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# Estimate of the Antifungal Activity and Phytotoxicity of ZnO Nanoparticles on *Magnaporthe oryzae* and Rice Cultivar Sakha 101 using Morphological, Biochemical and Molecular Markers

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**Rice** blast caused by *Magnaporthe oryzae* is the major biotic stress influences rice yield. The current investigation aimed to estimate the antifungal activity and phytotoxicity effect of different concentrations of ZnO nanoparticles (0.0, 10, 25, 50, 100 and 200 mg/L) on *M. oryzae* and rice cultivar Sakha101 using morphological, biochemical and molecular markers. Five ISSR markers and seven RAPD primers were utilized to estimate the potentiality effects of ZnO nanoparticles (NPs). The effect of different rates and applications of ZnO NPs used to control rice blast disease and improve grain yield were estimated in the field during 2017 and 2018 growing seasons. The results showed that, in vitro antifungal assay of ZnO NPs showed a significant decrease in colony formation in comparison to control. Foliar application of ZnO NPs at 25 mg/L was the most effective treatments for mitigation rice blast at five days before inoculation. While under field conditions, foliar spray with 25 mg/L at nursery increased the grain yield to 4.366 ton/fed in season 2017 and to 4.625 ton/fed in 2018. Foliar spray with ZnO NPs of rice offer a practical and useful approach to improve rice grain yield and reduce leaf blast disease when applied at optimal concentrations. ZnO NPs at lower concentrations (10 and 25 mg/L) enhanced seed germination and improved seedling growth, while the higher concentrations (100 and 200 mg/L) resulted in phytotoxicity. ZnO NPs treatments altered the expression patterns of seeds storage protein; induced newly synthesized isoforms and disappearance of existing ones. The obtained results using molecular markers confirmed that the lower concentrations of ZnO-NPs (10 and 25 mg/L) are considered as a good enhancement agent, as in case of rice cultivar Sakha101, using UBC 825 primer with 306 bp at concentration of 25 mg/L and OPA-9 primer with 948 bp at concentration of 10 mg/L. For *M. oryzae* the

same trend of effects was appeared in case of UBC 880 primer with 994 bp at concentration of 25 mg/L and OPO-10 primer with 268 bp at concentration of 10 mg/L. In general, these results suggested that lower concentrations of ZnO NPs could be applied as an effective nano fertilizer for sustainable agriculture and food safety, and moreover, utilized as antifungal agent for rice blast disease.

**Keywords:** Zinc oxide nanoparticles, *Magnaporthe oryzae*, *Oryza sativa*, Phytotoxicity, RAPD and ISSR markers and grain yield.

Agriculture sector faces various and unprecedented challenges, such as reduced crop yields due to biotic and abiotic stresses, including nutrient deficiency and pathogenic microbes, the development of nanotechnology has offered propitious applications for sustainable agriculture. The use of nanoparticles to improve plant growth and for the control of plant diseases is a recent practice (Rastogi *et al*., 2019). However, phytotoxicity due to exposure to NPs is an unresolved issue. Recent studies showed a strong attempt to understand the effect of nanoparticles on plant growth (Youssef and Elamawi 2018) suggested a positive impact of NPs on plant development with the potential to be used as future Nano-fertilizer.

Rice (*Oryza sativa* L.) is one of the most important food for more than half of the world; accordingly, its production should be doubled by 2050 to meet the rising global demand (FAO 2009). It is also considered as the main food for all human ages because of its seed's richness in proteins, minerals, vitamins and fibers.

Rice blast is caused by *Magnaporthe oryzae* Couch (anamorph: *Pyricularia oryzae* Cavara) (Couch and Kohn 2002). *M. oryzae* occurs in about 80 countries and attacks over 50 grass species (Ou 1985) It is responsible for approximately losses of 10–30% of worldwide rice production (Nalley *et al*., 2016). Most of the rice cultivars are susceptible to different fungus races. Fungicides are commonly used to control blast; however, these are becoming less acceptable as they increase the potential for build-up of resistance in *M. oryzae* to fungicides and also conflict with the public concern for fungicide residues on human health and environment. The fungus is highly variable so disease control is a challenge (Ou 1985).

Nanoparticles could be used as alternative methods to control this disease. Silver nanoparticles applied effectively in the control of rice blast and the prevention of deleterious infections in rice as reported by (Young *et al*., 2009; Elamawi and El-Shafey 2013 and Elamawi *et al*., 2018) Several researchers reported the antifungal activity by ZnO NPs on numerous fungi species; i.e. (He *et al.*, 2011; Gunalan *et al*., 2012; Wani and Shah 2012; Savi *et al*., 2013; Elamawi *et al*., 2016 and Sierra-Fernandez *et al*., 2017). To our knowledge, there are no reports about using ZnO NPs for control of *M. oryzae*.

Zinc is an essential micronutrient element for rice growth. It is indispensable 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). The zinc oxide nanoparticles are used as fertilizer (Singh *et al*., 2013). ZnO NPs on the form of nanoparticles may increase its availability to plants so its application as a fertilizer may enhance rice growth over the regular Zn fertilizer. Foliar application of Zn fertilizer improved both productivity and grain Zn concentration in rice (Kulhare *et al*., 2017).

ZnO is considered to be a "GRAS" (generally recognized as safe) substance by the FDA (2011). The GRAS classification, however, most commonly refers to materials in the micron to a broader range of size, as even such substances can develop toxicity when reduced to the nanoscale (Rasmussen *et al*., 2011). Consequently, a detailed evaluation of ZnO NPs toxicity in both in vitro and in vivo systems is needed, as well as identifying means to reduce unwanted toxicity. Phytotoxicity of ZnO NPs on seed germination and root development has been studied in different plant species including; lettuce, radish, and cucumber (Lin and Xing 2007). ZnO NPs reduced biomass, shrunk root tip and epidermis, and cortical cells became highly vacuolated and collapsed on ryegrass (Lin and Xing 2008). Toxicity of ZnO NPs to rice seedlings was evident and increased with increasing concentration of ZnO NPs (more than 250 mg/L) (Chen *et al*., 2015). On the other hand, ZnO NPs promoted seed germination, root and shoot length in the peanut (Prasad *et al*., 2012). ZnO NPs in lower concentration increased seed germination in wheat (Zhu *et al*., 2019). *Cyamopsis tetragonoloba* when exposed to ZnO NPs, showed improved plant biomass, root and shoot length, chlorophyll and protein synthesis and other growth parameters (Raliya and Tarafdar 2013). Lower concentrations of ZnO NPs (10 and 25 mg/L) enhanced seed germination and improved seedling growth, while higher concentrations (100 and 200 mg/L) resulted in phytotoxicity in the *Vicia faba* (Youssef and Elamawi 2018).

Recently, studies on the genotoxic effects of NPs on plants are appearing. Genotoxicity describes the property of chemical agents that damage the genetic information within a cell causing mutations, induced by nanoparticles in plants (Remédios *et al*., 2012). Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is used for DNA analysis in the field of genotoxicity in different plants as a sensitive method, capable of detecting variations in genome profiles (Kekec *et al*., 2010). The genotoxic and phytotoxic studies represent that ZnO at 2000 mg/l effects on buckwheat (*Fagopyrum esculentum*) seedling (Lee *et al*., 2013)

The objectives of the present investigation are to evaluate the impacts of ZnO nanoparticles on *M. oryzae* and *O. sativa* using morphological, biochemical and molecular markers. In addition, to study the potentiality effects of different rates and

applications of ZnO NPs as nano-fertilizer and anti-fungal agent to control rice blast disease and improve grain yield of rice cultivar Sakha101.

## Materials and Methods

*2.1. Preparation of ZnO nanoparticle:*

The ZnO nanopowder (M K Impex Corp Mississauga, Canada) with primary diameter of 30 nm was used to prepare five different concentrations of ZnO NPs (10, 25, 50, 100, and 200 mg/L). The preparation and characterization of ZnO NPs suspension were fully described in previous paper (Youssef and Elamawi 2018).

*2.2. Biological materials:*

Rice seeds of *Oryza sativa cv.* Sakha 101 were obtained from Rice Research and Training Center, Kafrelsheikh, Egypt. Rice blast fungus was isolated from infected leaves of Sakha101 rice cultivar from Kafrelsheikh governorate and identified as *M. oryzae* race ID-15 according to disease reaction pattern on the international differential varieties (Atkins *et al*., 1967).

*2.3 Estimate of antifungal activity of ZnO NPs against rice blast:*

Three different experiments were conducted to assess the potential application of ZnO NPs as antifungal agent and to improve rice productivity under *M. oryzae* infection.

*2.3.1. In Vitro Antifungal Assay of ZnO NPs:*

The antifungal activity of ZnO NPs against *M. oryzae* was examined based on colony formation test and sporulation capacity according to a modified method of (Jo *et al*., 2009). Briefly, conidia were collected from *M. oryzae* culture grown on Banana Dextrose Agar (BDA) medium and incubated at 25°C for 10 days. Conidial suspension was diluted with sterile distilled water to a concentration of 106 spores/ml. 500 µl of the conidial suspension were mixed with different concentrations of ZnO NPs (0.0, 10, 25, 50, 100 and 200) to a final volume of 1 ml per each treatment. All treatments were incubated at 28˚C for 24h. then, 50 µl aliquot of each treatment were spread on BDA and incubated at 28°C. The developed colonies were counted after 2, 4 and 6 days of incubation according to Elamawi and El-Shafey (2013). Each treatment was replicated three times. The Sporulation capacity was estimated by adding 10 ml of distilled water to each Petri dish, and then the spores were harvested by spatula. The number of spores/ml was counted using the hemocytometer.

*2.3.2. Assay of ZnO NPs capacity for controlling rice blast under artificial inoculation:*

In the current investigation, ZnO NPs capacity for controlling rice blast on Sakha 101 cultivar was estimated under greenhouse conditions. Three protocols and six different ZnO NPs concentrations (0.0, 10, 25, 50, 100 and 200 mg/L) were applied. The first protocol included soaking of rice seeds in the desired solutions for 48 h, the

second one involved foliar treatment for seedling with ZnO NPs five days before inoculation (DBI) with spores of *M. oryzae*. While, the third protocol for foliar treatment of rice seedling was carried out five days post inoculation (DPI). All protocols included inoculation of seedlings (21 days old) with spore suspension of *M. oryzae* at $1\times10^5$ spore/ml conc. Meanwhile, plants sprayed with water were kept as control for comparison. The inoculated seedlings were held in a moist chamber for 24 h and then moved to the greenhouse with at least 90% R.H. and 25-28$^o$C. The experiments were performed twice with three replicates for each treatment. Seven days after inoculation, the reaction was scored using the (0-9) scale of IRRI Standard evaluation system (Anon, 2013). Severity of infection was estimated by counting the total number of type (4) or more of blast lesions of 100 randomly selected leaves per pot.

*2.3.3. Effect of ZnO NPs on rice blast and rice grain yield under field conditions:*

For evaluating the ability of ZnO NPs to control rice blast as well as improving rice grain yield under natural rice blast infection, four protocols with three ZnO-NPs concentrations (25, 50 and 100 mg/L) were applied on susceptible rice cultivar Sakha 101. In the first protocol rice seeds were soaked in ZnO nanofluid for 48 h. The second protocol involved foliar application of ZnO nanofluid on rice seedlings at nursery (20 days age). While, in the third protocol foliar spray of ZnO nanofluid was carried out at 25 days post transplanting (DPT). Finally, foliar spray with selected ZnO concentrations at 25 DPT followed by a second spray at 50 DPT was the fourth tested protocol. Tricyclazole (Beam 75% WP) as a recommended fungicide for competing rice blast was applied as positive control as well as untreated plants as a negative control for comparison. Thirty days old seedlings were transplanted by four seedlings/hill in a distance of 20×20 cm. The experiments were designed in random complete block design with three replicates during 2017 and 2018 growing seasons at Rice Research and Training Center Research Farm at Sakha Agricultural Research Station, Egypt. Before transplanting, land was prepared as follow; Phosphorous fertilizer (21 kg P2O5/fed) and potassium sulphate (24 kg K2O/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 two equal doses at; land preparation and 30 days after transplanting (DAT). The rest of management issues were typically followed according to Rice Research and Training Center recommendations. Severity of infection was estimated by counting the total number of type (4) or more of blast lesions/100 leaves.

*2.4. Phytotoxicity evaluation of ZnO NPs using germination and seedling vigour testing:*

Rice seeds were immersed in a 10% sodium hypochlorite solution for 10 min to ensure surface sterility, followed by rinsing three times with distilled water. Then the seeds were allowed to sprout on filter paper saturated with the tested concentrations of ZnO NPs or distilled water as a control at 25±2$^o$C. Three replicates with 25 seeds/dish were applied. Germination percentages were calculated after 10

days of treatment according to the equation: Seed germination (%) = (Number of germinated seeds/ Number of total seeds) ×100. Seedling vigor parameters including; shoot length, root length and seedling vigor index (VI) were recorded after 20 days. VI was calculated according to Dhindwal *et al.* (1991) as VI = (shoot length + root length) x germination percentage.

*2.5. Effect of ZnO NPs on rice seeds protein PAGE analysis:*

Rice seeds of variety Sakha 101 were soaked for 48 h in the desired concentrations of ZnO NPs. Then protein was extracted by homogenizing 100 mg from treated and untreated seeds with 1000 µl sodium phosphate buffer (PH 7.2), including 2% sodium dodecyl sulfate (SDS) Dhindwal *et al*., (1991). The extracts were centrifuged at 10000 rpm for 20 min. The supernatant was kept at -20ºC until use. Then 50 µl of each sample were combined with the same sample buffer volume containing 0.125 M Tris/Hcl, pH 6.8, 2% (w/v) SDS, 10% sucrose, 1% (v/v) β-mercapto-ethanol and 0.15 (m/v) bromophenol blue followed by heat denaturation at 100ºC for 4 minutes. One dimensional SDS-PAGE of extracted proteins was carried out in a vertical slab gel using 12.5% (w/v) acrylamide according to Laemmli (1970). A volume of 40 µl was loaded in each well. In addition, 10 µl of a protein ladder ranging from 150 to 20 kDa (Thermo Fisher Scientific, Waltham, MA, USA) were loaded to allow the detection of the molecular weights of the separated proteins. Electrophoresis was carried out (using BioRad protean ® II x i cell vertical slab gel unit) at constant voltage of 100 v and 50 mA, until the bromophenol blue dye reached the bottom of the gel. The gel was directly placed in commassie brilliant blue R250 staining solution for 4 h and distained several times using the distaining solution until bands become clear. The gel was then visualized in white fluorescent light and photographed. The molecular weights of the produced bands by each sample, were calculated using the Lab Image Software version 2.7 program and then were compared with those of other treatments (Laemmli, 1970).

*2.6. Detection effect of ZnO NPs using ISSR and RAPD-PCR primers*

*2.6.1. Extraction and purification of genomic DNA:*

Seeds of rice cultivar Sakha101 were germinated and grown for two weeks on filter papers saturated with the desired ZnO NPs suspensions. Leaves representing at least three different plants per each treatment were grinded to a fine powder using liquid nitrogen, a porcelain mortar and pestle. Then 100 mg of the powder were transferred to pre warmed cetyltrimethyl ammonium bromide (CTAB) extraction buffer and incubated for 30 min at 65 °C. The total genomic DNA was isolated in accordance with the methods adopted by Clarke (2009), and the modifications done by Saad-Allah and Youssef (2018). In case of *M. oryzae*, the protocol mentioned before was effective in extracting genomic DNA from 50 mg of its hyphae. Firstly, the isolate of *M. oryzae* was inoculated into 50 ml liquid medium of potato dextrose containing the desired concentration of ZnO NPs (0.0, 10, 25, 50, 100, 200 mg/L) at 28°C in orbital shaker (120 rpm). The fresh mycelia mass of each treatment was harvested after two weeks of incubation using Whatman filter paper No. 3 and

immediately frozen in the liquid nitrogen. The frozen mycelia were ground into a fine powder using a sterile pestle and mortar. The mycelia powder was stored at -20°C until needed for DNA extraction. The DNA concentration and purity were determined by a Nanodrop ND-1000 spectrophotometer. The 260/280 absorption ratio ranged from 1.7 to 1.8, which indicates DNA purity. For molecular screening of potentiality effects of nano-ZnO, five ISSR and seven RAPD-PCR primers were used (Saad-Allah and Youssef, 2018). The details of the selected primers are listed in Table (1).

**Table (1): List of ISSR and RAPD-PCR primers used for screening the effect of ZnO NPs on *Magnaporthe oryzae* and rice cultivar Sakha101**

| No. | Primer code | Sequence (5'–3') |
|---|---|---|
| **ISSR primers** | | |
| 1 | UBC 825 | ACACACACACACACACT |
| 2 | UBC 845 | CTCTCTCTC TCT CTC TGG |
| 3 | UBC857 | ACA CAC ACA CAC ACA CTG |
| 4 | UBC 861 | ACCACCACCACCACCACC |
| 5 | UBC 880 | GGAGAGGAGAGGAGA |
| **RAPD primers** | | |
| 1 | OPA-9 | GGGTAACGCC |
| 2 | OPA-13 | CAGCACCCAC |
| 3 | OPB-11 | GTAGACCCGT |
| 4 | OPC-2 | GTGAGGCGTC |
| 5 | OPG-2 | GGCACTGAGG |
| 6 | OPO-10 | TCAGAGCGCC |
| 7 | OPZ-20 | ACTTTGGCGG |

OP = Operon Technologies Almeda, CA. UBC = Primer set #9. University of British Columbia, CA.

*2.6.2. ISSR markers.*

Five ISSR primers were used as generic primers in the PCR amplification of the ISSR regions in treated and control samples. The amplification mixture (20 µl) contained the indicated primers (1.0 µl), 2 µl genomic DNA (100 ng), sterilized double-distilled H2O (7 µl) and Dream Taq Green PCR Master Mix #k 1081 (Thermo Fisher Scientific, UK) (10 µl). The thermocycling conditions were as follows: an initial denaturation step for at 94 °C for 5 min; 40 cycles of 94 °C for 1 min, 48–52 °C for 30 s (based on the primer sequence), and 72 °C for 1 min; and a final extension step at 72 °C for 8 min (Saad-Allah and Youssef, 2018).

*2.6.3. RAPD-PCR:*

Seven random DNA oligonucleotide (10- base) primers were independently used for detection of DNA alteration upon exposure to ZnO NPs using the PCR technique. The PCR amplifications were performed using the indicated primers (1.0

µl) in a 20 µl reaction volume containing genomic DNA (100 ng), sterilized double-distilled H2O (7 µl) and 10 µl of Dream Taq Green PCR Master Mix #k 1081 (Thermo Fisher Scientific, UK). The thermal cycles were as follows: initial denaturation at 94 °C for 5 min; 40 cycles of 94 °C for 1 min, 37 °C for 30 s and 72 °C for 2 min; and a final extension for 8 min at 72 °C (Saad-Allah and Youssef, 2018).

*2.6.4. Estimate the Amplified DNA fragments and Data Analysis:*

After the completion of PCR, 10 µl of the PCR-amplified products were electrophoresed using 1.6 % agarose gel in 0.5 × TBE buffer in a submarine gel apparatus at 100 V for 1 h. The gels were visualized under UV light and imaged using a gel documentation system (CFW- M; Bio-Rad). All reactions were repeated twice, and only reproducible bands were scored. Thermo Scientific Gene-Ruler DNA ladders with a length of 1 kb or 100 bp (100 ng µl) were used as DNA size standards for detection band size (Saad-Allah and Youssef, 2018).

*2.7. Statistical analysis:*

All data were subjected to statistical analysis according to the procedures reported Kobata *et al*. (2018). The significance of differences among means was measured by Duncan's multiple range tests at $P<0.05$. The results expressed as mean ±SD of three replicates per each treatment and subjected to one-way analysis of variance (ANOVA) using SPSS v.16 (SPSS, Chicago, USA).

## Results

*3.1. Estimate of antifungal activity of ZnO NPs under Lab. conditions:*

Effect of ZnO NPs on colony formation and spores production of *M. oryzae* is illustrated in Figure 1. To our knowledge, there is no previous reports discussed the effect of ZnO NPs on M. oryzae. All the studied concentrations of ZnO NPs showed significant decrease in colony formation in comparison to control (Fig. 1A). Although, the growth inhibition under ZnO NPs exposure was not dose-dependent, the lowest numbers of colonies were recorded at 10 and 25 mg/L ZnO NPs (11.66 and 17.00, respectively). Interestingly, ZnO-NPs treatments accelerated the conidia production compared to control. The highest spore production (45 spore/L) was estimated after exposure to 100 mg/L ZnO NPs (Fig. 1B).

*3.2. Effect of ZnO NPs on rice leaf blast severity under artificial inoculation:*

The potentiality of the ZnO NPs to mitigate rice blast is presented in Table (2). Generally, all applied protocols; seed soaking in ZnO NPs solutions, foliar spray at 5 DBI and foliar spray at 5 DPI reduced blast lesions numbers compared to untreated control without noticeable phytotoxicity. Seed soaking and foliar spray at 5 DBI treatments significantly declined the disease severity. The lowest degree of rice blast severity (16 lesions/100 leaves) was obtained by foliar spray with 25 mg/L ZnO NPs at 5 DBI. Results of experiment under greenhouse indicated that foliar application of

ZnO NPs at 5 DBI with concentration 25 mg/L was the most effective treatment for mitigation rice blast without obvious phytotoxicity.

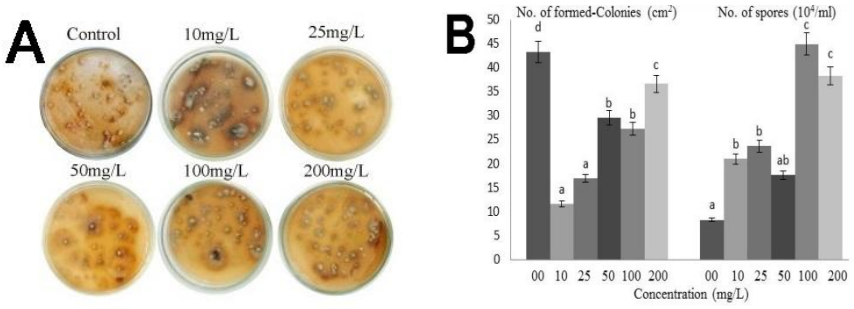


**Fig. (1): Effect of ZnO NPs on colony formation of *Magnaporthe oryzae* after 7 days on banana dextrose agar medium. (A) Frequency of Number of formed-Colonies ($cm^2$), (B) No. of spores/ml**

**Table (2): Effect of ZnO NPs on rice blast severity on Sakha101 cultivar under greenhouse conditions**

| Concentrations (mg/L) | Severity of infection (No. of lesions/100 leaves) | | |
|---|---|---|---|
| | Seed Soaking | 5DBI* | 5DPI** |
| Control (00) | 37.66i | 37.66i | 37.66i |
| ZnO NP 10 | 21.32d | 19.27c | 27.97g |
| ZnO NP 25 | 20.67d | 16.00a | 25.66f |
| ZnO NP 50 | 18.00b | 17.33b | 26.33f |
| ZnO NP 100 | 19.67c | 22.00de | 29.00gh |
| ZnO NP 200 | 28.53g | 25.26f | 30.13h |

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

*3.3. Effect of ZnO NPs on rice blast and grain yield under field conditions:*

The ability of ZnO NPs to control rice blast as well as improving rice grain yield under natural rice blast infection is summarized in Table 3. The effect of ZnO NPs on rice blast under field conditions showed the same trend of those recorded in greenhouse experiments. The most powerful ZnO NPs treatments against rice blast were foliar application with 25 mg/L at nursery and double spray at 25 DPT followed by 50 DPT. Foliar spray at 25 DPT followed by 50 DPT reduced the disease severity to 8.60 and 9.33 lesions/100 leaves during growing seasons 2017 and 2018, respectively. Foliar spray with 25 mg/L at nursery declined the disease severity to 9.70 and 10.67 lesions/100 leaves during 2017 and 2018 seasons, respectively. These treatments showed insignificant differences with the recommended fungicide tricyclazole during season 2017. While, during 2018 season, they exhibited low significance. The mean efficiency severity of infection

was recorded with both treatments spray nursery at 20 days and 25 DPT followed by 50 DPT at 25 mg/L (81.0 and 83.3 %, respectively). Consequently, the aforementioned treatments improved grain yield in both seasons. Foliar spray with 25 mg/L at nursery increased the grain yield to 4366 kg/fed in season 2017 and to 4625 kg/fed in 2018. While, double foliar spray with 25 mg/L ZnO NPs at 25 DPT followed by 50 DPT enhanced grain yield to 4307 kg/fed in 2017 seasons and to 4650 kg/fed in 2018. The mean grain yield increasing over control (Efficiency %) has been increased 15.22 and 14.56 % with both treatments at nursery and Two spray at 25DPT + 50DPT with concentration of 25 mg/L, respectively.

**Table (3): Effect of ZnO NPs on rice blast severity and grain yield on Sakha101 cultivar under field conditions during 2017 and 2018 growing seasons**

| Treatments | Conc. (mg/L) | Severity of infection (No. of lesions/100 leaves) | | Mean Severity of infection | Efficiency % | Grain Yield (kg/fed.) | | Mean Grain Yield (kg/fed.) | Efficiency % |
|---|---|---|---|---|---|---|---|---|---|
| | | 2017 | 2018 | | | 2017 | 2018 | | |
| Control | 0.0 | 55.67 i | 51.67i | 53.67 | - | 3870.00e | 3934.00e | 3902.00 | - |
| Seed Soaking | 25 | 15.17b | 19.50ef | 17.34 | 67.7 | 3917.33d | 4444.00c | 4180.66 | 7.14 |
| | 50 | 14.17b | 18.67e | 16.42 | 69.4 | 3916.67d | 4483.11b | 4199.89 | 7.63 |
| | 100 | 22.94c | 20.77f | 21.86 | 59.3 | 3918.00d | 4472.00b | 4195.00 | 7.51 |
| Spray nursery at 20 days | 25 | 9.70a | 10.67b | 10.19 | 81.0 | 4366.33b | 4625.34a | 4495.83 | 15.22 |
| | 50 | 13.14b | 14.00c | 13.57 | 74.7 | 4191.00c | 4496.00b | 4343.50 | 11.31 |
| | 100 | 21.17bc | 15.00cd | 18.05 | 66.4 | 3988.67d | 4495.14b | 4241.90 | 8.71 |
| One spray at 25DPT* | 25 | 19.00bc | 20.10f | 19.55 | 63.6 | 3873.33f | 4394.45d | 4133.89 | 5.94 |
| | 50 | 17.93bc | 16.57d | 17.25 | 67.9 | 3883.33f | 4371.34d | 4127.33 | 5.77 |
| | 100 | 35.80d | 25.67h | 30.74 | 42.7 | 3861.00f | 4356.23d | 4108.61 | 5.30 |
| Two spray at 25DPT + 50DPT * | 25 | 8.60a | 9.33b | 8.97 | 83.3 | 4307.00b | 4633.00a | 4470.00 | 14.56 |
| | 50 | 12.23b | 11.33b | 11.78 | 78.1 | 4126.67c | 4650.12a | 4388.39 | 12.47 |
| | 100 | 26.50c | 23.33g | 24.92 | 53.6 | 3976.67d | 4394.25d | 4185.46 | 7.26 |
| Tricyclazole | 0.5 g/L | 6.10a | 5.33a | 5.72 | 89.4 | 4510.00a | 4678.00a | 4594.00 | 17.73 |

*DPT**;** days post transplanting

*3.4. Effect of ZnO NPs on germination and seedling vigor:*

The effect of ZnO NPs on germination % and seedling vigor of Sakha 101 rice cultivar is represented in Figure (2). The results showed that increasing ZnO NPs concentration resulted in gradual increase in germination % followed by gradual decrease. In general, lower conc. of ZnO NPs (especially 10 and 25 mg/L) enhanced seed germination. On the other hand, higher conc. (200 mg/L) showed insignificant effect compared to control treatment (Fig 2A). Visual observation of rice

germination after three days of treatment showed that ZnO NPs accelerated germination process compared to the control (Fig. 2B). This observation needs more future studies about the influence of ZnO NPs on metabolic and regulation process of seed germination.

Concerning the root growth for Sakha 101, an obvious increase in root length was recorded as the ZnO NPs concentration was increased. The root reached the maximum value, 13.00 cm for Sakha 101 with 25 mg/L ZnO NPs treatment. A noticeable inhibition for root growth was detected at 200 mg/L treatment (8.64) (Fig. 2C).

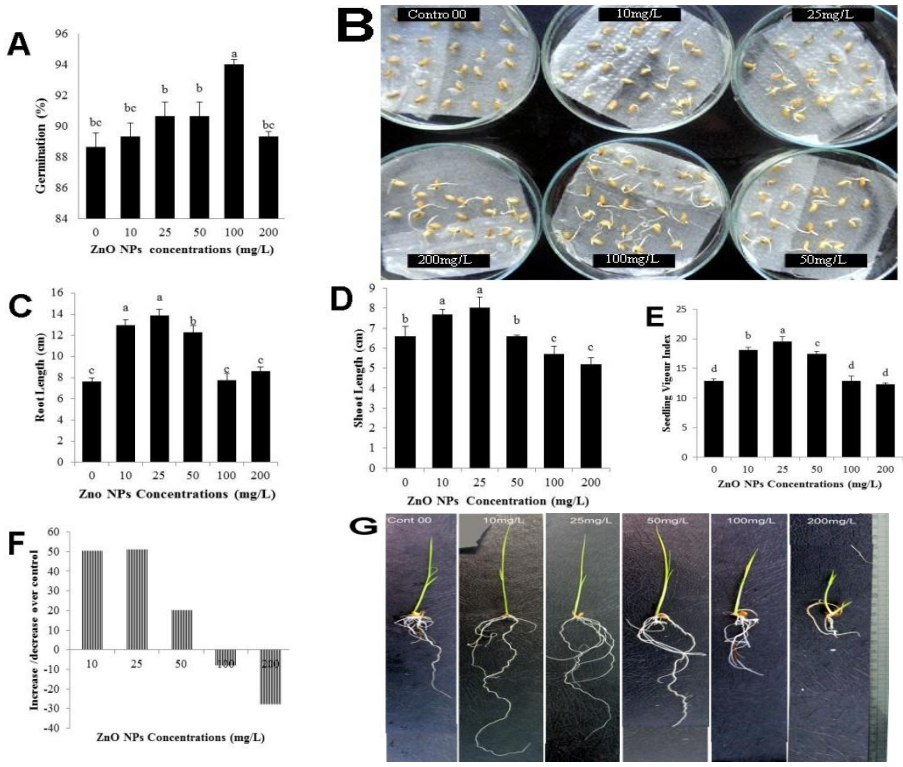


**Fig (2): Effect of different concentrations of ZnO nanoparticles on Sakha 101 rice cultivar. (A) Germination %, (B) Acceleration of germination after 4 days, (C) Root length (cm), (D) Shoot length (cm), (E) Seedling Vigor Index, (F) Increase/decrease over control related to seedling vigor, (G) Morphological characteristics of the treated seedlings after 20 days post sowing.**

Results of the current study indicated an obvious improvement in shoot growth with 25 mg/L ZnO NPs treatment, at which the highest shoot length value (8.03) cm

was recorded. On the contrary, a great drop in shoot length (5.21 cm) was detected at 200 mg/L treatment (Fig. 2D). The beneficial effects of lower-medium levels of ZnO NPs on seedling establishment were also confirmed using the vigor index, which reached a maximum value at 25 mg/L ZnO NPs (Fig. 2E), corresponding to a 100% increase relative to control conditions (Fig. 2F). At higher ZnO NPs levels, the vigor index (20.36) was gradually declined in a trend similar to that observed for the shoot and root growth (compared to Fig. 2C–D). At a morphological level, plants appeared taller when treated with10 and 25 mg/L ZnO NPs (Fig. 2G). At the highest ZnO NPs concentrations (100 and 200 mg/L), the root tips exhibited a dark coloration denoting signs of necrosis. Collectively, these results clearly show the beneficial effects of low-moderate concentrations of ZnO NPs on plant performance.

*3.5. Effect of ZnO NPs on rice cultivar Sakha 101 seeds protein using PAGE analysis:*

SDS–PAGE of rice seeds soaked for 48 h in different concentrations of ZnO NPs gave protein bands with molecular weights ranging from 134 kDa to 21 kDa. The total number of scored bands was 22, with 14 bands for control and 15 bands were recorded per each treatment (Table 4). The results showed that, ZnO-NPs induced variations in the protein banding pattern between control and treated seeds with 68.2 % polymorphism. Newly synthesized protein bands had been detected; the treatments from 10 to 100 mg/L induced the synthesis of the two bands (63 and 60 KDa), while the treatment with 200 mg/L of ZnO-NPs induced the synthesis of new six bands (120, 99, 89,77, 72. 61) in treated seeds of rice cultivar Sakha 101 (Fig. 3).

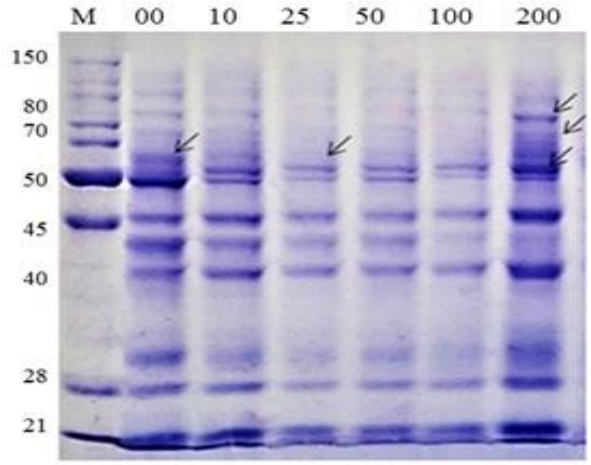


**Fig. (3): SDS-PAGE protein patterns of rice in response to different concentrations (mg/L) of ZnO-Nps exposure for 48 hr. (M) Protein marker. Arrows point to newly synthesized or appeared bands.**

The number of polymorphic protein bands varied among treatments. The highest value (11 polymorphic bands) was recorded for 200 mg/L ZnO NPs treatment showing 55% polymorphism compared to control, followed by 25 mg/L (4 polymorphic bands) with 25% polymorphism (Table 4). The other concentrations (10, 50 and 100 mg/L) showed three polymorphic bands with 18.75% polymorphism for each.

**Table (4): Protein banding pattern of rice cultivar Sakha 101 under different concentrations of ZnO NPs**

| Band number | M.wt. | ZnO NPs Conc.(mg/L) | | | | | | Frequency | Polymorphic bands |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.0 | 10 | 25 | 50 | 100 | 200 | | |
| 1 | 134 | + | + | + | + | + | - | 0.833 | Polymorphic |
| 2 | 132 | + | + | + | + | + | - | 0.833 | Polymorphic |
| 3 | 120 | - | - | - | - | - | + | 0.166 | Polymorphic |
| 4 | 104 | + | + | + | + | + | - | 0.833 | Polymorphic |
| 5 | 99 | - | - | - | - | - | + | 0.166 | Polymorphic |
| 6 | 91 | + | + | + | + | + | - | 0.833 | Polymorphic |
| 7 | 89 | - | - | - | - | - | + | 0.166 | Polymorphic |
| 8 | 79 | + | + | + | + | + | - | 0.833 | Polymorphic |
| 9 | 77 | - | - | - | - | - | + | 0.166 | Polymorphic |
| 10 | 72 | - | - | - | - | - | + | 0.166 | Polymorphic |
| 11 | 66 | + | + | - | + | + | + | 0.833 | Polymorphic |
| 12 | 63 | - | + | + | + | + | - | 0.666 | Polymorphic |
| 13 | 61 | - | - | - | - | - | + | 0.166 | Polymorphic |
| 14 | 60 | - | + | + | + | + | - | 0.666 | Polymorphic |
| 15 | 59 | + | - | - | - | - | + | 0.333 | Polymorphic |
| 16 | 51 | + | + | + | + | + | + | 1 | Monomorphic |
| 17 | 45 | + | + | + | + | + | + | 1 | Monomorphic |
| 18 | 39 | + | + | + | + | + | + | 1 | Monomorphic |
| 19 | 36 | + | + | + | + | + | + | 1 | Monomorphic |
| 20 | 28 | + | + | + | + | + | + | 1 | Monomorphic |
| 21 | 25 | + | + | + | + | + | + | 1 | Monomorphic |
| 22 | 21 | + | + | + | + | + | + | 1 | Monomorphic |
| Total No. of bands | | 14 | 15 | 14 | 15 | 15 | 15 | | |
| No. of Polymorphic bands/treatment | | 0 | 3 | 4 | 3 | 3 | 11 | | |
| Polymorphism % | | 0.0 | 18.7 | 25 | 18.7 | 18.7 | 55 | | |

*3.6. Effect of ZnO NPs on genomic DNA of Magnaporthe oryzae using ISSR and RAPD-PCR primers:*

The genomic DNA of *M. oryzae* was subjected to five ISSR markers and seven RAPD primers in order to determine the effect of the different concentrations of ZnO-NPs used in this study (Table 5 and Figure 4). All ISSR primers amplified and generated polymorphic alleles except UBC 857 which showed monomorphic alleles.

Meanwhile, out of seven RAPD-PCR primers used in this study, only three primers amplified and generated polymorphic bands.

**Table (5): Effect of different concentrations of ZnO NPs on genomic DNA of *Magnaporthe oryzae***

| Type of Primers | Total no. of bands | Mono-morphic bands | Unique bands | Poly. unique bands (+) | Poly. unique bands (-) | Poly. % | Mw (bp) | Concentrations of ZnO NPs (mg/L) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 0 | 10 | 25 | 50 | 100 | 200 |
| ISSR UBC 25 | 7 (1151-129) | 2 | 2 | 7 | 5 | 100 | 1151 | - | - | - | + | + | - |
| | | | | | | | 790 | + | + | + | + | + | - |
| | | | | | | | 658 | + | + | + | + | + | - |
| | | | | | | | 233 | + | + | + | + | + | - |
| | | | | | | | 135 | - | - | - | - | + | - |
| | | | | | | | 131 | - | - | - | + | - | - |
| | | | | | | | 129 | + | + | + | - | - | - |
| UBC 845 | 5 (658-153) | 0 | 1 | 5 | 4 | 100 | 658 | - | - | + | + | + | + |
| | | | | | | | 525 | - | + | + | + | + | + |
| | | | | | | | 335 | - | + | + | + | + | + |
| | | | | | | | 233 | - | + | + | + | + | + |
| | | | | | | | 153 | - | - | - | + | - | - |
| UBC 861 | 7 (1175-300) | 3 | 1 | 4 | 3 | 57.0 | 1175 | + | - | + | + | + | + |
| | | | | | | | 993 | + | - | + | + | + | + |
| | | | | | | | 837 | + | - | + | + | + | + |
| | | | | | | | 300 | + | - | - | - | - | - |
| UBC 880 | 12 (1337-399) | 1 | 7 | 11 | 5 | 91.7 | 1337 | - | - | + | - | - | + |
| | | | | | | | 1181 | - | - | + | + | + | + |
| | | | | | | | 1171 | + | - | - | - | - | - |
| | | | | | | | 994 | - | - | + | - | - | - |
| | | | | | | | 888 | + | - | - | - | - | - |
| | | | | | | | 541 | - | + | + | + | + | + |
| | | | | | | | 525 | + | - | - | - | - | - |
| | | | | | | | 399 | + | - | - | - | - | - |
| **RAPD-PCR** | | | | | | | | | | | | | |
| OPC-2 | 6 (2345-416) | 1 | 0 | 5 | 5 | 83.3 | 2345 | - | - | - | + | + | + |
| | | | | | | | 942 | - | - | - | + | + | + |
| | | | | | | | 686 | - | - | - | + | + | + |
| | | | | | | | 416 | - | - | - | + | + | + |
| OPG -2 | 8 (2397-309) | 6 | 2 | 2 | 0 | 25.0 | 2397 | - | - | - | - | + | - |
| | | | | | | | 2280 | - | - | - | - | - | + |
| OPO- 10 | 7 (2454-260) | 1 | 2 | 6 | 4 | 85.7 | 3454 | - | - | - | + | + | - |
| | | | | | | | 3357 | - | - | - | - | - | + |
| | | | | | | | 2303 | - | - | - | + | + | |
| | | | | | | | 268 | - | + | - | - | - | - |
| | | | | | | | 260 | - | - | - | + | + | + |

+: present, -: absent, Poly: polymorphism

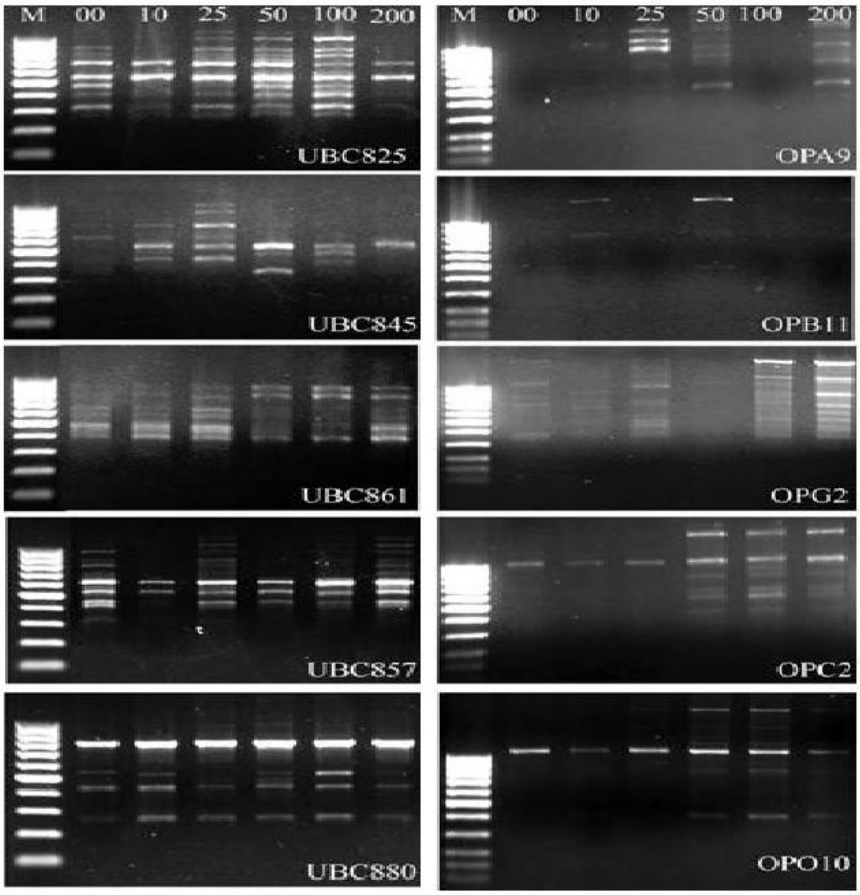


**Fig (4): Agarose gel profile of genomic DNA of *Magnaporthe oryzae* using ISSR on the left side and RAPD-PCR markers on the right side of image. Lane numbers represent serial number of control and different concentration (mg/L) of ZnO NPs. M= molecular marker ladder (100 bp).**

*3.6.1. ISSR markers:*

The total number of bands resulted from samples DNA amplification using UBC 825 was seven bands ranged from1151 to 129 bp. Seven polymorphic with unique bands were detected. On the same text, two unique bands were detected at molecular size 135 and 131 bp for 100 and 50 mg/L, respectively. The percentage of polymorphism was 100.0%. For UBC 845 primer, showed five polymorphic bands as total and ranged from 658 to 153 bp. Out of the five polymorphic bands only one

showed unique band and detected at molecular size 153 bp, and appeared for sample treated with 50 mg/L ZnO NPs. The percentage of polymorphism was 100.0%.

In case of UBC 857 primer, the total number of bands was four, all of them showed monomorphic bands and ranged from 870 to 241 bp. Hence, the percentage of polymorphism was 0%. For UBC 861 ISSR primer, the total number of bands was seven (three polymorphic without and four polymorphic with unique) and ranged from 1175 to 300 bp. One polymorphic unique band was detected at molecular size 300 bp, for the control (Sakha101) meanwhile was absent at all concentrations of ZnO NPs. All the bands were presented at molecular sizes with 1173, 993 and 837 bp at all different concentrations of ZnO-NPs except at 10 mg/L was absent. The percentage of polymorphism was 57%.

Finally, the total number of bands obtained by using UBC 880 was 12 bands ranged from1337 to 399bp (five polymorphic without and 11 polymorphic with unique). Seven unique bands were detected and out of them two unique bands were detected at molecular sizes 1337 and 994 bp at the lower concentration of ZnO-NPs (25 mg/L). Another unique band was detected at molecular size with 1337 bp at high concentration of ZnO-NPs (200 mg/L). The percentage of polymorphism was 91.7%.

*3.6.2. RAPD-PCR primers:*

Out of seven RAPD-PCR primers utilized in this investigation, only three primers amplified and generated polymorphic bands (OPC-2, OPG-2 and OPO-10) at all different concentrations of ZnO-NPs. The total numbers of bands obtained from samples DNA amplification using OPC-2 primer was 6 bands ranged from 2345 to 416 bp. Only one monomorphic band was detected and five were polymorphic with unique. The polymorphic unique bands were detected at molecular size with 2345, 942, 686, 595 and 416 bp and presented at all intermediate and high concentrations of ZnO NPs (50, 100 and200 mg/L). Meanwhile, these five polymorphic bands were absent at the lower concentrations of ZnO NPs (10 and 25 mg/L). The polymorphism percentage was 83.33%.

In case of OPG-2 primer, the total number of bands obtained was eight ranged from 2397 to 309 bp. Six monomorphic bands were detected and presented at all different concentrations of ZnO-NPs with molecular size (1522, 1028,733, 543, 451 and 309 bp). Two polymorphic unique bands were detected at 2397 bp with 100 mg/L and 2280 bp with 200 mg/L concentration of ZnO-NPs. For OPO-10 primer, the total number of bands obtained was seven bands and ranged from 3454 to 260 bp. Two polymorphic unique bands were detected at 3357 bp with 200 mg/L and 268 bp with 10 mg/L concentration of ZnO NPs. The polymorphic with unique bands were detected at molecular size with 3454, 2303 and 787 bp presented at all intermediate and high concentrations of ZnO-NPs (50 and 100 mg/L). The polymorphism percentage was 85.7%.

*3.7. Effect of ZnO NPs on genomic DNA of rice cultivar Sakha 101 using ISSR and RAPD-PCR markers:*

In this study, genomic DNA of rice cultivar Sakha 101 was extracted and subjected to five ISSR markers and seven RAPD primers (Table 6 and Figure 5). All ISSR primers amplified and generated polymorphic alleles at all different concentrations of ZnO-NPs. On the other hand, out of seven RAPD primers used in this study, only five primers amplified and generated polymorphic bands.

**Table (6): The effect of different concentrations of ZnO NPs on genomic DNA of rice cultivar Sakha101**

| Type of Primers | Total no. of bands | Mono-morphic bands | Unique bands | Poly. unique bands (+) | Poly. unique bands (-) | Poly.% | Mw (bp) | Concentrations of ZnO NPs (mg/L) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 0 | 10 | 25 | 50 | 100 | 200 |
| ISSR UBC 825 | 9(915-255) | 2 | 1 | 7 | 6 | 87.5 | 915 | + | + | - | + | + | + |
| | | | | | | | 822 | + | + | - | + | + | + |
| | | | | | | | 718 | + | + | - | + | + | + |
| | | | | | | | 616 | + | + | - | + | + | + |
| | | | | | | | 422 | + | + | - | + | + | + |
| | | | | | | | 309 | + | + | - | + | + | + |
| | | | | | | | 306 | - | - | + | - | - | - |
| UBC 845 | 5 (915-357) | 4 | 1 | 1 | 0 | 20.0 | 464 | - | - | - | - | + | - |
| UBC 857 | 4 (822-243) | 3 | 1 | 1 | 0 | 25.0 | 330 | + | - | - | - | - | - |
| UBC 861 | 7 (985-236) | 3 | 3 | 4 | 1 | 57.0 | 985 | - | - | - | + | - | - |
| | | | | | | | 892 | - | - | - | - | + | - |
| | | | | | | | 827 | - | - | - | + | - | - |
| | | | | | | | 236 | - | + | - | + | - | - |
| UBC 880 | 4(571-260) | 3 | 1 | 1 | 0 | 25.0 | 260 | - | - | - | - | - | + |
| RAPD-PCR | | | | | | | | | | | | | |
| OPA-9 | 10 (1709-295) | 8 | 1 | 2 | 1 | 20.0 | 948 | - | + | - | - | - | - |
| | | | | | | | 899 | + | | + | + | + | + |
| OPA-13 | 6 (3290-213) | 3 | 1 | 3 | 2 | 50.0 | 3290 | + | - | - | + | + | + |
| | | | | | | | 2188 | - | - | - | - | - | + |
| | | | | | | | 613 | + | - | - | + | + | + |
| OPC-2 | 5 (1156-338) | 0 | 0 | 5 | 5 | 100.0 | 1156 | - | + | + | + | - | - |
| | | | | | | | 1048 | - | - | + | + | - | - |
| | | | | | | | 637 | - | + | + | + | - | - |
| | | | | | | | 523 | - | + | + | + | - | - |
| | | | | | | | 338 | + | + | + | + | + | - |
| OPO-10 | 2(1347-1080) | 0 | 0 | 2 | 2 | 100.0 | 1347 | + | + | + | + | + | - |
| | | | | | | | 1080 | + | + | + | + | - | - |

+: present, -: absent, Poly: polymorphism

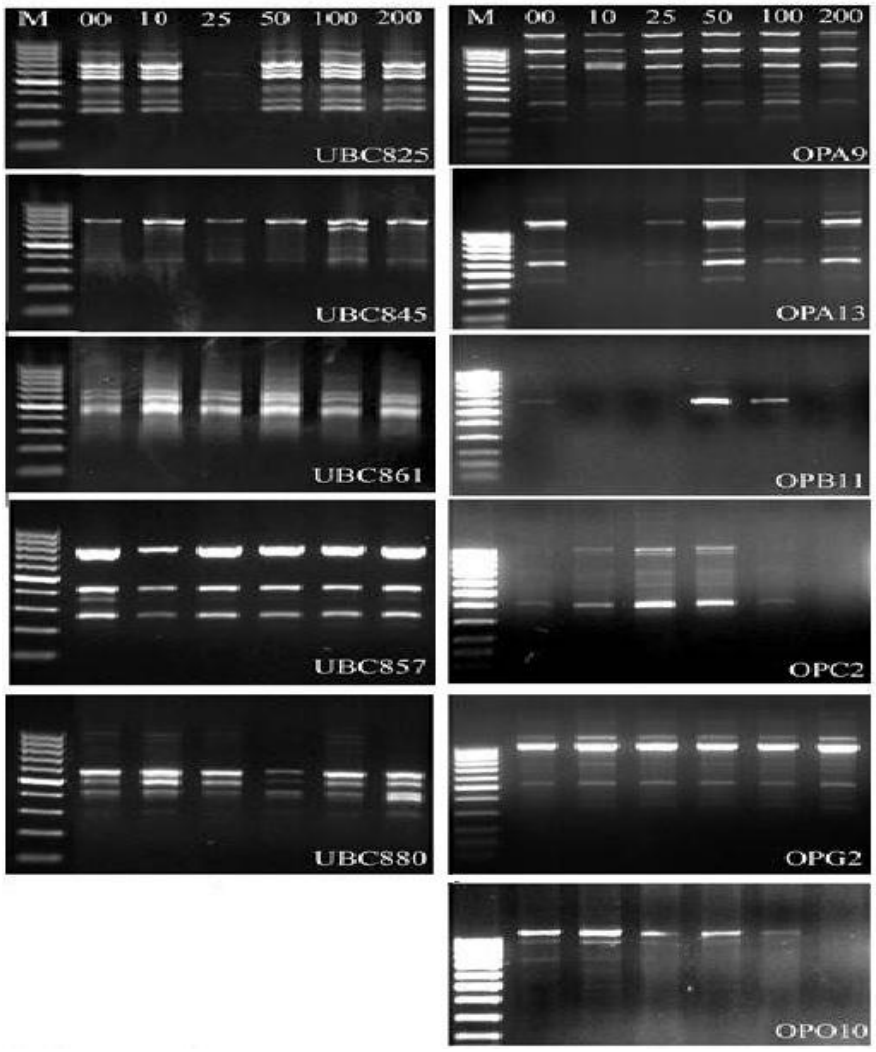


**Fig (5): Agarose gel profile of genomic DNA of rice cultivar Sakha 101 using ISSR on the left side and RAPD-PCR markers on the right side of image. Lane numbers represent serial number of control and different concentration (mg/L) of ZnO NPs. M= molecular marker ladder (100 bp).**

*3.7.1. ISSR markers:*

The total number of bands resulted from samples DNA amplification using UBC 825 was nine bands ranged from 915 to 255 bp. Only one unique band was detected

at molecular size 306 bp for sample treated with 25 mg/L ZnO-NPs. On the other hand, two monomorphic bands were detected and seven polymorphic with unique bands were appeared. The percentage of polymorphism was 87.5%. UBC 845 ISSR primer showed five polymorphic bands ranged from 915 to 357 bp and only one unique band was detected. The unique band was appeared at molecular size 464 bp for sample treated with 100 mg/L ZnO-NPs. In primer UBC 857, the total bands were four bands ranged from 822 to 243 bp. The three monomorphic bands were detected at molecular size 822, 412 and 243 bp while the only one polymorphic unique band was detected at molecular size 330 bp, for the control (Sakha101). The percentage of polymorphism was 25%. For UBC 861 ISSR primer, the total number of bands was seven (three monomorphic and four polymorphic with unique) and ranged from 985 to 236 bp. Three unique bands were detected at molecular size 985 and 827 bp and were appeared for sample treated with 50 mg/L ZnO-NPs and at 892 bp for 100 mg/L. The percentage of polymorphism was 57%. In case of UBC 880, the total number of bands was four (three monomorphic and one polymorphic with unique bands) that ranged from 571 to 260 bp. A unique band at molecular size 260 bp was appeared for sample treated with 200 mg/L ZnO-NPs.

*3.7.2. RAPD-PCR primers:*

Out of seven RAPD-PCR primers used in this study, only five primers amplified and generated polymorphic bands (OPA-9, OPA-13, OPC-2, OPG-2 and OPO-10) at all different concentrations of ZnO-NPs. The total number of bands obtained from samples DNA amplification using OPA-9 primer was 10 bands ranged from 1709 to 295 bp. Eight monomorphic bands were detected and two were polymorphic with unique.

The unique polymorphic band was detected at molecular size 948 bp, and appeared for sample treated with 10 mg/L ZnO-NPs. In primer OPA-13, the total number of bands was six (three monomorphic and three polymorphic with unique) and ranged from 3290 to 213 bp. The unique polymorphic band was detected at molecular size 2188 bp, and appeared for sample treated with 200 mg/L ZnO-NPs. The percentage of polymorphism was 50%.

In case of OPC-2 primer, the total numbers of bands were five, four of them showed polymorphic with unique and ranged from 1156 to 523 bp. The unique polymorphic bands were detected at molecular size 1156, 637 and 523bp, and appeared for sample treated with lower concentrations of ZnO-NPs (10, 25 and 50 mg/L) respectively. On the same text, one polymorphic unique band was detected at molecular size 1048 bp for sample treated with lower concentrations of ZnO-NPs (25 and 50 mg/L). The percentage of polymorphism was 100%. Related to OPG-2 primer, the total numbers of bands were six, all of them showed monomorphic bands and ranged from 1529 to 618 bp. Hence, the percentage of polymorphism was 0%. Finally, OPO-10 primer, showed only two polymorphic bands as a total and ranged from 1347 to 1080 bp and hence, the percentage of polymorphism was 100%.

## Discussion

Nanoparticles of different metal oxides can play important role to promote the growth and yield of plants as well as suppression of plant pathogens as a novel strategy (Petosa *et al*., 2017). The performance of Zn NPs was promised across the current study, it was observed that Zn NPs are more frequently associated with increases in growth and yield of rice when grown in pathogen-infection depending on concentration and time of applications. In all cases, the results were surprising given that lasting, often season-long, effect was achieved with a singular dose to young ages seedlings at nursery. These findings are extremely important for future field applications, in these small amounts of NPs could be applied under controlled and safe conditions to young seedlings, thereby reducing concerns over NPs exposure to humans and the environment.

Recently, the potential of ZnO for controlling pathogen growth was discussed. ZnO NPs are likely a more appropriate choice for fungal pathogen control than is Ag (Dimkpa *et al*., 2013). For example, ZnO NPs demonstrated higher inhibition against *F. graminearum* (Dimkpa *et al*., 2013), *Helmenthosporium oryzae* (Elamawi *et al*., 2016), *in vitro*. In a similar study, biosynthesized ZnO NPs (25 µg/mL) displayed higher inhibition rates against pathogenic bacteria and the fungal species (Jayaseelan *et al*., 2012). ZnO NPs significantly inhibited mycelia growth of *B. cinerea* and *P. expansum* in a plating assay (He *et al*., 2011). They referred theses inhibitory effects to interfering cell function and causing deformation in fungal hypha in *B. cinerea*, in addition to, inhibiting the development of conidia and conidiophores in *P. expansum*. Several mechanisms were proposed to explain the antimicrobial activity of ZnO NPs. The generation of hydrogen peroxide from the surface of ZnO NPs is considered as an effective mean for the inhibition of fungal growth. Another possible mechanism is the release of Zn2+ ions which can damage the cell membrane and interact with intracellular contents (Sirelkhatim *et al*., 2015). Navale *et al*., (2015) proposed that ZnO NPs and their photo catalytic properties contribute greatly to their antimicrobial activity causing structural changes of microbial cell membrane, oxidation stress and eventually the death of the cells (Sirelkhatim *et al*., 2015). In contrast to the present results, the stress caused by the ZnO NPs treatments can lead to an increase in conidia production by some fungi as mentioned by (Savi *et al*., 2013).

Foliar application is considered as a quick method to correct zinc unavailability and improvement resistance to plant diseases. Results under greenhouse and field conditions indicated that foliar application on young rice transplant lead to improve rice blast and yield under field conditions. ZnO NPs applied 5 DBI in artificial inoculation, nursery as well as at 25 followed by 50 DPT at concentration 25mg/L were the most effective treatments for reducing rice blast severity. Similar results were reported by Elamawi *et al*., (2016) on rice brown spot disease. In contrast to this investigation, Moreira *et al*., (2013) found that increasing Zn in leaf tissues was

associated with increasing rice susceptibility to brown spot post foliar application of ZnSO4.7H2O. It can be associated with the stimulation for production of non-selective toxins that become deleterious to the leaves. The mechanism of foliar uptake pathway for nanoparticles was well discussed by (Eichert *et al*., 2008 and Wang *et al*., 2013), their results revealed that the ZnO-NPs having smaller sizes could penetrate into plant leaves following the stomatal pathway, pass through different plant parts over the phloem sieve tubes. The nanoparticle size, concentration, application methods, environment conditions, and leaf structure are important factors to be considered for successful plant foliar uptake (Wang *et al*., 2013).

Several studies were conducted on Zn applications in rice crop contributing to increase grain yield under normal (Swamy *et al*., 2016) or salinity soil (Elamawi et al. 2016). This investigation clearly demonstrated that the ZnO NPs could be the good fertilizer and antifungal agent against pathogenic fungi that can help to avoid crop infection of *M. oryzae*.

Nanotoxicity of ZnO NPs to plants at the phytotoxicity and genotoxic level has been demonstrated by seed germination, seedling growth, isoenzyme systems, cytogenetic test (Youssef and Elamawi 2018). The results presented herein showed that, the effect of ZnO-NPs on germination was not varied significantly between different concentrations and the control. As well as, there is no reduction in the germination percentage due to ZnO NPs but enhancement was observed at lower concentrations (10 and 25 mg/L) (Fig. 1). It seems that ZnO NPs didn't affect seed germination in radish, rape, ryegrass, lettuce and cucumber except the corn seed as mentioned by Lin and Xing (2007), in soybean (López-Moreno *et al*., 2010) and on rice seedling (Boonyanitipong *et al*., 2011). In the current study, Zn NPs promoted plant growth as evidenced from improved shoot and root length at lower concentrations, while 100 and 200 mg/L ZnO NPs significantly inhibited the growth of rice seedlings. Similar results were reported by Bala *et al*., (2019) where the morphological characters as shoot and root length increased when rice seedlings were exposed to ZnO-NPs. This improvement may be recognized to the role of zinc in cell elongation, increased antioxidant responses, membrane function, and protein synthesis. As a consequence the Zn NP treated seeds, showed better potential for germination (Upadhyaya *et al*., 2017).

Toxicity of ZnO NPs to rice seedlings specially the root growth was evident and increased with increasing concentration of ZnO NPs. This could be attributed to an excess of Zn ions released by the NPs as reported by Lin and Xing (2008) or to an interaction between the NPs and the root surface. The interaction of ZnO NPs with plants could be influenced by the species of plants. Lin and Xing (2008) observed that ZnO NPs reduced biomass, shrunk root tip and epidermis, and cortical cells became highly vacuolated and collapsed on ryegrass. Also, (Chen *et al*., 2015) reported that ZnO NPs (250 mg/L) practically reduced root and shoot length on

ryegrass, (Boonyanitipong *et al*., 2011) observed reduction in number of roots and stunted the length of rice seedlings (*Oryza sativa L*). (Chen *et al*., 2015) explained that ZnO NPs have entered into the roots and shoots of rice seedlings. Where, dark dots in the roots and shoots under ZnO NPs treatment were observed and concentrated on the cell wall surface. This observation was also noticed by other researchers, ZnO NPs primarily adhered to root surfaces, and low upward translocation of ZnO NPs to shoots during the uptake of ZnO NPs by ryegrass (Lin and Xing 2008). In contrast, ZnO NPs were found to reduce seed germination in alfalfa, soybean, tomato, and cucumber at high concentration (4000 mg/L). However, corn germination was significantly reduced even at the minimum concentration of 500 mg/L. ZnO-NPs at lower conc. could be used to improve seedling vigor especially for sensitive genotypes.

Results of this study indicated that ZnO NPs has the potentiality to induce synthesis as well as disappearance of protein bands. There were unique proteins at molecular weight (120, 99, 89,77, 72. 61 KDa) in treated seeds of rice cultivar Sakha 101 at the concentrations 200 mg/L of ZnO-NPs. There are protein bands at molecular weight 63 and 60 KDa at the concentrations of ZnO-NPs 10, 25, 50 and 100 mg/L, on the other hand these bands were absent in control seeds, this occurs due to the effect of ZnO-NPs treatment on the expression of some genes causing to it turn on to encode proteins while in the control this gene turns off. In contrast, the application of ZnO-NPs may cause turn off to some genes while expressed in the control sample (0 mg/L), leading to the absence of some protein patterns under ZnO-NPs treatments while presence in control condition such as the protein pattern at molecular weight 59 KDa. Proteins are main products of active structural genes expression; their size and amino acids sequence are the direct results of expression process of nucleotide sequences of the genes; hence, any observed variation in protein banding pattern induced by any mutagen is considered a mirror for genetic variations (Hamoud *et al*., 2005). So, alteration of protein profile could be useful for exploring the potentiality effects of pollutants or chemicals as well as nanomaterials.

The presence of these new bands may exhibit alterations in the priming sites leading to new annealing conditions in addition to homologous recombination which leads to the appearance of new bands (Atienzar and Jha 2006). Rice Sakha 101 plants were more sensitive to the effects of ZnO NPs as more band alterations were observed when compared to the control. The only difference between the treated and control plants were the presence or absence of ZnO-NP which support that the changes in the DNA were caused by the effect of the ZnO-NP. It was observed that the more effective concentrations on the plant genome were 10 and 25 mg/L compared to control.

From field experiment, the best values to improve rice grain yield and reduce leaf blast were obtained with ZnO NPs at a concentration of 10 and 25 mg/L, this indicates that the lower concentrations of ZnO NPs (10 and 25 mg/L) are considered

as a good enhancement agent in case of rice cultivar such as UBC 825 primer with 306 bp at concentration of 25 mg/L and OPA-9 primer with 948 bp at concentration of 10 mg/L. For *M. oryzae*, the same trend of effects was appeared in case of UBC 880 primer with 994 bp at concentration of 25 mg/L and OPO-10 primer with 268 bp at concentration of 10 mg/L.

## Conclusions

It can be concluded that the foliar application of ZnO NPs on rice cultivar Sakha101 offers a practical and useful approach to improve the performance of rice plant and increasing productivity as well as mitigate leaf blast when applied at optimal concentration and appropriate time. Biochemical and molecular markers are considered as an effective tools and evidence of enhancement effects for lower concentrations of ZnO NPs on rice cultivar Sakha101. Meanwhile, it showed inhibition effects on *M. oryzae* causing rice blast.

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# تقدير التاثير المضاد لجزيئات الزنك المتناهية الصغر على الفطر *Magnaporthe* oryzae و سميتها علي صنف الارز سخا ١٠١ باستخدام المعلمات المورفولوجيه والبيوكيماوية والجزيئية

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يعتبر مرض اللفحة للأرز المتسبب عن الفطر *Magnaporthe oryzae* من اهم الامراض المدمرة للأرز في مصر حيث تؤثر سلبيا على محصول الأرز. تهدف هذه الدراسة الي تقدير تأثير المستويات المختلفة (صفر,١٠, ٢٥, ٥٠ , ١٠٠ و ٢٠٠ مليجرام/ لتر من جزيئات الزنك المتناهية الصغر علي الفطر المسبب لمرض لفحه الارز و صنف الارز سخا 101 باستخدام المعلمات المورفولوجية و البيوكيميائية و الجزيئية. استخدمت خمسة معلمات ISSR وسبعة معلمات RAPD و ذلك لدراسة التاثيرات المحتملة لاستخدام جزيئات الزنك المتناهية الصغر. تم تقييم تأثير المعدلات المختلفة و طريقه المعاملة لجزيئات الزنك المتناهيه الصغر علي مرض لفحه الارز و تحسين محصول الارز خلال موسمي الزراعة ٢٠١٧ ٢٠١٨. و اظهرت النتائج التأثير المضاد لجزيئات الزنك المتناهية الصغر على الفطر في المعمل حيث اظهرت نقصا معنويا في عدد المستعمرات المتكونة مقارنه بالكنترول. وكانت معامله الرش بتركيز ٢٥ مليجرام / لتر من جزيئات الزنك النانونيه اكثر المعاملات في تقليل الاصابه بمرض اللفحه عند معاملتها قبل تلقيح النباتات بالفطر بخمسة ايام وذلك تحت ظروف الصوبة. بينما تحت ظروف الحقل كانت معامله الرش بتركيز 25 مليجرام/ لتر فى المشتل عند عمر ٢٠ يوم افضل المعاملات و ادت الي تحسين محصول الحبوب حيث بلغ ٤,٣٦٦ طن/للفدان في موسم ٢٠١٧ و الي ٤,٦٢٥ طن/للفدان في موسم ٢٠١٨. التركيزات المنخفضة من جزيئات الزنك النانونيه (١٠ و ٢٥ ملجم / لتر) شجعت من إنبات البذور وتحسين نمو البادرات ، في حين أن التركيزات الأعلى (١٠٠ و ٢٠٠ ملجرام / لتر) أسفرت عن تسمم النبات. المعاملة بجزيئات الزنك النانونيه غيرت أنماط التعبيرعن البروتين المخزن في البذور، حيث ادت الي استحثاث تكوين حزم بروتينيه جديده واختفاء تلك الموجودة. واظهرت نتائج

متشابهة باستخدام المعلمات الجزيئية على ان التركيزات المنخفضة من جزيئات الزنك النانونيه تعتبر كعامل تنشيط واستحثاث جيد ؛ كما فى حالة صنف الارز سخا ١٠١ باستخدام المعلم الجزيئي UBC825عند ٣٠٦ قاعدة وزن جزيئي لتركيز ٢٥ ملليجرام/ لتر والمعلم الجزيئي OPA-9 عند ٩٤٨ قاعدة وزن جزيئي لتركيز ١٠ ملليجرام/لتر. اما بالنسبة للفطر فقد ظهر نفس اتجاه التأثير كما في حالة المعلم الجزيئي UBC880 عند ٩٩٤ قاعدة وزن جزيئي لتركيز ٢٥ ملليجرام/لتر والمعلم الجزيئي -OPO ١٠ عند ٢٦٨ قاعدة وزن جزيئي لتركيز ١٠ ملليجرام/لتر. فى العموم فان هذه النتائج تشير إلى ان رش المجموع الخضري بجزيئات الزنك النانونيه تعتبر طريقة عملية ومفيدة للحد من الإصابة بمرض لفحه الأوراق وتحسين محصول حبوب الأرز عند تطبيقها بالتركيزات المثالية. ايضا يمكن تطبيق جزيئات الزنك النانونيه كأسمدة فعالة ومبيدات فطريه للزراعة المستدامة وسلامة الأغذية.