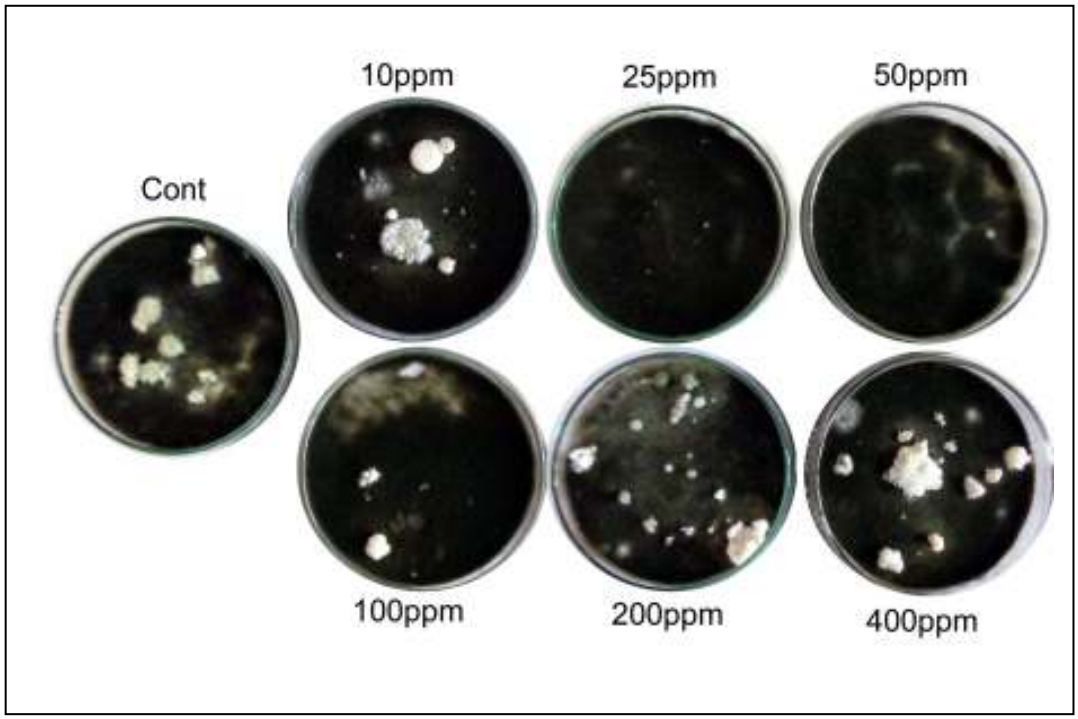

Fig. 3: Effect of ZnO-nanoparticles on Number of formed-colonies of *H. oryzae* on PDA medium after 7 days.

Table 3: Effect of ZnO-NPs on brown spot infection on Giza 177 variety under greenhouse conditions.

Concentration of ZnO-NPs ppm	Infection % of brown spot leaves		
	Seed Soaking	5DBI*	2DPI**
Control	68.33b	68.33b	68.33bc
ZnO-NP10	28.66a	9.33a	35.33a
ZnO-NP 25	23.66a	11.66a	40.33a
ZnO-NP 50	28.33a	16.66a	45.66ab
ZnO-NP 100	25.00a	16.00a	54.66bc
ZnO-NP 200	21.66a	17.66a	58.00bc

*** DBI; days before inoculation, **DPI; days post-inoculation**

Pathological measurements under field conditions: ZnO-NPs at concentrations 10, 20, 30 ppm were selected according to the results of Lab. and greenhouse assay which showed that the effective ZnO-NPs concentration ranged from 10 to 50 ppm. Under saline soil, foliar spray of ZnO-NPs was applied twice at mid tillering and panicle initiation stages on three rice varieties Giza 177, Giza 178 and Giza 179. The effect of ZnO-NPs against *H. oryzae* at different concentrations was presented in Table (4). In general, the brown spots infection % and severity (number of spots/ 100 leaves) were reduced at 20 ppm of ZnO-NPs solution in both seasons regardless varieties response (Table 4). Rice varieties had certain variation in both seasons regarding diseases infection. Giza 178 rice variety produced the lowest infection % and severity of infection of brown spot. In contrary, the other two varieties Giza 177 and Giza 179 showed high infection percentage and severity of brown spot infection in both seasons. ZnO-NPs application generally had a positive impact on the severity of brown spot infection over the control treatment. Furthermore, zinc sulfate treatment showed the lower severity of brown spot infection control. Foliar application at the concentration of 20 ppm ZnO-NPs decreased the severity of brown spot infection followed by the concentration 10 and 30 ppm.

Effect of ZnO-NPs on seed discoloration: Discolored grains were decreased for Giza 179 and Giza 178 varieties compared to Giza 177 rice variety. These results are in agreement with the findings of El-Wahsh (1997) who mentioned that percentage of discolored grains differed from one cultivar to another. Concerning the effect of different concentration of ZnO-NP, various ZnO-NPs concentrations significantly affected the seed discolored in both seasons. The ZnO-NPs concentration at 20ppm reduced discolored grains in both seasons comparing with untreated check control. ZnO-NP at 30 and 10 ppm and $ZnSO_4$ 2% as well as untreated check control were at the same level of significant in 2013 seasons (Table 4). The interaction between rice varieties and zinc treatments had significant effect on infection %, Severity of infection and discolored grains percentage in both seasons (Table 5). The interaction effect markedly provide the integration of Giza 178 and Giza 179 with ZnO-NPs of 20 ppm to lower infection %, infection severity of brown spot and discolored grains % in both seasons (Table 5).

The smaller size of NPs facilitates easy entry into the microbial cell membrane and enables inhibition mechanisms to occur inside the cell. ZnO-NPs generate hydrogen peroxides which chemically interact with membrane proteins and lipid bilayers (Aneja 2003). The antimicrobial activity of these NPs may involve both the production of reactive oxygen species (ROS) and the accumulation of NPs in the cytoplasm on the outer membranes. ROS causes membrane dysfunction and cell death by oxidizing the membrane lipids (Akhtar *et al.* 2012 and Sirelkhatim, 2015).

Table 4: Infection %, severity of infection % of brown spot and discolored grains % of rice varieties as affected by ZnO-NPs foliar application during 2013 and 2014 seasons

Factor	Infection %		Severity of infection (No. of spots/ 100 leaves)		Discolored grains %	
	2013	2014	2013	2014	2013	2014
Varieties (A):						
Giza 177	59.00a	59.33a	223.2 a	220.9 a	47.7 a	49.5 a
Giza 178	22.87b	23.47b	78.4 b	84.4 b	24.6 b	24.7 b
Giza 179	63.07a	61.07a	219.2 a	218.3 a	28.7 b	29.2 b
F test	**	**	**	**	**	**
Treatment (B):						
Control	64.11a	59.33a	231.6 a	242.0 a	37.2 a	37.9 a
$ZnSO_4$ 2%	49.44b	47.78ab	193.3 b	200.1 b	36.6 a	36.6 a
ZnO-NP 10ppm	44.11bc	46.00b	147.6 cd	144.6 c	33.4 a	33.7 b
ZnO-NP 20ppm	36.89c	41.56b	137.8 d	128.9 d	24.9 b	27.6 c
ZnO-NP 30ppm	47.00b	45.11b	157.8 c	157.1 c	36.1 a	36.7 a
F test	**	**	**	**	**	*
Interaction (A*B)	**	**	**	**	**	**

Table 5: Effect of the interaction between ZnO-NPs foliar application and rice varieties during 2013 and 2014 seasons

Interaction	season	Infection %			Severity of infection (No. of spots/ 100 leaves)			Discolored grains %		
		Giza177	Giza178	Giza179	Giza177	Giza178	Giza179	Giza177	Giza178	Giza179
Control	2013	79.3a	34de	79ab	306.7a	131gh	288ab	54.0a	23.7fgh	34cde
$ZnSO_4$ 2%		61.3c	24.7efg	62.3	275.0b	73.3i	252c	49.7a	28.0ef	32de
ZnO-NP 10ppm		55.0c	14.0fg	63.3bc	177.0e	43.7j	213d	41.7b	23.0fgh	35.7bd
ZnO-NP 20ppm		50.3c	12.3g	48.0cd	176.7e	61.0ij	149fg	39.3bc	18.3gh	17.0h
ZnO-NP 30ppm		49cd	29.3ef	62.7c	169.3ef	113h	189e	53.7a	30def	24.7fg
Control	2014	76.0a	28.7de	73.3a	312a	105.3f	277a	55.7a	25.0fg	33.0de
$ZnSO_4$ 2%		58.7ab	23.3e	61.3ab	275b	66.7g	256b	48.3b	28.0f	33.3de
ZnO-NP 10ppm		55.3ab	20.0e	62.7ab	181de	42.7g	219c	42.7c	22.3gh	36.0d
ZnO-NP 20ppm		60.0ab	14.0e	50.7bc	187d	66.7g	160e	45.7bc	19.0h	18.0h
ZnO-NP 30ppm		46.7bcd	31.3cde	57.3ab	179de	111f	184de	55.3a	29.0ef	25.7fg

Agronomy characteristics results under field conditions: Dry matter, leaf area index and chlorophyll content were maximum with Giza 179. However, Giza 178 and Giza 179 were identical regarding the above mentioned traits. Giza 177 was proved as salt sensitive variety for the mentioned growth traits. Giza 178 had the longest period to days to heading (Table 6). On the other hand, Giza 179 was found to have the shortest period in the current study. Zinc as nano-particles is being effective in improving rice growth as it seems in Table 6. Foliar application of ZnO-NPs was superior to $ZnSo_4$ particularly at the levels of 20 and 30 ppm. The growth parameters were gradually increased up to 30 ppm but it was significant up to 20 ppm. The values of growth parameters with control in terms of proved the imperative need of rice to zinc application. Also, ZnO-NPs application as a foliar showed certain positive role in improving rice growth under saline soil. The positive role of ZnO-NPs on rice growth, it may be attributed to its role in reducing Na^+ uptake. There is a close link between Zinc and auxin levels in plants that support the role of Zinc application. The findings are in the conformity with Khan *et al.* (2009), Shehata *et al.* (2009) and Amira (2011). Regarding days to heading was significantly shortened, especially with Zn level elevating (Table 6).

All yield attributes significantly differed among tested rice varieties (Table 7). Giza 178 and Giza 179 had the highest mean values of yield attributes in both seasons. While, Giza 177 was showed lower performance under such stress. The same trend was obtained in the yield attributes of varying rice variety. ZnO-NPs treatments significantly influenced the yield component. The highest values of yield component were recorded with concentration of 30 ppm ZnO-NPs in both seasons (Table 7). ZnO-NPs at concentration 20 and 30 ppm were at the same trend of plant height, panicle length, tillers and panicles numbers, panicle weight, 1000-grain weight and filled grains $panicle^{-1}$. Markedly, ZnO-NPs application showed high affinity to minimize the sterility of grains.

Table 6: Growth characteristics of rice varieties as affected by ZnO-NPs foliar application during 2013 and 2014 seasons.

Factor	Dry matter (g/m^2)		Leaf area index (LAI)		Chlorophyll content (SPAD)		Days to Heading (day)	
	2013	2014	2013	2014	2013	2014	2013	2014
Varieties(A):								
Giza 177	384.3b	437.4b	2.91b	2.88b	39.68b	42.11b	93.4b	96.3b
Giza 178	576.0a	647.2a	4.09a	4.67a	41.36a	43.13a	101.5a	101.4a
Giza179	570.2a	625.7a	3.93a	4.22a	41.31a	42.70a	91.1c	91.4c
F test	**	**	**	**	*	**	**	**
Treatments(B):								
Control	420.8c	511.6b	3.45b	3.43c	39.97b	41.66b	95.8a	96.9a
$ZnSO_4$ 2%	497.0b	559.4ab	3.61ab	3.46c	40.67ab	42.68a	95.2b	96.7ab
ZnO-NP 10ppm	502.2b	567.7a	3.63a	3.99b	40.47ab	42.54ab	95.3b	96.3bc
ZnO-NP 20ppm	553.4a	605.8a	3.76a	4.32ab	41.22ab	43.00a	95.2b	96.2c
ZnO-NP 30ppm	577.3a	605.9a	3.77a	4.42a	41.56a	43.36a	95.2b	95.7d
F test	**	**	**	**	*	**	*	**
Interaction(A*B):	NS	NS	NS	NS	NS	NS	NS	NS

Table 7: Yield attributes of rice varieties as affected by ZnO-NPs foliar application during 2013 and 2014 seasons.

Factor	Plant height (cm)		No. of tillers (m^2)		No. of panicles (m^2)		Panicle length (cm)	
	2013	2014	2013	2014	2013	2014	2013	2014
Varieties (A):								
Giza 177	74.77c	76.30c	369.8b	380.3c	344.3b	338.5b	17.90b	16.56c
Giza 178	80.17a	83.50a	473.6a	514.2a	419.5a	438.3a	19.02a	18.79a
Giza179	78.42b	78.00c	464.0a	483.9b	407.4a	425.8a	18.83a	17.77b
F test	**	**	**	**	*	**	**	**
Treatments (B):								
Control	75.70d	75.81c	403.9d	438.5b	361.0b	367.3c	18.43c	17.37b
$ZnSO_4$ 2%	77.51c	78.90b	425.6c	447.4b	374.3b	385.4c	18.48bc	17.42b
ZnO-NP 10ppm	77.90c	80.20a	436.7bc	452.1b	377.6b	391.6bc	18.63ab	17.73ab
ZnO-NP 20ppm	78.70b	80.81a	450.6ab	478.0a	421.5a	418.2ab	18.66ab	17.90ab
ZnO-NP 30ppm	79.20a	80.80a	462.4a	481.4a	417.5a	441.9a	18.72a	18.11a
F test	**	**	**	**	**	**	**	*
Interaction(A*B):	**	**	NS	NS	NS	NS	NS	NS

The 20 and 30 ppm levels of ZnO-NPs were at the same trend regarding all yield attributes. At the same time the treatments of $ZnSo_4$ and 10 ppm ZnO-NPs had the same level of significant in all measured yield attributes. Furthermore, the level 10 of 20 ppm was significantly better under such condition since soil is suffering from Zn deficiency. The control treatment gave the lowest values of yield attributes except number of unfilled grains (Table 9). The favorable impact of Zn application mainly contributed in improving rice growth and salinity withstanding as well as Zn deficiency relieving. The results are in the conformity with those reported by Shehata *et al.* (2009) and Amira, (2011). Zn application improves ion selectivity toward of K^+ uptake Zn might increase nucleotide formation and corresponding biochemical. Additionally, Zn has vital role under saline soil since, it has high capacity to keep the membrane integrity ensuring water uptake under high osmatic pressure outside plant cell. Zn application might be improved K+ uptake that provides grain filling improving (Sims 1986).

Yield and harvest index of investigated rice variety showed great variation in this concern in both seasons. Giza 179 yielded the higher yield and harvest index in both year of study (Table 10). Giza 179 and Giza 178 rice varieties were identically in this concern. Giza 177 yielded lower yields and harvest index in both seasons. Yield and harvest index were significantly and positively affected by Zn treatments in both seasons (Table 10). Grains and straw yield were increased as Zn Nano-particle was increased. The highest grain yield with ZnO-NP at concentration of 30 ppm was recorded in both seasons. The ZnO-NP at 20 and 30 ppm was identical. At the same time, $ZnSo_4$ and 10 ppm of ZnO-NPs showed the same yield and heaviest index. High yield at 20 and 30 ppm of ZnO-NPs is primarily due to improving rice growth, yield components; panicle numbers, panicle weight, filled grains and 1000-grain weight.

Table 8: Effect of the interaction between ZnO-NPs foliar application and rice varieties on plant height and No. of filled grains/panicle during 2013 and 2014 seasons.

Interaction	season	Plant height (cm)			No. of filled grains/panicle		
		Giza 177	Giza178	Giza179	Giza 177	Giza178	Giza179
Control	2013	72.0j	77.9ef	77.2fg	70.0g	95.0cd	90.9de
$ZnSO_4$ 2%		74.5i	79.9b	78.0e	77.5fg	100.7c	95.2cd
ZnO-NP 10ppm		75.2hi	79.9b	78.5de	79.4f	112.2b	95.7cd
ZnO-NP 20ppm		75.6h	81.6a	78.9cd	84.3ef	113.9ab	96.5cd
ZnO-NP 30ppm		76.6g	81.7a	79.5bc	85.2ef	121.9a	98.1cd
Control	2014 season	68.9f	81.8c	76.8e	75.7g	108.2de	104e
$ZnSO_4$ 2%		76.1e	82.8bc	77.7de	82.5f	114.5bcd	114.8bc
ZnO-NP 10ppm		78.8d	84.3ab	77.6de	82.8f	114.3bcd	113.3cd
ZnO-NP 20ppm		79.1d	84.1ab	79.1d	86.3f	124.20a	118.7abc
ZnO-NP 30ppm		78.7d	84.7a	78.9d	88.1f	124.70a	120.7ab

Table 9: Yield components of rice varieties as affected by ZnO-NPs foliar application during 2013 and 2014 seasons.

Factor	Panicle weight (g)		1000-grain weight (g)		No. of filled grains/panicle		No. of unfilled grains/panicle	
	2013	2014	2013	2014	2013	2014	2013	2014
Varieties (A):								
Giza 177	2.13b	2.15b	23.98a	23.33a	79.3c	83.1c	29.84a	25.23a
Giza 178	2.20a	2.31a	17.92c	17.03c	108.7a	117.1a	15.63b	11.73b
Giza179	2.31a	2.33a	23.70b	22.21b	95.3b	114.3b	16.54b	12.70b
F test	*	*	**	**	**	**	*	*
Treatments (B):								
Control	2.08c	2.00c	21.30d	20.27c	85.3d	95.9c	30.37a	22.67a
$ZnSO_4$ 2%	2.17b	2.22b	21.67c	20.79b	91.1c	103.9b	16.95b	14.61b
ZnO-NP 10ppm	2.21b	2.25b	21.92b	20.84b	95.7bc	103.5b	16.68b	14.50b
ZnO-NP 20ppm	2.29a	2.41a	22.16a	21.14a	98.2ab	109.7a	14.38c	12.22c
ZnO-NP 30ppm	2.31a	2.44a	22.27a	21.23a	101.8a	111.0a	12.89c	12.11c
F test	*	*	**	**	**	**	**	**
Interaction:(A*B)	NS	NS	NS	NS	*	*	NS	NS

Table 10: Grain yield, straw yield and harvest index of rice varieties as affected by ZnO-NPs foliar application during 2013 and 2014 seasons.

Factor	Grain yield (t/ha)		Straw yield (t/ha)		Harvest index (HI)	
	2013	2014	2013	2014	2013	2014
Varieties (A):						
Giza177	2.31b	2.43b	3.30b	3.32b	0.411b	0.422b
Giza178	5.15a	5.41a	6.42a	6.87a	0.444a	0.440a
Giza179	5.00a	5.41a	6.54a	7.08a	0.432a	0.433a
F test	**	**	**	**	*	*
Treatments (B)						
Control	3.51c	3.86c	4.74c	5.21c	0.421b	0.423c
$ZnSO_4$ 2%	3.89b	4.20b	5.15b	5.41c	0.424b	0.434b
ZnO-NP 10ppm	4.01b	4.33b	5.34b	5.78b	0.428b	0.428b
ZnO-NP 20ppm	4.61a	4.77a	5.90a	6.12a	0.434ab	0.435ab
ZnO-NP 30ppm	4.73a	4.93a	5.97a	6.27a	0.440a	0.441a
F test	**	**	**	**	*	*
Interaction(A*B):	*	*	**	*	NS	NS

The response of rice plants to Zn application was reported by khan *et al.* (2007), Khan *et al.* (2009), Shehata *et al.* (2009) and Amira, (2011). The interaction effect of varieties and Zn treatments exhaled significant effect on plant height, filled grain panicle^{-1}, grain and straw yields in both seasons. Sprayed-Giza178 with 30 ppm recorded higher mentioned parameters. Giza178 with 20 ppm or/ and 30 ppm were at a par. Data corresponding to the interaction effect confirm the capacity of zinc oxide nanoparticles to improve rice salinity withstanding even with salt sensitive rice variety Giza 177, especially with high concentration of ZnO nanoparticles 30 ppm (Table11). Rice varieties treated with foliar ZnO-NPs exhibited positive response in comparison to $ZnSo_4$ or untreated control. Lin and Xing 2008 reported the upward movement of ZnO-NPs

which is effective in regulating the plant growth. The mechanism of foliar uptake pathway for aqueous solutes and water–suspended nanoparticles was well discussed by Eichert *et al.* (2008) in the context of *Allium porrum* and *Vicia faba*. The results suggest that the stomatal pathway differ fundamentally from the cuticular foliar uptake pathway. When ZnO-NPs are used for foliar application, their performance is strongly determined by the size range specification of the ZnO particles present in the formulation (Moran, 2004). Particle size may affect agronomic effectiveness of Zn fertilizers. Decreased particle size also increases the specific surface area of a fertilizer (Mortvedt, 1992). In addition, ZnO-NPs is having less hydrophilicity and being more dispersible in lypophilic substances, also it can penetrate through the leaf surface (DaSilva *et al.* 2006). Moreover, the mobility of the nanoparticles is very high which ensures the nutrient to reach all plant parts (Gonzalez-Melendi *et al.* 2008).

Table 11: Effect of the interaction between ZnO-NPs foliar application and rice varieties on grain and straw yield during 2013 and 2014 seasons.

Interaction	seasons	Grain yield (t/ha)			Straw yield (t/ha)		
		Giza 177	Giza178	Giza179	Giza 177	Giza178	Giza179
Control	2013	1.70h	4.45e	4.39e	2.53g	5.82d	5.88d
$ZnSO_4$ 2%		2.08g	4.70de	4.90d	2.90fg	5.97d	6.58c
ZnO-NP 10ppm		2.15g	4.85d	5.03cd	3.23f	6.08d	6.72bc
ZnO-NP 20ppm		2.70f	5.85a	5.28bc	3.84e	7.08ab	6.78abc
ZnO-NP 30ppm		2.90f	5.90a	5.40b	4.00e	7.15a	6.76abc
Control	2014	1.78h	4.98cd	4.83d	2.52h	6.55e	6.55e
$ZnSO_4$ 2%		2.16gh	5.33bc	5.10cd	2.97g	6.58de	6.68cde
ZnO-NP 10ppm		2.48fg	5.15cd	5.36bc	3.33g	6.98b-e	7.02bcd
ZnO-NP 20ppm		2.83ef	5.81a	5.67ab	3.87f	7.08bc	7.42ab
ZnO-NP 30ppm		2.91e	5.80a	6.09a	3.90f	7.17b	7.73a

CONCLUSION

It be concluded that the ZnO Nanoparticle foliar application at the concentration of 20 ppm applied as foliar twice at maximum and panicle initiation stages could be recommended for rice growing under high salinity and alkalinity soils. Furthermore, improving rice growth and yield by was completely coincided with reducing diseases infection in the terms of brown spot infection and discolored grains. Zinc plays a vital role for various metabolic pathways, and tolerance of abiotic and biotic stresses in plant systems. A zinc application fertilizer is imperative to fetch higher rice yields under salt stress. The potential benefits of nanotechnology have been widely reported but fate of nanomaterials on agriculture or environment is not well studied.

REFERENCES

Akhtar M.J., A. M. Kumar, M.M. Khan, J. Ahmad and S.A. Alrokayan (2012). Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. International Journal of Nanomedicine, 7: 845-57.

Amira, M. Oksha (2011). Effect of zinc and phosphorus fertilizer on rice crop under saline soil conditions. MSc. thesis, Fac. of Agric., kafr EL-Sheikh, Univ., Egypt.

Aneja K.R. (2003). Experiments in Microbiology, Plant pathology and Biotechnology, fourth Edition. New Age International (P) Limited, Publishers, 102-106,190,246-275.

Batsmanova L. M., L. M. Gonchar, N. Y. Taran, and A. A. Okanenko (2013). Using a colloidal solution of metal nanoparticles as micronutrient fertilizer for cereals, Proceedings of the International Conference Nanomaterials, vol. 2, no. 4, 2 pages,

Black C. (1983). Methods of soil analysis, Part I and Part II. Amer Argon.Inc. Publ. Madison, Wisconsin, USA.

Chitra K, and G. Annadurai (2013). Antimicrobial, activity of wet chemically engineered spherical shaped ZnO nanoparticles on food borne pathogen. Inter. Food Res. J.; 20(1):59-64.

Da Silva, L. C., M. A. Oliva, A. A. Azevedo, and M. J. De Araujo (2006). Response of resting plant species to pollution from an iron pelletization factory. Water, Air and Soil Pollution 175: 241–256.

Dietz, K.J. and S. Herth (2011). Plant nanotoxicology. Trends Plant Sci., 16: 582-589.

Dimkpa C.O., J.E. McLean, D.W. Britt and A.J. Anderson (2013). Antifungal activity of ZnO nanoparticles and their interactive effect with a biocontrol bacterium on growth antagonism of the plant pathogen *Fusarium graminearum*. Biometals 26(6):913–924

Duffy B. (2007). Zinc and plant disease. In: Datnoff LE, Elmer WH, Huber DM. (eds) Mineral Nutrition and Plant Disease. St Paul, MN, USA, APS Press, 155–172.

Eichert, T., A. Kurtz, U. Steiner, and H. E. Goldbach. 2008. Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water suspended nanoparticles. Physiologia Plantarum 134: 151–160.

Elamawi R. M. and R. A. S. El-Shafey (2013). Inhibition effects of silver nanoparticles against rice blast disease caused by *Magnaporthe grisea*. Egypt. J. Agric. Res., 91 (4).

Elamawi, R.M. and R. E. Al-Harbi (2014). Effect of biosynthesized silver nanoparticles on *Fusarium oxysporum* fungus the cause of seed rot disease of faba bean, tomato and barley. J. Plant Prot. and Path., Mansoura Univ., Vol. 5 (2): 225 – 237.

El-Wahsh S.M., Fayzalla S.A. and A.A. Hassan (2008). Effect of nitrogen level, seed moisture content and storage periods on the fungal seed discoloration in some rice cultivars. J. Agric. Sci. Mansoura Univ., 33(3): 1871-1887.

El-Wahsh, S.M. (1997). Studies on both brown spot and blast diseases of rice in Egypt. Ph.D. thesis, Fac. of Agric., kafr EL-Sheikh, Tanta Univ. Egypt.

El-Wahsh, S.M.; R.A. Ebaid, M. I. Abo-Youssef, and B.A. Zayed (2005). Combining ability effect of some rice varieties and their crosses for brown spot resistance and some physiological characters in saline soil. J. Agric. Res., 83 (5B): 521 – 538.

Gomez, K.A. and A.A. Gomez. (1984). Statistical Procedures for Agricultural Research. 2nd, John Wiley and Sons, USA.

Gonzalez-Melendi, P., R. Fernandez Pacheco, M. J. Coronado, E. Corredor, P. S. Testillano, M. C. Risueno, C. Marquina, M. R. Ibarra, D. Rubiales, and A. Perez-De-Luque (2008) .Nanoparticles as smart treatment-delivery systems in plants: Assessment of different techniques of microscopy for their visualization in plant tissues. Annals of Botany 101: 187–195.

He L., Y. Liu, A. Mustapha and M. Lin (2011). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. Microbiological Res.166:207-215.

Khan M.U., M. Qasim and I. Khan (2007). Effect of Zn fertilizer on rice grown in different soils of Dera Ismail khan. Sarhad. J. Agric., 23(4):1033-1040.

Khan, R., A.R. Gurmani, M.S. Khan and A.H. Gurman (2009). Residual, direct and cumulative effect of zinc application on wheat and rice yield under rice-wheat system. Soil & Environment. 28 (1): 24-28.

Kirk, G.J.D., and J.B. Bajita (1995). Root induced iron oxidation, pH changes, and zinc solubilization in the rhizosphere of lowland rice. New Phytol. 131:129–137.

Lin, D., and B. Xing. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. Environmental Sci. Tech. 42: 5580–5585.

Milani N., G. M. Hettiarachchi, J. K. Kirby, D. G. Beak, S. P. Stacey, M. J. McLaughlin (2015). Fate of Zinc Oxide nanoparticles coated onto macronutrient fertilizers in an alkaline calcareous soil. PLOS ONE.10 (5).

Moran, K. (2004). Micronutrient product types and their development. International Fertiliser Society– Proceeding 545. York, England: International Fertilizer Society.

Moreira W. R., F. A. Rodrigues and H. S. Duarte (2013). Effect of zinc on the development of brown spot in Rice. J. Phytopathol. 161: 749–751.

Mortvedt, J. J. (1992). Crop response to level of water soluble zinc in granular zinc fertilizers. Fertilizer Research 33: 249–255.

OECD (2010). Environment, Health and Safety Publications, Series on the safety of manufactured nanomaterials No.27 List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship.

Ou, S.H. (1985). Rice disease. Commonwealth Mycological Institute, Kew, Surrey, England, 2nd ed. 380pp.

Phogat, N. S. A. Khan, S. Shankar, A.A. Ansary and I. Uddin (2016). Fate of inorganic nanoparticles in agriculture. Adv. Mater. Lett. 2016, 7(1), 03-12

Piper N., (1950). Soil and plant analysis. Inc. Soc., Publ.Inc., New York, USA.

Ponnampperuma F.N. (1984). Effects of flooding on soils. In: Kozlowski TT (ed) Flooding and plant growth. Academic, Orlando, pp 1–44 programme for the testing of manufactured nanomaterials.

Quijano-Guerta C., Kirk G.J.D., Portugal A.M.. Bartolome V.I. and G.C. McLaren (2002). Tolerance of rice germplasm to zinc deficiency. Field Crops Res. 76:123–130.

Raikova O. P., L. A. Panichkin, and N. N. Raikova (2006). Studies on the effect of ultrafine metal powders produced by different methods on plant growth and development. Nanotechnologies and information technologies in the 21st century," in Proceedings of the International Scientific and Practical Conference, pp. 108–111, Moscow, Russia.

RRTC (Rice Research and Training Center) (2015). Annual rice national campaign report of rice program. Field Crops Research, Agric. Research Center, Ministry of Agriculture, Egypt.

Sehly, M.R., Z. H. Osman and E.A. Salem (2002). Rice diseases In: T. Castello, pp 198-247. Rice in Egypt. Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt.

Selivanov V. N. and E. V. Zorin (2001). Sustained Action of ultrafine metal powders on seeds of grain crops. Perspekt. Materialy, 4:66–69.

Servin, A, W. Elmer, A. Mukherjee, R. De la Torre-Roche, H. Hamdi, J. C. White, and P. B. Dimkpa, C. (2015). A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nanopart. Res.17:92.

Shehata S.M., B.A. Zayed, E. S. Naeem, S. E. Seedek and A. A. E. Gohary (2009). Response of rice (*Oryza sativa*) to different levels of zinc and sulfur under saline soil. Egypt J. of Appl. Sci, 24 (12B):

Sims J.T. (1986). Soil pH effects on the distribution and plant availability of manganese, copper, and zinc. Soil Sci. Soc. Am. J. 50:367–373

Sirelkhatim, A.S. Mahmud, A. Seeni, N. M. Kaus, L. C. Ann, S. K. Bakhori, H. and D. M.Hasan (2015), Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Lett., 7(3):219–242.

Wani A.H. and M.A. Shah (2012). A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. J. of Appl. Pharm. Sci. 02 (03); 40-44.

Yoshida, S., Forno, D.A., Cock, J.H. &Gomez, K.A.(1976). Laboratory manual for physiology studies of rice .IRRI, Los banos, Philippines.

Young, K. J., Byung H. Kim and G. Jung (2009). Antifungal activity of silver ions and nanoparticles on Phytopathogenic Fungi. Plant Disease, 1037-1043.

تـأثير جزيئـات أكسـيد الزنـك النانونيـة علـي مـرض التبقـع البنـي وإنتاجيـة الأرز تحـت ظـروف الأراضـي المتـأثرة بالملوحة

رباب ممدوح العماوي [١] , شريف ماهر بسيوني [٢] , وليد محمد الخبي [٢] و بسيوني عبد الرازق زايد [٢]

[١] قسم بحوث أمراض الأرز - معهد بحوث أمراض النباتات - مركز البحوث الزراعية – مصر

[٢] قسم بحوث الأرز- معهد بحوث المحاصيل الحقلية –مركز البحوث الزراعية- مصر

تشير الدراسه الحاليه الي تـأثير جزيئات اكسيد الزنك النانونية علي نباتات الارز بالإضافه لدورها في مكافحه مرض التبقع البني في الارز المتسبب عن الفطر *Helmenthosporium oryza*. تم تقيم التأثير المثبط للنمو الفطري لجزيئات الزنك النانونية ذات الحجم ٢٥ ال ٣٥ نانومتر بتركيزات مختلفه, حيث أظهرت مزارع فطر التبقع البني المعامله: إنخفاض في إنبات الجراثم و عدد المستعمرات المتكونه, و عدد الجراثيم لكل مللي مع التركيز ٢٥ و ٥٠ جزء في المليون معمليا. أشارت نتائج الصوبة أن معاملة نقع البذور و الرش ٥ أيام قبل العدوي بالجراثيم الفطريه بجزيئات أكسيد الزنك النانونية أدت إلي تقليل نسبه الإصابه بمرض التبقع البني بدون إختلافات معنويه بين التركيزات. بينما معاملة الرش بعد العدوي بالجراثيم الفطرية بيومين عند التركيزات المنخفضه ١٠ و ٢٥ جزء في المليون كانت قادره علي تقليل نسبه الاصابه بمرض التبقع البني.

تحت ظروف الحقل المتأثر بالملوحه , أجريت تجربتين حقليتين بالمزرعة البحثية لمحطة البحوث الزراعية بالسرو ، بمحافظة دمياط خلال موسمي صيف ٢٠١٣ ، ٢٠١٤م. إشتملت التجربه علي ثلاثه أصناف من الأرز و ثلاث تركيزات من جزيئات الزنك النانونية (١٠, ٢٠, و ٣٠ جزء في المليون) رشا و كبريتات الزنك ٢ % (معامله نقع بذره ٤٨ ساعه) بالإضافه للكنترول بدون معاملة , تم الرش عند منتصف مرحلة التفريع وعند مرحله بدأ نشوء الدالية. أظهرت النتائج أن المعاملة بجزيئات الزنك النانونية عند تركيز ٢٠ جزء في المليون قللت من معدل الإصابه بمرض التبقع البني علي أوراق الأرز و الحبوب الملونه علي كل الأصناف المختبره. وقد أظهرت نتائج كل من موسمي الدراسة تباين أصناف الأرز المختبرة معنويا فيما بينها من حيث مقاييس النمو الخضري ومحصول الحبوب ومكوناته. أعطى الصنف جيزة ١٧٨ و ١٧٩ أفضل نمو خضري عند تركيز ٢٠ جزء في المليون من جزيئات الزنك النانونيه. وحدث تحسن في اداء الصنف جيزه ١٧٧ كصنف حساس للملوحه فى معظم الصفات المدروسة.

أوضحت نتائج الدراسة أيضا أن المعاملة بجزيئات الزنك النانونيه أدت إلى تقليل تأثير الإجهاد الملحي وأيضا تحسين النمو الخضري ومحصول الحبوب ومكوناته لأصناف الأرز المختبرة تحت ظروف الأراضي الملحية و تقليل معدل الاصابه بمرض التبقع البني. تشير الدراسه الحاليه إلي تأثير جزيئات الزنك علي نباتات الأرز مع الأخذ في الإعتبار إستخدامها مستقبليا "كأسمده نانويه" بالإضافه لدورها في مكافحه مرض التبقع البني في الأرز.