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Silver nitrate and zirconium oxide nanoparticles as management of wheat damping-off caused by *Fusarium graminearum*

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Abstract

The fungi were identified as: *Fusarium graminearum, Fusarium moniliforme and Fusarium solani. F. graminearum* isolate caused the highest percentage of damping- off (100%) in two wheat varieties (Al-Rasheed and Tamuze-2) followed by *F. solani*. According to these result, *F. graminearum* were used in all next experiment. The higher inhibition rate of fungi (50%) and spores 55.102% were found at 200mg/ml concentration of Zirconium oxide nanoparticles. While 10mg/ml of silver nitrate nanoparticles caused reduction in fungal and spores with inhibition rate 52.083, 57.142mg/ml respectively. Damping-off caused by *F. graminearum* was reduced by different concentrations of Zirconium oxide nanoparticles in both varieties. The response (to this nanoparticles) of Al-Rasheed variety was more sensitive to resistance damping off compared with Tamuze-2 variety. There were reductions in damping-off caused by *F. graminearum* by different concentrations of silver nitrate nanoparticles in both varieties.

The growth of Al-Rasheed variety was more sensitive to Zirconium oxide nanoparticles compared with Tamuze-2 variety. The results were different using silver nitrate nanoparticles, the reductions of germination percentage, germination rate and germination value of Al-Rasheed variety were not important at all concentrations. The same feature were found in mean germination time, mean daily germination and promoter indicator, at 0.1-0.01mg/ml while all higher concentration were significance reduction in them. The reductions of Tamuze-2 variety were dose depending manner. These results provide evidence of the promise management of damping off using silver nitrate nanoparticles and zirconium oxide nanoparticles with less effect on plant growth.

Keyword: Damping-off, Management, Nanoparticles, Silver; Wheat, Zirconium.

Introduction

Wheat (*Triticum aestivum L.*) is an important staple food crop across the world (Aslam *et al*., 2014). In terms of total production tonnages used for food, it is currently second to rice as the main human food crop and ahead of maize, (Brouns *et al*., 2013). Wheat like any other crop, suffers from many diseases caused by fungi, bacteria, viruses, nematodes and other abiotic disorders. Fungal diseases are one of several biotic constraints or restrictions to winter wheat yields. Fusarium is a cosmopolitan genus of filamentous ascomycete fungi (Nectriaceae) which includes many species that has high economic importance of plant diseases and has a wide range of host plants cause major damage in cereals, fruits and vegetables، (O'Donnell *et al*., 2000). *Fusarium* diseases include wilts, blights, rots, and cankers of many horticultural, fields, ornamental, and forest crops in both agricultural and natural ecosystems, (Ilgen *et al*., 2008). It can also cause pre- and post-emergence damping-off upon seed germination. However, for reasons such as slow to shed the seed coat, for example, inoculate contacting and infecting the emergent tissues will often cause the new shoot to rot at the soil line. Rotting of young germinated shoots at the soil line and breaking or falling over at this point typifies symptoms of post-emergence damping-off (Peterson, 2008).

Several common methods have been used to control these pathogens, each of them have one or other limitations. It is difficult to control fungal growth because fungi has developed resistance to many conventional fungicides such as: benzimidazoles and dicarboximides (Elad *et al*., 1992). To control these resistance, it is important to explore new anti-fungal agents, which may replace current control strategies (Ocamb *et al*., 2007).

Nanotechnology has got important application

for enhancing agricultural productivity, along with other emerging technologies such as biotechnology including genetics, plant breeding, disease control, fertilizer technology, precision agriculture, and other allied fields (Sastry *et al*., 2010; Jha *et al*., 2011). Nanotechnology can be used for combating the plant diseases, (especially crop), either by controlled delivery of functional molecules or as diagnostic tool for disease detection, (Scott and Chen, 2003). Sometimes this include controlled release of encapsulated pesticide, fertilizer and other agrochemicals in protection against pests and pathogens (Ghormade *et al*., 2011).

Recently, the term nanopesticides is used to describe any pesticide formulation that intentionally includes entities in the nanometer size, (below 100 nm), is designated with a "nano" prefix (e.g., nanohybrid, nanocomposite), and/or is claimed to have novel properties associated with the small size. On this basis nanopesticides include a wide variety of products, (European Commssion Joint Research Center, 2010).

Today nano-silver provides protection against fungi and bacteria; therefore, it is used to produce safer foods, (Seabra and Durán 2015). Some substantial experiments have been conducted on the antibacterial properties of Ag NPs (Rai *et al*., 2009; Xiu *et al*., 2012), but less work has been done on the its nanopesticides properties, some recent studies reported its antimycotic activity on some fungi such as wood rotting fungi, *Fusarium* species and other phytopathogenic fungi (McClements and Decker, 2000). Some study concentrate on the effect of these nano-metals on plant growth and found they were very safety or even they enhanced the growth but other research found the opposite,

Yin *et al*., (2012) studied the effects of Ag NPs on germination of eleven wetland plants species (*Lolium multiflorum, Panicum virgatum, Carex lurida, C. scoparia, C. vulpinoidea, C. crinita, Eupatorium fistulosum, Phytolaca americana, Scirpus cyperinus, Lobelia cardinalis, and Juncus effusus*) and found that Ag NPs (40 mg L with (6nm) in size) enhanced the germination rate of one species (*E. fistulosum*). There were increasing in inhibition of seedling growth, seedlings failed to develop root hairs, had highly vacuolated and collapsed cortical cells, broken epidermis and root cap, while using similar concentration but larger paticles size (25nm) lead to decreasing in Ag NPs effect. Moreover, effects of silver nanoparticles on plant growth parameters of common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) were studied by (Salama, 2012). The results of her showed that small concentrations (20ppm) of silver nanoparticles had a stimulating effect in shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of the two tested crop plants. Krishnaraj *et al*., (2012)found that no severe toxic effects were observed in silver nanoparticles treated plants in the morphological studies under scanning electron microscopy (SEM). On contrast, phytotoxicity of Oryza sativa was studied, (Mazumdar and Ahmed, 2011), by directly exposing it to silver nanoparticles solutions. Transmission Electron Microscope (TEM) revealed that various particle sizes deposited inside the root cells. It was found that during penetrations of particles inside the cell of root, they damaged the cell wall as well as vacuoles to enter, (Mazumdar and Ahmed, 2011).

This study aims to diagnosis some species of fusarium sp. that may cause damping off diseases to wheat and attempt to control it by some metal nanoparticles and if it has any effect on germination of wheat.

Materials and Methods

Identification of fungi and pathogenicity test: Five pure unknown fungi isolates were get from Dr. Hadi M. Aboud, the chief scientific researcher in the center of agriculture research/ Ministry of Science and Technology. He isolated them from infected wheat, cucumber and roses in season 2013. Fungi were cultured on potato - dextrose agar (PDA). For identification of *Fusarium* species, single spore isolating was growing on PDA medium. The culture were incubate on 28 Cº for eight days. The identifying had done using the keys of: (Booth, 1971: Gerlach and Nirenberg, 1982; Nelson *et al.,* 1983; Burgess *et al.,* 1994; Nirenberg and O'Donnell, 1998; O'Donnell *et al*., 2000; Leslie and Summerell, 2006).

Dry seeds of *T. aestivum* were taken from Ministry of Science and Technology- Seed Technology Center. These were: Al Rasheed variety and Tamuze-2 variety. Germination percentage of them were 100% for both varieties. For pathogenicity test mycelium agar plug technique, (Kammoun- Gargouri *et al*., 2009) was used. An agar plug (0.8cm diameter) with mycelium was cut from the periphery of 7 day-old cultures grown on PDA. The agar plug was then placed on the center of petri dishes containing 5 sterilized seeds (Seeds immersed in a 1% sodium hypochlorite solution for 5min and rinsed three times with sterilized distilled water) of each variety of wheat.

An agar plug without fungus was used as a control treatment. Five replicate were get for each treatment. Each replicate contain ten seeds. The

seeds were germinated in 28 C^o for ten days. The following symptoms were observed:

- Seedlings fail to emerge (pre-emergence damping off).
- Seedlings collapse, submerged in a mass of whitish fungal growth.

The number of dead seeds or dead seedling were determined after seven days to calculate total percentage of damping- off as fallowing:

Damping- off%= [(S-s)/S]*100

Where is: S=average of germinated seed in control plates, s= average of germinated seed in plates treated with nanoparticles.

Preparations nanoparticles: Zirconium oxide nanoparticles was procured from Eprui nanoparticles & microspheres company-china. the supplier data were: color: white, shape: tetragonal, particle size: 20-40nanometer, assay 99.0%. Silver nitrate nanoparticles was procured from Alpha Acer (USA). The supplier data were: black color, spherical shape, particles size was 22-30nanometer. Sterilized distilled water was used to prepare different concentrations of: ZrO_2 200, 20, 2mg/ml and $AgNO_3$ 10, 1, 0.1, 0.01mg/ml.

Antifungal activity: The antifungal activity of all nanoparticles were evaluated against one species of *Fusarium* (isolate number 1). Different concentrations of nanoparticles were incorporated into PDA medium just before pouring in sterilized Petri dishes. Negative control of distill water were tested at all experiment. All experiment has run in three replicate. Petri dishes were inoculated in the center with 3 mm of fungal plugs. Incubated at 28 ± 2°C for 8-10 days. The radial growth of the colony was measured. Inhibition% of mycelial growth was calculated as fallowing:

Inhibition% = [(R1- R2)/R1] x100

Where: R1= the radius of normal growth in control plates; R2= the radius of inhibited growth.

The spore suspension was collected from above culture. The spore suspensions in sterile distilled water, (10 ml per petri dishes), were centrifuged. A hemocytometer was used to calculate % Spores inhibition rate:

Spores inhibition rate=[X-x] /X

Where: X is the average of spor number in control plate. x is the average op spores number in plates treated with nanoparticles.

The effect of nanoparticles on pathogenicity of Fusarium: This was don according to mycelium agar plug technique. Seeds were soaked in nanoparticles suspensions at various concentrations for 72hrs. Five seeds per petri dishes, were inoculated with different concentrations of nanoparticles. There was negative control and there were three replicate for each treatment. The symptoms of damping off that describe above were observed and the number of dead seeds or dead seedling were determined after seven days. Total percentage of damping- off was calculate.

The effect of nanoparticles on germination parameters of *T. aestivum*: Sterilized similar size seeds of two variety of wheat were soaked in nanoparticles suspensions at various concentrations for 72 hrs. There was negative control of distill water for all experiment. All experiment has run in three replicate. All seeds were incubating at laboratory conditions (27±1C^o, 12hrs. light: 12hrs. dark). The number of germinated seeds was recorded daily. A seed was considered germinated when the radicle showed at least 2mm in length.

1. Germination percentage (GP, %): GP = 100 × GN / SN

Where: GN = the total number of germinated seed. SN = the total number of seeds tested, (Feizi *et al.,* 2013).

2. Germination rate (GR): GR =Σ Gi /I.

Where: Gi = the number of seeds germinated on day I, (Al-Kaisi *et al.,* 2011).

3. Mean germination time (MGT): MGT=Σ Gi × i / Σ G. Where: i = the number of days since the day of sowing (day 0), and Gi = the number of seeds germinated on day i. Only seeds that germinated were included in the calculation, (Feizi *et al.,* 2013).

Results and Discussion

Pathogenicity test: In current study, the fungi were identified as: *F. graminearum, F. moniliforme and F. solani. F. graminearum* isolate caused the highest percentage of damping- off (100%) in Al-Rasheed and Tamuze-2 variety followed by *F. solani* isolate D (50%) and *F. solani* (isolate B) respectively, while the lower pathogenicity caused by *F. moniliforme* and *F. solani* (isolate C and E) in Al-Rasheed (20 %) and *F. moniliform* (*isolate C*), *F. solani* (isolate B, D and E) in Tamuze-2 variety (30%), (Table 1).

According to these result, isolate (A) of *F. graminearum* were used in all next experiment. Several report found that many species of Fusarium (including *F. graminearum, Fusarium moniliform* and *Fusarium solani*) caused damping off and root rot of wheat which reduces germination, seedling stand and yield, (Abo-Elnaga 2012; Kammoun–Gargouri *et al.,* 2009).

Table (1): Percentage of pathogenicity of fungi on two variety of *T. aestivum.*

Isolates no.	sources	Identified fungi	Al-Rasheed variety	Tamuze-2 variety
A	Wheat	*Fusarium graminearum*	100	100
B	wheat	*Fusarium solani*	40	40
C	cucumber	*Fusarium moniliforme*	20	30
D	cucumber	*Fusarium solani*	50	30
E	roses	*Fusarium solani*	20	30
F		CT-	0	0

CT-: untreated seeds.

Antifungal activity of nanoparticles:

1. Zirconium oxide nanoparticles: In current study, the higher inhibition rate 50% and spores' inhibition rate 55.102% were found at 200mg/ml concentration while the lowest of them 12.5 and 32.653% were found at 2 and 20mg/ml concentration respectively, (Table 2).

Table (2): Effect of Zirconium oxide nanoparticles on Inhibition rate and Spores inhibition rate of *F. graminearum.*

Con. (mg/ml)	% fungal Inhibition rate	% Spores inhibition rate
200	50	55.102
20	14.583	32.653
2	12.5	40.816
CT-	0	0

Con.; concentrations; CT-: untreated fungi

2. Silver nitrate nanoparticles: Table (3), showed the higher fungal and spore inhibition rate at 10mg/ml. They were 52.083 and 57.142mg/ml while the lowest fungal and spore inhibition rate were 12.5 and 34.693% respectively.

Table (3): Effect of Silver nitrate nanoparticles on Inhibition rate and Spores inhibition rate of *F. graminearum*

Con. (mg/ml)	% fungal Inhibition rate	% Spores inhibition rate
10	52.083	57.142
1	39.583	46.938
0.1	12.5	38.775
0.01	12.5	34.693
CT-	0	0

Con.; concentrations; CT-: untreated fungi.

In this study, the fungal Inhibition rate and spores inhibition rate was increase with the increasing in concentration of all industrial synthetic NPs, in most cases it, probably, happens due to the high density at which the solution was able to saturate and cohere to fungal hyphe and to deactivate plant pathogenic fungi. Upon treatment with Ag NPs, DNA loses its ability to replicate, resulting in inactivated expression of ribosomal subunit proteins, as well as certain other cellular proteins and enzymes essential to ATP production. It has also been hypothesized that Ag+ primarily affects the function of membrane-bound enzymes, such as those in the respiratory chain, (Kim *et al.,* 2012). Other study found that NPs led to changes in microbial community composition, biomass, extracellular enzyme activity, (Colman *et al.,* 2013), conidial germination, hyphal growth and sclerotial germination growth with different severe damage (Kim *et al.,* 2009; Lamsal *et al*. 2011; Min *et al*., 2009; Nel *et al.,* 2006). The result of the effect of silver nanoparticles on fungi was agree with Min *et al*., (2009) who suggested the possible use of silver nanoparticles as an alternative to chemical pesticides for the eradication of phytopathogens even though there were some parameters to be evaluated for practical use.

These may involve the evaluation of phytotoxicity and antimicrobial effects in hosts, and development of delivery systems of silver nanoparticles into host tissues colonized by phytopathogens.

The effect of nanoparticles on pathogenicity of *F. graminearum*:

3. Zirconium oxide nanoparticles: damping-off caused by *F. graminearum* were reduced by different concentrations of nanoparticles in both varieties but the highest one were found at 20 mg/ml and 200 mg/ml of Al-Rasheed and Tamuze-2 varieties respectively. The result found that the response to nanoparticles of Al-Rasheed variety was more sensitive to resistance damping off compared with Tamuze-2 variety, (Table 4).

Table (4): Effect of Zirconium oxide nanoparticles on percentage of pathogenicity of F. graminearum) of two variety of *T. aestivum*.

Con. (mg/ml)	Damping-off %	
	Al-Rasheed variety	Tamuze-2 variety
200	17.857	20.690
20	14.286	31.034
2	25.000	34.483
CT-1	100	100
CT-2	0	0

Con.; concentrations; CT-1: untreated seeds exposed to fungi; CT-2: untreated seeds without exposed to fungi.

4. Silver nitrate nanoparticles: there were reduction in damping-off caused by *F. graminearum* by different concentrations of nanoparticles in both varieties but the highest one were found at 10 and 0.01mg/ml and 1-10mg/ml in Al-Rasheed and Tamuze-2 varieties respectively, (Table 5).

Table (5): Effect of Silver nitrate nanoparticles on percentage of % Pathogenicity of *F. graminearum* of two variety of *T. aestivum.*

Con. (mg/ml)	Damping-off %	
	Al-Rasheed variety	Tamuze-2 variety
10	46.667	44.828
1	50	48.276
0.1	50	55.172
0.01	46.667	51.724
CT-1	100	100
CT-2	0	0

Con.; concentrations; CT-1: untreated seeds exposed to fungi; CT-2: untreated seeds without exposed to fungi.

Although, Zirconium oxide nanoparticles was more effective to resistance the pathogen compared with silver nanoparticles according to current results, both of them seem to be promising and effective antifungal agent against the pathogenic fungal strains.

A few studies were found about antifungal effect of Zirconium oxide nanoparticles but the antifungal effects of silver nanoparticles were studied by other researcher against *Humicola insolens* (MTCC 4520), *Fusarium dimerum* (MTCC 6583), *Mucor indicus* (MTCC 3318) and *Trichoderma reesei* (MTCC 3929), (Vivek *et al.,* 2011), *Candida albicans* (ATCC 5027), *Saccharomyces cerevisiae* (ATCC 5027), (Nasrollahi *et al.,* 2011) and these effect depends completely on the particle size, surface area, shape of particles and methods of synthesis of particles which are change the physiological and chemical properties of these particles led to morphological, structural and physiological changes in microbes, (Nel *et al.,* 2006) or even suppression of enzymes and toxins used by the fungal pathogens (Vahabi *et al.,* 2011)

Effect of nanoparticles on germination parameters of *T. aestivum*:

5. Zirconium oxide nanoparticles

Al-Rasheed variety: The changing of germination percentage and mean germination time were not significance at all concentrations. The significance reduction were found at all concentrations in: germination rate, germination value and promoter indicator. Mean daily germination reduced by 200mg/ml of nanoparticles, ($P<0.05$), all lower concentrations were not significant, (Table 6).

Tamuze-2 variety: The changing of germination percentage, germination rate and germination value were not significance at all concentrations. The significance reduction were found at: all concentrations in mean germination time, (200 and 2) mg/ml in mean daily germination and (2 mg/ml) in promoter indicator. All other treatment of them were not significant, (Table 7). The result found that Al-Rasheed variety was more sensitive to nanoparticles compared with Tamuze-2.

Table (6): Effect of Zirconium oxide nanoparticles on Germination percentage, Germination rate, Mean germination time, mean daily germination, Germination Value and Promoter Indicator of Al-Rasheed variety of *T. aestivum*.

Con. (mg/ml)	% GP	GR	MGT	MDG	GV	PI
200	73.333	0.205 B	4.667 A	733.333 B	266.667 B	1.750 B
20	76.667	0.509 B	3.267 A	766.667 A	600.000 B	2.500 B
2	86.667	0.717 B	4.133 A	866.666 A	866.667 B	2.666 B
CT-	100	1.340 A	4.200 A	1000 A	1833.333 A	3.750 A

Data shows means; Con.: concentration; CT-: untreated; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; GV: Germination Value; PI: Promoter Indicator; Similar letters are not significance at ($P < 0.05$) vertically.

Table (7): Effect of Zirconium oxide nanoparticles on Germination percentage, Germination rate, Mean germination time, mean daily germination, Germination Value and Promoter Indicator of Tamuze-2 variety of *T. aestivum.*

Con. (mg/ml)	% GP	GR	MGT	MDG	GV	PI
200	66.667	0.632A	2.533B	666.667B	833.333A	3.167A
20	83.333	1.009A	2.833B	833.333A	983.333A	3.750A
2	70.000	1.178A	2.033B	700.000B	866.667A	2.417B
CT-	100.000	0.935A	3.967A	1000.000A	1333.333A	4.333A

Data shows means; Con.: concentration; CT-: untreated; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; GV: Germination Value; PI: Promoter Indicator; Similar letters are not significance at ($P < 0.05$) vertically.

6. Silver nitrate nanoparticles

Al-Rasheed variety: The reductions of germination percentage, germination rate and germination value were not significance at all concentrations. The same feature were found in 0.1-0.01mg/ml of mean germination time, mean daily germination and promoter indicator, while all higher concentration were significance reduction in them, (Table 8).

Tamuze-2 variety: There were reduction in all treatment dose depending manner, (Table 9).

Table (8): Effect of Silver nitrate nanoparticles on Germination percentage, Germination rate, Mean germination time, mean daily germination, Germination Value and Promoter Indicator of Al-Rasheed variety of *T. aestivum.*

Con. (mg/ml)	% GP	GR	MGT	MDG	GV	PI
10	70.000	0.977A	2.567B	700.000B	866.667A	2.417B
1	80.000	0.978A	2.967B	800.000B	888.889A	2.333B
0.1	93.333	0.942A	3.767A	933.333A	1133.333A	3.167A
0.01	96.667	1.116A	4.067A	966.667A	1200.000A	3.000A
CT-	100.000	1.305A	3.800A	1000.000A	1333.333A	3.750A

Data shows means; Con.: concentration; CT-: untreated; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; GV: Germination Value; PI: Promoter Indicator; Similar letters are not significance at ($P < 0.05$) vertically.

Table (9): Effect of Silver nitrate nanoparticles on Germination percentage, Germination rate, Mean germination time, mean daily germination, Germination Value and Promoter Indicator of Tamuze-2 variety of *T. aestivum*.

Con. (mg/ml)	% GP	GR	MGT	MDG	GV	PI
10	66.667	1.236A	2.067B	666.667B	1083.333A	2.250B
1	73.333	0.959B	2.867A	733.333B	577.778B	3.417A
0.1	86.667	1.036B	3.500A	866.667A	1166.667A	2.750B
0.01	83.333	0.900B	3.500A	833.333A	883.333B	3.083B
CT-	96.667	1.653A	3.100A	966.667A	1933.333A	4.750A

Data shows means; Con.: concentration; CT-: untreated; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; GV: Germination Value; PI: Promoter Indicator; Similar letters are not significance at ($P < 0.05$) vertically.

These results provide evidence of the promise management of damping off using silver nitrate nanoparticles and zirconium oxide nanoparticles with less effect on plant growth. The best nano-pesticide was zirconium oxide nanoparticles. Several studies about the effect of these nano-metals on plants growth had done. They found that the negative effect (if it was there) was due to different physical and chemical properties and these depend completely on their size, shape of crystals, concentrations and species of plant, (Gruyer *et al*., 2013), or even varieties (in current

study), (Stoimenov *et al*., 2002) these nanoparticles may be able to easy penetrate inside the cell of root, they damaged the cell wall as well as vacuoles or even nucleus membrane and could have caused damage to DNA or it might have also been due to the inhibition of DNA synthesis at S-phase, (Mamta *et al*., 2009); (Hackenberg *et al*., 2011; Mazumdar and Ahmed, 2011)

Conclusions

F. graminearum has the highest percentage of damping- off in two above varieties. Silver nitrate nanoparticles and zirconium oxide nanoparticles can inhibit the fungal growth and sporulation and decrease pathogenicity of it but they have some negative effect on different parameters of plant growth. These was depend on concentrations and plant varieties.

More study is necessary to establish the influence of ZrO_2 nanoparticles in other phytopathogenes (fungi, bacteria. viruses, nematodes, pests). And because off most studies is concentrate on the toxic effect only on seed germination and root growth, the best understanding is available in the literature regarding the all stages of growth of wheat including biochemical changes that take place during the uptake and accumulation of these nanoparticles and if there any effect on chemical composition of plants.

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