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EVALUATION OF EFFECT OF ZnO NANO-PARTICLES AND THEIR INTEGRATION WITH THE BIOCONTROL AGENT *TRICODERMA HARZIANUM* FOR CONTROL OF DAMPING OFF DISEASE CAUSED BY *RHIZOCTONIA SOLANI* ON TOMATO PLANTS

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Abstract

The soil borne fungus *Rhizoctonia solani* is the causative agent of damping-off disease that causes severe economic losses in several crops including tomato world wide. Currently, the Nano particles have become an accepted biocide, aimed to combat plant pathogens. However, ZnO nano particles has not been examined alone or in combination with the biocontrol factor *Trichoderma harzianum* against *R. solani*. Hence, experiments were conducted to determine the influence of ZnO nano particles (synthesized and commercial) alone or along with *T. harzianum*in protection of tomato plants against damping-off disease. All of the tested of ZnO NPs exhibited influential antifungal activity against *R. solani*. The most effective compound was the commercial ZnO NPs with concentration of 15 µg/mL. This antifungal activity was also appeared against *T.harzianum*. Germination of tomato seeds was not affected by ZnO NPs. However, growth parameters of tomato seedlings were affected. The treatment of soaking tomato seeds in ZnO nano particles suspension (15 µg/mL) for three days, then sowing them in compost inoculated with *T. Harzianum* and *R. solani* inoculums in addition to spray the ZnO nano particles suspension on the emergent tomato seedlings was the only one among the treatments that significantly proved a protection to tomato seeds and seedlings from the pathogen *R. solani*. These results showed that ZnO NPs could be used in combination with the biological factor *T. harzianum* as an effective resistance approach in tomato for control of damping-off disease.

Key words : Tomato-damping-off, *R. solani*, ZnO NPs, *T. harzianum*.

Introduction

In Iraq, damping-off, caused by several fungal pathogens including *Rhizoctonia solani*, is a destructive disease of a wide range of plant hosts comprising vegetable crops. Tomato is the second most important vegetable crop that negatively affected by this disease that induces two type of symptoms. Pre-emergence of seedlings, including killing of seeds before their germination, where they become rot, decompose, and be unable to germinate or the seeds are killed after germination and forming seedlings. Soft decomposing patches appear on their hypocotyl, which later become brown or black and extend rapidly to include the entire hypocotyl and radicle of the seedlings before their emergence on soil surface (Horst 2013; Cram 2003; Landis 2013).On the other hand, the symptoms of post-emergence of seedlings involve wilt, decomposition and rotting of the seedlings after they appear above the soil surface. In addition, patches of brown rotten structure may appear on the stems of seedling at the level of the soil surface leading to become the injured area thin and rough resulting in the fall of seedling and death. If the infected seedlings are not dead, they will be often poorly developed and shriveled (Lamichhane *et al*., 2017).

To control this disease, application of the fungicides is considered the most effective current control strategy. However, as a result of excessive usage of these fungicides, the environmental risks to human, Animal and flora have become a major concern in recent years (Whipp & Lumsden 1991).Additionally, the uncontrolled utilization of these chemical agents can investigate resistance development for fungal plant pathogen against the fungicides (Saharan *et al.,* 2013). Therefore, alternative approaches to combat the causative agents of devastating plant diseases including the damping-off are being demanded (Gogos *et al.,* 2012; Khot *et al*., 2012).

One of these approaches is the presence of microbiological agent that antagonize the growth of phyto fungal pathogens. *Trichoderma harzianum* is an example of these agents that directly inhibit growth of numerous fungal pathogens, including *R. solani* due to

producing different types of antibiotics and analyzed enzymes. It has also a high competitive ability with plant pathogens and is able to parasite some of them as well as its capacity for induction of systemic resistance responses in plant against pathogens (Benítez *et al*., 2004; Harman 2006).

Another approach that may influence the health of plants is the presence of Nano-particles (NPs), which atleast one of their dimension is between 1-100 nm in size. This type of particles gain an enormous attention as new agricultural chemicals. This is related to their utilization in plant nutrition and protection because of their small size and large surface area, which lead to stimulate of unique reactive groups, and novel chemical properties that enhance the nanoparticles to be more efficient against the harmful microbes (Bouwmeester *et al.,* 2009;Ghormade *et al.,* 2011; Khot *et al.,* 2012).

Among of these nanoparticles, metal-based nanoparticles are widely applied in crop protection (Wani & Ahmad, 2013). ZnO NPs is one of these metal nanoparticles that mainly used as fertilizer and showed also a wide spectrum of antimicrobial activity (Dizaj *et al*., 2014). This activity results in numerous commercial purposes (Aydin & Hanley 2010; Vandebriel & De-Jong 2012).However, few studies have been conducted related to investigation effect of ZnO NPs against phyto fungal pathogens. For incidence,a significant reduction in growth of *Penicillium expansum* and *Botrytis cinerea* fungi that cause the post-harvest fruit molds were found as a results of use ZnO NPs (He *et al.*, 2011). Likewise, other two post-harvest fruit molds pathogen, *Aspergillus niger* and *A. flavus* were inhibited by this NP (Jayaseelan *et al.,* 2012).As well as of these features, Gajjar *et al*. (2009) and Dimkpa *et al*. (2013) mentioned that the ZnO NPs are less toxic to plants and beneficial soil bacteria compared to other metal-NPs.

However, there has not been found an investigation related to use of ZnO NPs against *R. solani* or use them in combination with the biological agent *T. harzianum* to control the damping-off disease of tomato plant. Thus, in this study we investigate the potential role of synthesized and commercial ZnO NPs in controlling the fungal pathogen *R. solani*. Subsequently, we also assessed the interactive influences between the ZnO NPs and *T. harzianum* on antagonism of the *R. solani* growth.

Materials and method

Isolation and identification of *R. solani*

Samples of tomato seedlings, showed dark brown rot on basal stems of seedlings were collected from tomato plastic houses of Agriculture college, University of Kerbala, Iraq. The diseased samples were washed with tap water, cut into 0.5-1 cm long segments that were surface sterilized via sodium hypochlorite (2%), and washed with distilled water. They were then placed on water agar media (WA) and incubated at 25±2 for three days in darkness. After colony emergence, they were purified using tip mycillia technique on petri dishes containing the growth medium potato dextrose agar (PDA) and incubated at 25±2 for 5-7 days. The fungus associated with symptomatic tomato seedlings was identified as *Rhizoctonia solani* based on its cultural and morphological characteristics (Watanabe 2010; Parmeter & Whiteny 1970; Moni *et al*., 2016).

Evaluation of activity of the biocontrol agent *T. harzianum* against the pathogen *R. solani*

The dual culture method, described by Baker & Cook (1974), was applied to assess the antagonism activity of the biological factor *T. harzianum* against the fungus *R. solani*. The Petri dishes were divided equally using marker. The first section was inoculated with a 5 mm in diameter disk collected from the edge colony of 7 days old *T. harzianum* culture while the second section was inoculated with same size disk obtained from a 7 days old culture of *R. solani*. Furthermore, control Petri dishes contained one of those fungi were prepared. All treatments were repeated three times and all Petri dishes were then incubated at 25±2°C. The antagonism activity was recorded after the growth of fungi reached to the edge of control Petri dishes by following the key of Bell *et al.* (1982) that consists of 5 degree:

1. The biological agent growth covers the whole area of the Petri dish, including the growth of the pathogenic agent.
2. The biological agent growth covers 2/3 area of the Petri dish, while the growth of the pathogenic agent covers the rest 1/3 area.
3. The biological agent growth covers 1/2 area of the Petri dish, while the growth of the pathogenic agent covers the rest 1/2 area.
4. The pathogenic agent growth covers 2/3 area of the Petri dish, while the growth of the biological agent covers the rest 1/3 area.
5. The pathogenic agent growth covers the whole area of the Petri dish, including the growth of the biological agent.

Note that the biological agent is considered effective if the rate of antagonism 1 or 2.

Synthesis of Zinc oxide (ZnO) nano particles

The ZnO nano particles used in this study were produced following the instructions described

previously by Chen *et al.* (2009). In a typical synthesis, an aqueous solution of $Zn(CH_3COO)_2$ was prepared with concentration 30/L of distilled water. A 10 g of starch and 20 ml of $C_2H_4(OH)_2$ were added to the solution that was heated under controlled magnetic bar stirring and the pH was adjusted to 8 with NaOH (2N). The solution was then incubated at room temperature for 24 h. Next, the sediment was collected by centrifugation at 4000 rpm for 7 min and washed several time with distilled water then dried at 50°C. Subsequently. It was burned at 400°C and stored at room temperature until use. The synthesized ZnO nano particles were characterized via Fourier transform infrared spectroscopy (FTIR), Atomic force microscopy (AFM) and X-Ray Diffractometer. The average size of nanoparticles was ~ 70 ± 10nm.

Assessment the antifungal activityof the synthesized and commercial ZnO nano particles against the pathogen *R. solani*

The methodology conducted by Alwan *et al.* (2011) and Altaee (2017) was followed to evaluate the antifungal activity of the ZnO nano particles against the causative agent *R. solani.* Two different ZnO nano particles, the synthesized and the commercial purchased from MK Nano company, were added to PDA media before sterilization in various concentration (15, 10, 5, 2.5 and 1 µg ZnO /mL PDA). The Petri dishes of control contained PDA medium only. The *R. solani* inoculum (5mm in diameter disks collected from 7 days old pure colonies) was placed in the center of petri dishes that were incubated at 25 ± 2 °C in darkness. Each treatment was replicated four times. The test wasended when the mycelium growth of *R. solani* touched the edges of the control Petri dishes. The inhibition percentage was calculated by measuring the colonies diameter (cm) in treated and not treated Petri dishes using the following equation:

$$\text{Inhibition}\% = \frac{\mathbf{C} - \mathbf{T}}{\mathbf{C}} \times 100$$

Where T is the average fungal colony diameter in the treated Petri dishes and **C** is the average fungal colony diameter in the control Petri dishes.

Evaluation of effect of ZnO nano particles on the fresh and dry weight of the pathogen *R. solani*

Impact of different concentrations (15, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1 µg /mL) of the commercial ZnO nano particles were evaluated on the fresh and dry weight of the fungus *R. solani.* The treatments were prepared in potato dextrose broth (PDB) media in 250 ml flasks. The fungal inoculum (5mm in diameter disks) was obtained from 7 days old fungal cultures. Two disks of the fungal inoculum was put in each 250 mL flask containing 100 mL of PDB media and different concentrations of the ZnO nano particles. The control flasks contained PDB medium only. These flasks were incubated at 25±2 °C in darkness for 7 days. Each treatment was replicated three times. The fresh weight of the fungal growth in all treatments was recorded then the dry weight was measured after three days of dehydration at 50°C in an oven. The inhibition percentage of the fresh and dry weight of *R. solani* was estimated using the same formula above.

Determination impact of the effective concentrations of ZnO nano particles on the biocontrol agent *T. harzianum*

The most effective concentrations (15, 10, 5 and 2.5 µg /mL)of the ZnO nano particles against *R. solani* were tested against *T. harzianum* using the same methodology described by Alwan *et al.* (2011) and Altaee (2017) that was followed above. This was to value possibility applying a direct combination of the biological factor and the ZnO nano particles to combat the pathogen *R. solani*.

Determination impact of the effective concentrations of ZnO nano particles on seeds and seedlings of Tomato plant

Toxicity of the best effect ZnO nano particles concentrations (15, 10, 5 and 2.5 µg ZnO/mL distilled water) on the pathogen *R. solani* were assessed on seeds germination and seedlings growth of tomato plants. Tomato seeds were surface sterilized using sodium hypochlorite (2%) and washed three times successively with distilled water then placed in Petri dishes (10 seeds per dish) containing sterilized Whatman® filter paper. The tomato seeds were subsequently watered with the prepared concentrations of ZnO nano particles every two days. However, tomato seeds in control dishes were watered with distilled water only. Every treatment was repeated 4 times. Later all of these dishes were incubated in growth chamber at 21 °C temperature and 16 h light/8 h dark. After two weeks, the germination percentage of tomato seeds was calculated based on the following formula:

$$\text{Germination}\% = \frac{\textbf{Number of seeds germinated}}{\textbf{Total number of seeds}} \times 100$$

The length of hypocotyl and radicle of tomato seedlings were measured and the dry weight of these seedlings was also recorded after three days of dehydration at 50 °C.

Evaluation of activity of ZnO nano particles, *T. harzianum* and the combination between them in control of tomato damping-off disease caused by *R. solani*

This experiment was accomplished in the horticultural canopy of Agriculture College, University of Kerbala in 30/4/2018. The compost was autoclaved two times under conditions 121 °C at 15 psi for 1 hour. It was then distributed equally in disinfected plastic pots (8 cm in diameter). The inoculum of the pathogenic fungus *R. solani* was prepared using procedure of Buttner *et al.* (2004) that comprises mixing contents of 5 pure *R. solani* culture Petri dishes with 1000 mL of distilled water using electrical blender for two minutes. A 15 mL of inoculum was added to each pot. On the other hands, the inoculum of the biocontrol factor *T. harzianum* was prepared by following the protocol of Purwati *et al.* (2008). The spores were collected of pure *T. harzianum* cultures (10 days old) using distilled water. They were then counted using a hemocytometer, diluted with distilled water, and poured into autoclaved compost with concentration $1x10^7$ (spore of *T. harzianum* /g of compost). The inoculated compost was then incubated at 25±2 °C for three days before be utilized in this experiment. The tomato seeds were sterilized using 2% solution of sodium hypochlorite before conducting the following treatments:

1. Soaking the sterilized tomato seeds in ZnO nano particles suspension (15 µg/mL) for three days then sowing them in the inoculated *T. harzianum* compost and adding the *R. solani* inoculum.
2. Soaking the sterilized tomato seeds in ZnO nano particles suspension (15 µg/mL) for three days, sowing them in the inoculated *T. harzianum* compost, adding the *R. solani* inoculum and spraying the ZnO nano particles suspension on the emergent tomato seedlings.
3. Sowing directly the sterilized tomato seeds in the inoculated *T. harzianum* compost, adding the *R. solani* inoculum and spraying the ZnO nano particles suspension on the emergent tomato seedlings.
4. Soaking the sterilized tomato seeds in the fungicide Beltanol-L solution (1mL/L) and sowing them in the inoculated *R. solani* compost.
5. Sowing the sterilized tomato seeds in the inoculated *R. solani* compost as a negative control treatment.
6. Sowing the sterilized tomato seeds in non-inoculated compost as a positive control treatment.

After two weeks of seeds sowing, the germination percentage of tomato seeds in treatments conducted was calculated following the same formula applied previously.

After four weeks of seeds sowing, the incidence percentage of damping-off disease was recorded by following the equation:

$$\textbf{Diseases incidence\%} = \frac{\textbf{Number of inf ected seedlings}}{\textbf{Total number of seedlings}} \times 100$$

Results and Discussion

Evaluation activity of the biocontrol agent *T. harzianum* against the pathogen *R. solani*

The dual culture approach showed that the biological agent *T. harzianum* has a high antagonism ability (Figure 1), less than two degree by scale of Bell *et al.*(1982), against the phyto pathogenic fungus, *R. solani*. This is due to several antagonistic mechanisms such as producing a number of antibiotics including Trichodermin, Peptaibols, Alkylpyrones, Alamethicine and Trichorzianines that inhibit of kill the phyto pathogens (Dias 2012). Furthermore, it secretes some enzymes such as β-glucanases, chitinases, cellulases and proteases that disintegrate the cell walls of the pathogens. Worth to mention that the mycelia of the biological factor *T. harzianum* covered the pathogen mycelia. This indicates to occurring an interference status between both mycelia as a result of the biological agent's parasitism on the pathogenic fungus, where itsmycelia is a smaller diameter that wrap spirally around the mycelia of the pathogens (Harman 2006; Hermosa *et al.,* 2012).

Figure 1: Antagonism ability of *T. harzianum* (left side) against *R. solani* (right side)

Assessment of antifungal activity of the synthesized and commercial ZnO nano particles against the pathogen *R. solani*

The growth of the pathogen *R. solani* was for 7 days under investigation with different commercial and synthesized concentrations of ZnO NPs. This investigation demonstrated that the inhibition was a dose-dependent significantly ($p > 0.05$) at all levels comparing to the control. However, the commercial Zn NPs were significantly more inhibitory to pathogen growth than the synthesized at each of the examined concentrations (Table 1 and Figure 2).

Table 1: Inhibition percentage of *R. solani* growth influenced by different commercial and synthesized concentrations of ZnO NPs

Type/Conc.(µg/ml)	15	10	5	2.5	1	0
Commercial	100	100	100	100	62.85	0
Synthesized	76.15	75.71	73.5	72.05	70.58	0

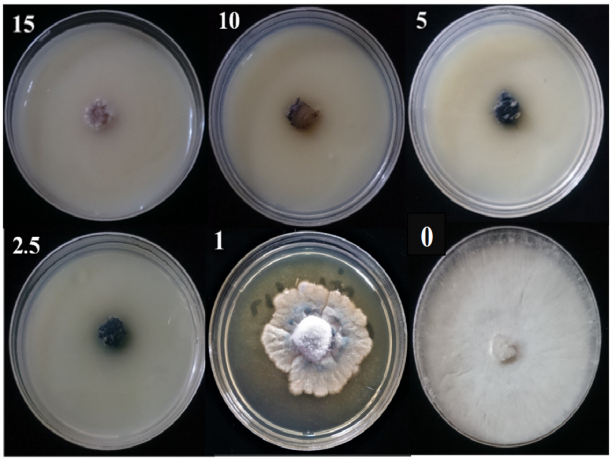

Figure 2: Effect of the commercial concentrations of ZnO NPs on *R. solani* growth

Evaluation of effect of ZnO nano particles on the fresh and dry weight of the pathogen *R. solani*

The results of this evaluation (Table 2) showed that ZnO NPs have a significant effect ($p > 0.05$) on the fresh and dry weight of *R. solani*. The two types of weights of the pathogenic fungus were 0 g at concentrations (15, 10, 5, 2.5, 1 and 0.5) µg/ mL. Also, the lowest concentrations 0.25and 0.1 µg / mL showed an inhibitory effect, but less at a mean fresh weight 1.66 and 1.62 g respectively, whereas it was in the comparison treatment (pathogenic fungi only) 2.33 g. On the other hand, the dry weight of the two concentrations above were 0.58 and 0.64 g respectively while in the control treatment was 0.69 g. It should be mentioned that the minimal inhibitory concentrations (MIC) of the examined ZnO NPs that were defined as the lowest concentration completely inhibits fungal growth was 0.5 µg/ mL in the liquid medium PDB while it was 2.5µg/ mL in the sold medium PDA.

Table 2: The fresh and dry weight(g) of *R. solani* growth affected by different commercial concentrations of ZnO NPs

Type/Conc. (µg/ml)	15	10	5	2.5	1	0.5	0.25	0.1	0
Fresh	0	0	0	0	0	0	1.66	1.62	2.33
dry	0	0	0	0	0	0	0.58	0.64	0.69

Determination impact of the effective concentrations of ZnO nano particles on the biocontrol agent *T. harzianum*

To probe the effect of the best ZnO NPs concentrations in this study (15, 10, 5, 2.5 and1µg/mL) on growth of the biological factor *T. harzianum*, it was challenged with these NPs concentrations in PDA medium. The results (Figure 3) indicates that fungal growth was impaired significantly ($p > 0.05$) at all concentrations. At higher doses (15, 10, 5, 2.5 µg/mL), aerial mycelium of *T. harzianum* growth was eliminated completely and its hyphal spread was significantly reduced, 100 % less growth comparing to the control (Figure4). In contrast, the fungal growth, challenged with a lower level (1µg/mL) of NPs, was less inhibited (61.11%). Furthermore, the nano-particle ZnO treatments at all concentrations raised the production of a greenish yellow pigment by the fungus. However, the role of this pigment in the metabolism of the biocontrol fungus is not understood at this point.

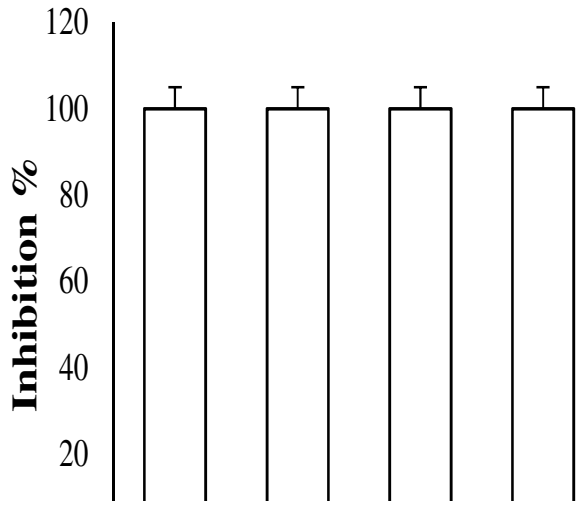

Figure 3: Concentration-dependent influence of exposure of *T. harzianum* to ZnO NPs (µg Zn/mL) for 7 day in PDA. Values are averages and standard deviations (n = 3)

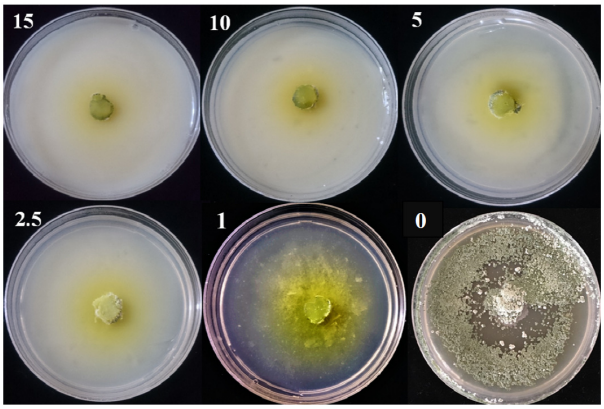

Figure 4: Effect of the commercial concentrations of ZnO NPs on *T. harzianum* growth

The effect of ZnO NPs on the pathogen *R. solani* and the biocontrol agent *T. harzianum* is due to the mechanism of forming free radicals on the surfaces of nano particles, as well as the formation of toxic compounds such as hydroxyl and superoxide, which cause damage to the lipids in the cells membrane of bacterial and fungal micro-organisms. This lead to leakage and collapse of cell membranes and inhibition of their function resulting in the transmission of high energy from the inside of the cells to the outside ultimately with cells death (Patra & Goswami 2012). The NPs also degrades fungal filaments and prevents the formation of conidia and conidiophore, which ultimately leads to the death of fungal filaments (He *et al.,* 2011).

Worth to mention that the effect of ZnO nano particles was Inhibitor (Fungistatic) and not lethal (Fungicidal) to the growth of *R. solani* and *T. harzianum* fungi, as they were able to grow from a PDA-mediated with the ZnO NPs to a PDA free of this NPs. This result is in agreement with previous study related to use ZnO NPs against the phytopathogen *Fusarium graminearum* that showed similar manner of action (Dimkpa *et al.* 2013).

Determination impact of the effective concentrations of ZnO nano particles on seeds and seedlings of Tomato plant

The seeds of tomato plant challenged with four effective concentrations (15, 10, 5 and 2.5) of ZnO NPs germinated successfully(Table 3) without a significant difference with those seeds in control, indicating that the NPs are not affected negatively on seeds germination process and seeds can be treated with NPs for use in disease management.

Although the averages of hypocotyl long and dry weight were decreased in most of treatments (Table 3), the significant differences between treatments and control were not found. Furthermore, the averages of the seedlings dry weight in 15-concentration treatment (0.028 g) was the closest to control. However, the hypocotyl long (2.375 cm) was higher than in control (2.232 cm). In contrast, the average of the seedling radicles long was reduced significantly ($p>0.05$) in all treatments except in the 15-concentration treatment (0.712 cm) that was the closest to control (0.897 cm) and without significant differences. These outcomes indicate to fact that the 15-concentration was the best which means it is in agreement with results of previous experiment that showed effectiveness of this concentration against the pathogen *R. solani*. Therefore, this concentration was adopted in the final experiment.

Table 3: The parameters of tomato seed and seedlings affected by different commercial concentrations of ZnO NPs

Parameters/Conc. (µg/ml)	**15**	**10**	**5**	**2.5**	**0**
Seed germination (%)	100	100	100	100	100
Radicles long (cm)	0.712	0.600	0.447	0.337	0.897
Hypocotyl long (cm)	2.375	2.045	1.797	1.870	2.232
Dry weight (g)	0.028	0.028	0.026	0.029	0.089

These results are consistent with the results of many previous studies. For example, the effect of zinc oxide nano particles were evaluated on seed germination rate and root length of cucumber and maize. It was observed that this nano particle in concentration (1 g / L) caused a significant reduction in root lengths of cucumber by 51% and maize by 17% but did not affect seeds germination rate of both hosts (Zhang *et al.* 2015). In another study about evaluation of the effect different concentrations of the same nanoparticles on some growth parameters of the sweet leaf plant *Stevia rebaudiana* showed a positive effect on improving these growth characteristics of the plant compared to plants not treated with this nano particle (Javed *et al.* 2017).

Evaluation of activity of ZnO nano particles, *T. harzianum* and the combination between them in control of tomato damping-off disease caused by *R. solani*

The results (presented in table 6) showed that all the treatments achieved more protection to tomato seeds from the infection of the fungus *R. solani* by increasing the germination rate significantly ($p> 0.05$). The highest treatment was No. 4 reached 75% followed by treatment No. 3, which did not differ from the positive comparison treatment that was 70%. Moreover, the treatment No. 1 and 2 were 65% and 60% respectively; while in the negative comparison was 55%.On the other hand, the incidence percentage of tomato damping-off was reduced significantly in treatment No. 2, which was

83.33% while in the negative comparison treatment was 90%. However, the rest of the treatments were not efficient in protecting the tomato seedlings from the fungus *R. solani*. It is clear from these results that treatment No. 2 was the only one among the treatments that significantly increased protection of the tomato seeds and seedlings from the pathogen infection *R. solani*.

These results indicate to facts that application of ZnO nano particles alone, or the use of *T. harzianum* alone or their use together can provide more protection to the seeds and seedlings of the tomato from the infection by *R. solani*. These facts are agreed with results of numerous previous studies that showed the ability of ZnO NPs in control of many pathogenic fungi such as *Penicillium expansum, Fusarium graminearum, F.oxysporum, Botrytis cinerea, Alternaria alternate* and *Rhizopus stolonifer* (He *et al.,* 2011; Wani & Shah 2012).

The fungus, *T. harzianum*, is well-known as an effective biological agent due to its antagonism ability against many of plant pathogens. This is as a result of its high competitiveness and production of many antibiotics, as well as a number of enzymes that analyze cell walls of pathogens. It is also able to parasite some phyto pathogens (Benítez *et al.,* 2004; Harman 2006). Additionally, it stimulates plant defenses against pathogens and has positive effects on plant growth and development (Harman *et al.,* 2004).

The mode of integration between different control methods has proved highly efficient in combating many plant pests including pathogenic micro organisms (Agrios, 2005). Although nano particles and biocontrol agents demonstrate a high ability in controling plant pathogens but their integration studies are very few. Thus, the data of the present research have clearly shown that the integration between ZnO NPs and *T. harzianum* would offer a different strategy of plant disease control that can improved damping-off disease suppression and raised tomato yield and enhanced the reliability of biological control agents under diverse climatic environments.

References

Agrios G. (2005) Plant Pathology, 5th Edition. Academic Press, USA.

Altaee, A.K. (2017) Identification of the causative agent of kohlrabi wilt disease and possibility control it by bio and non-bio agents and evaluation of their effect on growth parameters. Master degree thesis, Agriculture College, Kufa University, Iraq.

Alwan, S.L., H.H. Khudair, K.K. Alzubaidy (2011) Test the effect of some herbicides (Chevaler, Tupac and Lintor) in the growth of bacteria *Bacillus circulans. Journal of Biological Sciences,* 3(2): 21-28.

Aydin, S.B. and L. Hanley (2010) Antibacterial activity of dental composites containing zinc oxide nanoparticles. *Journal Biomed Mater Research B*, 94: 22–31.

Baker, K.F. and R.J. Cook (1974) Biological Control of Plant Pathogens. American Phytopathological Society, Saint Paul, pp: 35-50.

Bell, D.K., H.D. Wells and C.R. Markham (1982) In Vitro Antagonism of *Trichoderma* species Against Six Fungal Plant Pathogens. *Phytopathology,*72: 379-382.

Benítez, T., A. M. Rincón, M. C. Limón and A. C. Codó (2004) Biocontrol mechanisms of *Trichoderma* strains. *International microbiology Journal*, 7: 249-260.

Bouwmeester, H., S. Dekkers, M.Y. Noordam, W.I. Hagens, A.S. Bulder, C.D.Heer, S.E.C.G. Voorde, S.W.P. Wijnhoven, H.J.P. Marvin and A.J.A.M. Sips (2009) Review of health safety aspects of nanotechnologies in food production. *Regul Toxicol Pharmacol,* 53: 52-62.

Buttner, G., B. Pfahler and B. Marlander (2004) Greenhouse and field techniques for testing sugar beet for resistance to Rhizoctonia root and crown rot. *Plant Breeding*, 123: 158-166.

Chen, B., H. Zhang, N. Du, D. Li, X. Ma andD. Yang (2009) Hybrid nanostructures of Au nanocrystals and ZnO nanorods: Layer-by-layer assembly and tunable blue-shift band gap emission. *Materials Research Bulletin,*44: 889–892.

Cram, M.M. (2003) Damping-Off. *Tree Plant Notes* 50: 1–5.

Dias, D.A., S. Urban and U.A. Roessner (2012) Historical overview of natural products in drug discovery. *Journal of Metabolites*2:303-336.

Dimkpa, C.O., J.E. McLean, N. Martineau, D.W. Britt, R. Haverkamp and A.J. Anderson (2013) Silver nanoparticles disrupt wheat (*Triticum aestivum* L.) growth in a sand matrix. *Environmental Science Technology*, 47: 1082–1090.

Dizaj, S.M., F. Lotfipour, M. Barzegar-Jalali, M.H. Zarrintan and K. Adibkia (2014) Antimicrobial activity of the metals and metal oxide nanoparticles. *Materials Science and Engineering C*, 44: 278–284.

Gajjar, P., B. Pettee, D.W. Britt, W. Huang, W.P. Johnson and A.J. Anderson (2009) Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440. *Journal of Biological Engineering*, 3: 9.

Ghormade, V., V.D. Mukund and M. P. Kishore (2011) Perspectives for nano-biotechnology enabled

protection and nutrition of plants. *Biotechnology advances*, 29(6): 792– 803.

Gogos, A., K. Knauer and T.D. Bucheli (2012) Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *Journal of Agricultural Food Chemistry,* 60: 9781–9792.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp., *Phytopathology*, 96 (2):190-194.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito (2004) *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2: 43–56.

He, L., Y. Liu, Z. Mustapha and M. Lin (2011) Antifungal activity ofzinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiology Research* 166: 207–215.

Hermosa, R., A. Viterbo, I. Chetand E. Monte (2012) Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology*, 158: 17–25.

Horst, R. K. (2013) Damping-off. Westcott's plant disease handbook. Springer Netherlands, Dordrecht, pp: 177.

Javed, R., M. Usman, B. Yücesan, M. Zia andE. Gürel (2017) Effect of zincoxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of Stevia rebaudiana Bertoni. *Plant Physiology Biochemistry*, 110: 94–99.

Jayaseelan, C., A. Abdul Rahuman, A.V. Kirthi, S. Marimuthu, T.Santhoshkumar, A. Bagavan, K. Gaurav, L. Karthik and K.V. Bhaskara-Rao (2012) Novel microbial route to synthesize ZnO nanoparticles using *Aeromonashydrophila* and their activity against pathogenic bacteria and fungi. *Spectrochim Acta A Mol Biomol Spectrosc*, 90: 78–84.

Khot, L.R., S. Sankaran, J.M. Maja, R. Ehsani and E.W. Schuster (2012) Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Protection,* 35: 64–70.

Lamichhane, J.R., C. Dürr, A. A. Schwanck, M. Robin, J. Sarthou, V. Cellier, A. Messéanand J. Aubertot (2017). Integrated management of damping-off diseases. *A review Agronomy for Sustainable Development*, 37(10): 1-25.

Landis, T. D. (2013) Forest nursery pests: dampng-off. *For Nurse Notes*, 2: 25–32.

Moni, Z.R., M. Ali, M.S. Alam, M.A. Rahman, M.R. Bhuiyan, M.S. Mian, K.M. Iftekharuddaula, M.A. Latif and M.A. Khan (2016) Morphological and Genetical Variability among Rhizoctonia solani isolates causing sheath blight disease of rice. *Rice* Science, 23(1): 42-50.

Parmeter, J.R. and H.S. Whitney (1970). Taxonomy and nomenclature of the imperfect state. In: J.R. Parmeter, Editor, *Rhizoctonia solani*: Biology and Pathology, University of California Press, Berkely, 7–19.

Patra, P. and A. Goswami (2012) Zinc nitrate derived nano ZnO: Fungicide for disease management of horticultural crops. *International Journal of Innovative Horticulture,* 1:79-84.

Purwati R. D., N. Hidayah, O. Sudjindro and O. Sudarsono (2008) Inoculation Methods and Conidial Densities of Fusariumoxy sporumf. sp. cubense in Abaca. *HAYATI Journal of Biosciences*, 15 (1):1-7.

Saharan, V., A. Mehrotra, R. Khatik, P. Rawal, S. S. Sharma and A. Pal (2013) Synthesis of chitosan basednanoparticles and their in vitro evaluation against phytopathogenic fungi. *International Journal of Biological Macromolecules*, 62: 677–683.

Vandebriel, R.J. and W.H. De-Jong (2012) A review of mammalian toxicity of ZnO nanoparticles. *Nanotechnology, Science and Applications,* 5: 61–71.

Wani, A. H. and M.A. Shah (2012) A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. *Journal of Applied Pharmaceutical Science*, 2(3): 40–44.

Wani, I.A. and T. Ahmad (2013) Size and shape dependent antifungal activity of gold nanoparticles: a case study of Candida. *Colloids Surf B Biointerfaces*, 101: 162–170.

Watanabe, T. (2010) Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, 3rd Edition. CRC Press LLC, Florida,

Whipps, J.M. and R.D. Lumsden (1991) Biological control of *Pythium* species. *Biocontrol Science and Technology*, 1: 75-90.

Zhang, R., H. Zhang, C. Tu, X. Hu, L. Li, Y. Luo and P. Christie (2015) Phytotoxicity of ZnO nanoparticles and the released Zn (II) ion to corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) during germination. *Environmental Science and Pollution Research*, 22: 11109.