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Assessment of the nanosized particles of ZnO and MgO and some cultivars in control of *Alternaria solani* causing tomato early blight

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ABSTRACT

The aims of this study were to isolate and identify the causal microorganism causing the early blight on tomato and evaluate the efficiency of nanosized particles of ZnO and MgO and three tomato cultivars to control it. Morphological and molecular approaches were employed in diagnosis of the fungal pathogen. Influence of three concentrations (1,3,5 g/L) of two nanoparticles (ZnO and MgO) were assessed on the pathogen. Subsequently, the three concentrations of ZnO NPs with the three cultivars of tomato were evaluated under field conditions using plastic tunnels in management of the pathogen. The result of the morphological and molecular identification showed that the fungus caused the disease was *Alternaria solani*. Furthermore, all concentrations of ZnO nanoparticles proved to be effective against the causative agent achieving an inhibition percentage exceeded 50%. However, same concentrations of MgO NPs did not show efficiency in inhibition of the same pathogen. As well as, all three tomato cultivars were susceptible to pathogen of early blight, although they exhibited various percentages of disease severity with superiority to HLACV. that reached the least percentage (21.84%). Additionally, the results demonstrated that the two concentrations (3 and 5g/L) of ZnO NPs were the most effective in causing significant decrease in the percentage of diseased severity to 17.04 and 20.13% respectively comparing with control treatment that was 30.6%. Moreover, a significant integration was attained between the nanosized ZnO particles and the three cultivars in reduction of the disease severity percentage. Consequently, the best combination was between the two concentrations (3 and 5 g/L) and Hlacv. that caused a reduction to 13.20 and 15.72 % respectively compared with 29.51% in control treatment and 30.49% in the fungicide treatment. These results indicate clearly to possibility of replacing the fungicides with the ZnO NPs particularly at concentrations 3 and 5 g/L and the Hla tomato cultivar in the integrated management of early blight caused by *A. solani* under the circumstances of tunnels and plastic houses in Iraq and this can participate in conserving the environment.

Key words : *Zno, Mgo, Alternaria Sonani, Tomato early blight.*

Introduction

Tomato (*Solanum lycopersicum* L.) is an annual herbaceous plant belonging to the Solanaceae family. Its crop is one of the most important vegetable crops worldwide (Khalil, 2004). It grows widely in open fields or covered farming using greenhouses or under plastic tunnels in many countries, including Iraq (FAOSTAT, 2018). However, this crop has confronted many agricultural problems that have caused large losses in yield and quality. One of these problems is diseases such as early blight

caused by*Alternaria solani* fungus.

This disease is considered one of the most common leaf diseases worldwide appearing on the members of the family Solanaceae (Vander-Walls *et al.*, 2001; Abdulmoohsin, and Husain (2014). Symptoms of this disease are concentrated on leaves, branches and fruits forming concentric irregular spots surrounded by yellow halo. As the infection progresses, the entire leaf and branch become infected, resulting in death and falling of them. Moreover, necrotic lesions emerge on the stems and fruits leading to tissues rot. The high relative humidity due to dew, rain and excessive irrigation particularly under covered farming practice of tomato crop are considered the main factors that can assist in occurrence and development of the disease (Trigiano *et al.*, 2004).

Excessive and frequent application of fungicides have caused development resistant strains of phytopathogens as well as their toxic influences on humans, animals and beneficial organisms (Hameed, 2019; Slomy *et al.*, 2019). As a result of these disadvantages, the plant protectors have sought to discover and assess environmentally friendly control approaches. Resistant varieties, for example, are one of the best tactics to control plant diseases (Agrios, 2005). Additionally, application of alternative means such as the employment of nanoscale sizes particles (at least one of their dimensions of 1-100 nanometers) has become increasingly attractive for use in various aspects of agriculture including plant protection. This is due totheir high efficiency against harmful microorganisms. For example, the nanoparticles of gold, silver, titanium dioxide and zinc monoxide proved highly effective capabilities in killing or inhibiting many phytopathogens (Chauhan *et al.*, 2014; Anusuya and Sathiyabama, 2015).

Thus, due to the importance of this disease on tomato crop in Iraq and no fully effective approachis available incontrol it, this study aimed to assess the efficacy of the nano-particles of ZnO and MgO and three tomato varieties and the integration between them in managing of this disease.

Materials and Methods

Isolation and identification of the fungus causing the early blight on tomato

Samples of the diseased tomato leaves showing symptoms of early blight were collected from greenhouses and plastic tunnels at the College of Agriculture /University of Karbala during the growing season of 2018. These symptomatic samples were washed thoroughly with distilled water, dried and cut into small pieces (1-1.5 cm long) after removing of the dead parts and then disinfected with a solution of sodium hypochlorite (NaOCl) at concentration 2% for three minutes. The pieces were then washed thoroughly with sterile distilled water, dried and placed on Water Agar medium plates (five sterile pieces/plate). All the plated were then incubated under 25 ± 2 ° C in darkness. After 2-3 days, the fungal colonies were purified using the hyphal tip technique by transferring disc (5 mm in diameter) from the edge of each colony to a plate containing the Potato Dextrose Agar (PDA) medium (Burns, 2009). The fungal isolates accompanying with the infected leaves wereidentified initially based on their morphological features that includedthe colonies structure, color, shapes and measurement of the fungal mycelia, conidia and conidiophores. The pure isolates were then preserved in the refrigerator under temperature of 4 °C for further analysis.

The DNeasy Plant Mini Kit (QIAGEN N.V., Hilden, Germany) was used to extract the genomic DNA mycelial growth of the pure fungal colonies. The polymerase chain reaction (PCR) was employed using the universal primer set ITS1 and ITS4 (White *et al.*, 1990) for amplification of the internal transcribed spacers of ribosomal DNA. The PCR products were sequenced at Macrogen, Inc. (Seoul, South Korea). The nucleotide sequences collected were compared with other sequences of fungi deposited atGenBank sequence database of NCBI using the Basic Local Alignment Search Tool program (BLAST). Subsequently, the generated sequence was assigned with a specific accession number in GenBank database after submission.

The effectiveness of ZnO and MgO nanoparticles on the isolated causative agent of tomato early blight

The toxicmedia method (Hameed, 2019) was employed for the purpose of examination the effect of ZnO and MgO nanoparticles on the radial growth of the fungus isolated. Three concentrations (1,3,5 g/L) of each type of nanoparticles were added separately to PDA media that was then autoclaved for 20 minutes, after which it was distributed in plate (approximately 20 mL/plate). As well as, PDA media

plates free of nanoparticles were prepared to be employed as control. These plates were then inoculated with discs (0.5 cm in diameter) collected from a 7-day-old pure colony of the pathogen. Furthermore, PDA media plates containing the fungicide Beltanol (1 ml/L) were prepared for comparison with. All treatments and controlwerereplicated four times. They were then incubated at 25 ± 2 ° C in dark. After growth of the pathogenic fungus reached to the edge of control plates (free of nanoparticles and fungicide), percentage of inhibition was calculated according to the following equation:

$$\text{Inhibition \%} = \frac{\text{Average of fungal growth in control plates} - \text{Average of fungal growth in treatment plates}}{\text{Average of fungal growth in control plates}} \times 100$$

(Abbott, 1925; Ahmed *et al.*, 2006)

Evaluation the efficacy of the integration between ZnO nanoparticles and some tomato cultivars in management of the early blight disease under plastic tunnel conditions

This experiment was carried out in infectious plastic tunnels at College of Agriculture /University of Karbala during growth season of 2019. Plastic pots (4 kg size) were filled with organic fertilizer and planted with tomato seedlings (4 weeks old) of Hla, Siemens 242 and Boob Cat commercial cultivars (one seedling per pot). The tomato seedlings were monitored daily and watered whenever required. The experiment was accomplished according to Randomized Complete Block design (R.C.B.D) and applied the least significant difference (L.S.D) analysis. Each treatment was repeated eight times in each cultivar and the following treatments were carried out:

(1) Spraying of the tomato seedlings of the three cultivars separately with ZnO NPs at concentration 1 g/L
(2) Spraying of the tomato seedlings of the three cultivars separately with ZnO NPs at concentration 3 g/L
(3) Spraying of the tomato seedlings of the three cultivars separately with ZnO NPs at concentration 5 g/L
(4) Spraying of the tomato seedlings of the three cultivars separately with Beltanol fungicide at concentration 1 ml/L
(5) Spraying of the tomato seedlings of the three cultivars separately with water only as a control.

The percentage of the early blight disease incidence on the three tomato cultivars was calculated using the following equation:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

Additionally, the percentage of the disease severity was recordedaccording to Duarte *et al.* (2010) 's scale (Figure 1) with some modifications andapplication of the equation below:

$$\text{Disease severity (\%)} = \frac{\Sigma\ (\text{Score of disease} \times \text{Number of plants})}{\text{Total number of seedlings} \times 10} \times 100$$

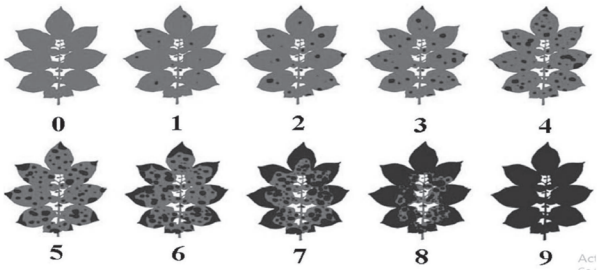

Fig. 1. As cale for measurement ofthe early blight severity caused by A. solani; 0=0%, 1=1%, 2=3%, 3=5%, 4=10, 5=20%, 6=40%, 7=60%, 8=80%, 9=100%.

Results and Discussion

Isolation and identificationof the fungal agent causing early blight disease on tomato

The isolation process was conducted from tomato leaves displaying symptoms of brown spots with irregular or circular edges, which develop to become dark with a skin appearance and combine together to include large areas of infected leaf ending with dryness and fall (Figure 2 A+ B). After purification of the fungus associated with diseased tomato leaves, its morphological properties were examined. The colonies emerged consisted of divided and branched fungal mycelia that was part of it immersed in the media and the other was superficial in dark olive or black to dark gray color with some scattered white growth (Figure 2 C). The fungal conidia were observed in dark brown color in different sizes. The larger conidia were usually ovaloften divided with 4–6 transverse and occasionally with 1-2 longitudinal septawith a relatively longellip soidal tapering to a beak that was straight or little curved. On the other hand, the smaller conidia were usually circular divided by longitudinal and trans-

verse septa or only transverse septa (Figure 2 D). The conidiophores were dark brown in color, septate and in straight or flexuous shape. Based on these cultural and microscopic features, which were compared with those descriptions mentioned previously (Ellis, 1971; Watanabe, 2010; Narayanasamy, 2011) the fungus was initially identified as *Alternaria solani*. It should be mentioned that the 20 fungal isolates collected randomly from all samples of the infected leaves belonged to the same fungus.

This morphological identification was confirmed by molecular analysis that showed a high similarity >99% between nucleotide sequences of the representative isolate (Accession number: MN121432.1) and several global isolates stored in GenBank such as those with accession numbersMG273689.1, KT721914.1, KC478609.1 and AY154716.1. This molecular identification is first record generated based on the molecular marker (ITS) which confirmed the *A. solani* as one of fungal pathogen causing early blight disease on tomato crop in Kerbala province, Iraq. This result is in compatible with several previous studies that indicated to *A. solani* as a pathogen causingthe early blight on tomato crop worldwide (Thomma, 2003; Simmons, 2007; Roopa, 2012; Adhikari *et al.*, 2017)

Fig. 2. Pathogenic, cultural and microscopic characterizations of *A. solani* causing early blight on tomato. A and B the Early and late stage of disease symptoms; C the upper (left) and lower (right) surfaces of the pathogen colony; D conidia of the pathogen

The effective ness of ZnO and MgO nanoparticles on the isolated causative agent of tomato early blight, *A. solani*

The three concentrations (1, 3, 5g/L) of ZnO NPs showed a good inhibition capacity against*A. solani*growth reached 50, 62.50, 65.62% respectively (Figure 3A + B + C). However, the same concentrations of MgO NPs demonstrated a very low inhibition capability amounted to 0, 4.25, 8.37% respectively (Figure 3 E + F+ G). However,the fungicide Beltanol (Figure 3 H) caused a full inhibition 100% to growth of the fungal pathogen, while it was in control plate 0% (Figure 3D).

Generally, the inhibition mechanism of ZnO nanoparticles against microorganisms belongs to their direct interference with the cell wall, which affectson cell optional transmission, leading to entry of nanoparticles to inside and stimulating the oxidation process that ultimate with growth inhibition. Additionally, ZnO NPs have proven its high toxicity against many harmful microorganisms including those cause plant diseases (Lahuf *et al.*, 2019).

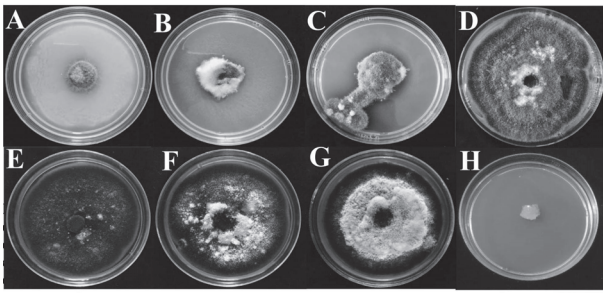

Fig. 3. The effect of ZnO and MgO nanoparticles on the radial growth of fungus *A. solani* causing early blight on tomato. In images(A, B, C)are showing the influence of the concentrations of 5, 3, 1 g/L of ZnONPs while in images (E,F, G)are presenting the effect of the same concentrations of MgO NPs. Image (D) is the fungal growth in control (without any nanoparticles or fungicide). Image (H) is the effect of the fungicideBeltanol on *A. solani.*

Evaluation the efficacy of the integration between ZnO nanoparticles and some tomato cultivars in management of the early blight disease under plastic tunnel conditions

The results displayed that the three tomato cultivars used in this study were sensitive to the early blight pathogen by achieving 100% disease incidence. In contrast, the same cultivars exhibited different capabilities in reducing of the disease severity percentage. The most significant reduction was achieved byHlacultivar (21.84%) followed by Boob Cat (23.26%) and Siemens (27.92%) cultivars (Figure 4).

The results also revealed (Figure 5) that the concentrations (Co.) 3 and 5 g/L of ZnO nanoparticles achieved a significant reduction of the disease severity amounting to 17.04 and 20.13% respectively compared to 30.6% in control treatment. It should be noted that these concentrations had also a significant advantage in comparison with the treatment of the fungicide (Beltanol), which was 26.18%.On the other hand, the concentration 1 g/L decreased the

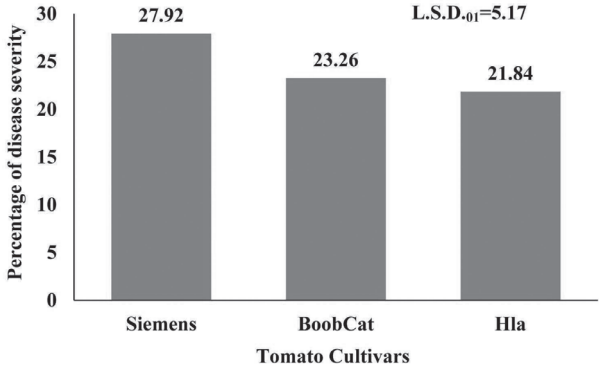

Fig. 4. The average percentage of the early blight severity on tomato cultivars examined

severity of the disease to 27.77%, but it was not a significant comparing to the treatment of control.

In addition to these results, the statistical analysis showed that there was a positive interaction between some concentrations of ZnO nanoparticles and the three tomato cultivars against the pathogen by reducing the severity percentage of the early blight. The best interactions were between concentrations 3 and 5 g/L of ZnO NPs and Hla cultivar, which decreased disease severity into 13.20 and 15.72% respectively comparing to the treatments of

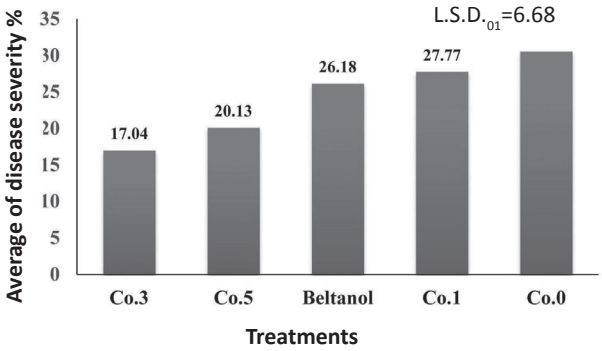

Fig. 5. The effect of different concentrations of ZnO NPsand the fungicide Beltanolon the percentage of the early blight severity

control (concentration of 0 g/L) that was 29.1% and the treatment of Beltanol fungicide which was 30.49%. As well as, the interaction between the concentration 5 g/L and Boob Catcultivar achieved 14.17%. Furthermore, the interaction between the concentration 3 g/L and the Siemens caused a significant reduction reached 18.54%.

The above results indicate clearly to a conclusion that of ZnO nanoparticles can be among control methods used to control of*A. solani*, the causal agent of early blight on tomato. This fact is consistent with the results of many previous studies that reveled the efficiency of ZnO NPs against many plant pathogens such as *Penicilliume xpansum*, *Fusarium graminearum*, *Botrytis cinerea*, *Alternaria alternate*, *F. oxysporum, Rhizoctonia solani* and *Rhizopus stolonifer* (Wani and Shah, 2012; Hameed, 2019).

It should be stated that the ZnO nanomaterial, in addition to their efficacy asanti-growth of harmful microorganism, they are characterized by low toxicity to plants treated as well as their secondary benefits such as increasing soil fertility. Hence it is suggested that they can beapplied with other nanomaterials as substitutes tofungicides to control plant pathogens (Hameed, 2019) and this can participate significantly in conserving of the environment from disadvantages of application of the pesticides.

The integration among different control methods has proven to be highly efficient against many phytopathogens (Abdulmoohsin and Husain 2014). Although the nanoparticles and cultivars prove to be highly capable in controlling many plant pathogens, investigation of their integration is not fully studied. For example, Lahuf *et al.* (2019) discovered the possibility of integrating between ZnO nanoparticles with some varieties of sunflower crop against *Rhizoctonia solani*, causing seed rot and damping-off disease. They found two positive effects resulting of

Table 1. Effect of the integration between ZnO nanoparticles and tomato cultivars in the percentage of the early blight severity

Treatments (g/L)Cultivars	Co. 0	Co.1	Co.3	Co.5	Beltanol
Siemens	33.30	32.40	18.54*	30.49	24.90
BoobCat	28.99	30.63	19.36	14.17*	23.15
Hla	29.51	20.28	13.20**	15.72**	30.49
L.S.D.$_{01}$	**11.57**				

NB: Co. abbreviation is referring to concentration of ZnO NPs. One star sign refers to significant differences between the ZnO NPs treatments and control treatment only (concentration 0) while the two stars referring to significant differences between the ZnO NPs treatments and both of the control and the fungicide treatments.

this integration. The first was the direct impact on the growth of the pathogen and the second was the indirect effect through the possibility of stimulating a systemic resistance. This agrees with outcome of Anusuya and Sathiyabama, (2015) study who found spraying of turmeric plant with ²γ-D-glucan nanoparticles can cause stimulation of systemic resistance against pathogen of the rhizome rot disease. The integration control technique has received a considerable awareness of plant protection scientists due to its high performance in plant disease management and it can contribute significantly in decreasing of pesticides application. This can lead to decline their negative influence on the environment. Further studies are required to investigate the effect of ZnO NPs and its interaction with tomato cultivars on pathogens infecting tomato crop.

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