Antifungal Effect of Nanomaterials and Cyanobacteria Against *Rhizoctonia solani* the Causative of Bean Root-Rot

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ABSTRACT---Bean is considered as an important legume crop in Egypt for local consumption and exportation. Many soil-borne pathogenic fungi can infect bean roots; from those, *Rhizoctonia solani* causes damping-off and root rot and affects the yield greatly.

In this study, three nanoparticle materials; zinc oxide (ZnO), magnetite (Fe_3O_4) and zinc ferrite ($ZnFe_2O_4$) were tested for their antifungal effect against the tested fungus. ZnO nanoparticles (ZnO NPs) showed obvious inhibition percentage and destruction to the cell walls and plasmolysis of the internal organs of the tested fungus. Fe_3O_4 and $ZnFe_2O_4$ NPs showed no inhibition percentage but they affected the morphological characters of the tested fungus and the germination percentage of the treated seeds.

Cyanobacteria (blue-green algae) were used as bioagents against the pathogenic fungus, we used the filtrates of the three blue green algal species, *i.e. Spirulina platensis*, *Nostoc muscorum*, *Anabaena flos-aquae* in managing *R. solani In vitro* and in the greenhouse. It was observed that the tested concentrations of the three Cynaobacterial filtrates significantly inhibited the growth of the tested fungus. The reduction in the linear growth was increased gradually by increasing the concentration of the filtrate. In addition, the tested cyanobacteria significantly reduced the infection by the tested fungus and improved crop parameters of bean plants.

Treatment of bean seeds with cyanobacteria filtrates and nanoparticle materials significantly increased the activity of peroxidase and polyphenoloxidase enzymes .

Keywords : Bean, *Rhizoctonia solani*, Cyanobacteria, Metal oxide and mixed oxide nanoparticles, Peroxidase, Polyphenoloxidase, Crop parameters.

1. INTRODUCTION

Bean crop (*Phaseolus vulgaris* L.*)* is sensitive to various soil borne fungi; causing damping-off of seedlings, root rots, stem rot, ashy stem blight and wilt. The main pathogens reported in Egypt are *R. solani, F. solani, M. phaseolina*, meanwhile, they are widespread, and the most important and the most aggressive, causing damping-off and root rots of several plant species [1] (El-Mougy *et al.*, 2007). These diseases affect adversely seed germination, seedling emergence and final plant density and cause great losses in the yield and its quality [2,3] (Abeysinghe, 2007 and Mukankusi *et al.*, 2010).

The host range of *R. solani* is wide-ranging. The pathogen is capable of causing seedling damping-off, root rot, collar rot and foliage blight on a variety of susceptible agriculturally important crops [4,5] (Baker, 1970 and Anderson, 1982)

[6] Abd-El- Khair *et al.* (2011) made known that Rhizoctonia root rot in bean, caused by *R. solani*, is one of the most economically important root diseases of beans. It has a broad host range that includes most annual and many perennial plant species. *R. solani* survives between crops as sclerotia and as mycelia in the soil. Young plants are more susceptible to infection than older plants.

Recently, resistance to commercially available fungicides by phytopathogenic fungi has been increased and has become a serious problem [7,8,9] (Dekker and Georgopoulos, 1982; Brent and Hollomon, 1995; and Goffeau, 2008). So, the search for new fungicides and alternatives is an important goal of the presented study to manage the newly emerging resistant strains of fungal pathogens [10] (Kanhed *et al.*, 2014). The solution would be utilization of cyanobacteria as a bioagent or nano-materials which show a promising antimicrobial activity. The improved antimicrobial activity of nanomaterials compared to their salts is due to their distinctive properties, *i.e.* large surface area to volume ratio.

[11]Burja, *et al.* (2001) reported that the wide spectrum of cyanobacterial secondary metabolites exhibits a wide range of bioactivities that may be more close to the natural environment, such as antibacterial, antifungal, antiviral.

[12] Sawai (2003) proved that nanoparticles of Fe_2O_3 and ZnO exhibited strong antimicrobial activity.

This study was carried out to shed light on the efficiency of three types of Nanomaterials ; zinc oxide (ZnO), magnetite (Fe_3O_4) and zinc ferrite ($ZnFe_2O_4$) and three species of cyanobacteria; *Spirulina platensis*, *Nostoc muscorum*, *Anabaena flos-aquae* on controlling *R. solani* the causative of bean root-rot.

2. MATERIALS AND METHODS

2.1. Source of *R. solani* cultures

The fungus understudy, *Rhizoctonia solani* Kuhn was isolated from bean rotted roots, purified and identified at the Department of Plant Pathology, Fac. of Agric., Cairo University by [13] Abada *et al* (2016) and [14] Attia (2016). The same authors studied also the pathogenic potentialities of the fungus.

2.2. Preparation of blue green algal biomass

Blue green algae (*Nostoc muscorum* and *Anabaena flos-aquae*) were grown on media described by [15] Watanabe (1951) at the Lab. of Phycol. , Dept. of Botany, Fac. Sci., Cairo Univ., Giza, Egypt , under continuous fluorescent white light whose intensity was kept at 200 Lux and temperature 28 ºC.

In regard to *Spirulina platensis* , it was grown on Zarrouk media [16] (Zarrouk, 1966) under temperature 30 °C and continuous fluorescent light . After 21 days, we started to separate the biomass from the cultural medium by centrifuging (40 min. , 800 g , 10 c) , the supernatant was sterilized using 0.25 µm syringe filter.

2.3. Nanomaterials

2.3.1. Chemicals

Solutions of reacting materials were prepared in deionized water provided by a Milli-Q water purification system. All Glassware were washed with aqua regia (HCl: HNO_3 = 3:1 (v/v)) and then rinsed with Milli-Q water. Zinc acetate dihydrate $Zn(CH_3COO)_2 2H_2O$, iron nitrate nonahydrate $Fe(NO_3)_3 \cdot 9H_2O$, sodium hydroxide NaOH and ethanol were supplied by WINLAB Company for Laboratory Chemicals (>99 %), India. Fe_3O_4 NPs were purchased from Sigma-Aldrich, St. Louis, Missouri, USA. The particles were characterized and identified by the company and their particle size is ≤ 50 nm.

2.3.2. Synthesis of nanomaterials

Zinc oxide nanoparticles (ZnO NPs) and zinc ferrite nanoparticles ($ZnFe_2O_4$ NPs) were synthesized in facile way and described as follow: Concentrated solution of NaOH (0.05 M) solution in a mixture of water :ethanol (1:1) is slowly added to 0.01 M solution of $Zn(CH_3COO)_2.2H_2O$ in a mixture of water :ethanol (1:1). The final solution was stirred and heated at 70 °C for two hours. For preparation of $ZnFe_2O_4$ NPs, 0.05 M of NaOH solution in a mixture of water : ethanol (1:1) is slowly added to 1:1 molar ratio solution of $Zn(CH_3COO)_2.2H_2O$ and $Fe(NO_3)_3 \cdot 9H_2O$ in a mixture of water : ethanol (1:1). The final solution was stirred and heated at 95 °C for three hours under condensation. Depending on the concentration of NaOH as well as the applied temperature, the stated method will allow the precipitation of NPs instead of precipitation of hydroxides. The producing ZnO and $ZnFe_2O_4$ NPs were collected and purified by centrifugation at 3000 rpm for 15 min. The process of centrifugation and re-dispersion in d.d. water was repeated many times. The washed particles were collected and dried in a hot air oven at temperature 60°C for 8 hrs. The synthesized NPs were characterized by transmission electron microscopy (TEM) and X-Ray diffractometery (XRD). In an attempt to eliminate any toxicity comes from organic chemicals, stabilizing and capping agents are avoided in the preparation of recommended NPs.

2.3.3. Characterization of nanomaterials

(a) Transmission electron microscope (TEM)

TEM images were performed using a JEOL 200 CX (Akishima, Tokyo, Japan) at 200 kV. A droplet of each sample (ZnO & $ZnFe_2O_4$ NPs) was placed on a copper grid and allowed to dry before being examined in the transmission electron microscope. The TEM images were analyzed using the Image-Pro Plus and Gatan Digital Micrograph program (Yubinbango103-0027 Nihonbashi, Chuo-ku, Tokyo, Japan).

(b) Powder XRD analysis

Powder X-ray diffraction (XRD) patterns were recorded for prepared samples (ZnO & $ZnFe_2O_4$ NPs) with a PANalytical: X'Pert PRO diffractometer using Cu Kα radiation source for investigation of the crystalline structure and phase transition.

2.4. Inhibitory effect of cyanobacteria filtrates on *R. solani*

This study was performed using Petri dishes containing PDA medium and stock with different concentrations from each of the cyanobacteria filtrates, plates containing PDA without cultural filtrates were used as check. The plates were incubated at 30 ^{0}C, the linear fungal growth was measured when the fungal growth completely covered any Petri dish of any treatment by measuring the mean of growth diameters [17] [17] [17] (Cobb *et al.*, 1968).

2.4.1.Effect on dry weight of the tested fungus

R. solani was grown in 100 ml conical flasks filled with 30 ml potato dextrose broth and each was inoculated with a disc taken from a 7-day-old PDA culture. Cultures were incubated at 30°C for 14 days. Fungal biomass dry weight was determined after filtration and drying at 70°C for 48 hrs.

2.5. The inhibitory effect of nanomaterials on the tested fungus

Gradual concentrations, *i.e.* (20, 100, 250 µg/ml) of ZnO, Fe_3O_4 and $ZnFe_2O_4$ NPs, were used in PDA media. The PDA media were sterilized in an autoclave for 20 min at 121 ^{o}C and air pressure 1.5 bars , poured in Petri-dishes and then discs (5 mm diameter) were taken from the margin of *R. solani* culture (7-day-old) and then transferred each in the centre of the Petri dish. Petri dishes with PDA only were used as check for *R. solani*. All the dishes were incubated at 30 ^{o}C. Three replicate plates were used for each concentration.

2.6. Microscopic examination of the treated fungi

This work was carried out in (TEM lab FA-CURP), Fac.Of Agric., Cairo University Research Park. Tissue samples were sliced into ~ 1 mm slices. Slice tissue was processed for TEM by fixation in glutaraldehyde and osmium tetroxide, dehydrated in alcohol and embedded in an epoxy resin. Microtome sections were prepared at approximately 500-1000 µm thickness with a Leica Ultracut UCT ultramicrotome. Thin sections were stained with tolodin blue (1X) then sections were examined by camera Lica ICC50 HD. Then ultra thin sections were prepared at approximately 75-90 µm thickness and were stained with uranyl acetate and lead citrate, then examined by transmission electron microscope JEOL (JEM-1400 TEM) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632 x 1632 pixel formate as side mount configuration.This camera uses a 1394 fire wire boared for acquision [18] (Bozzola and Russel, 1999).

2.7. Greenhouse experiments

The soil and pots (30 cm-diameter) used in the greenhouse experiments were treated with formalin solution made up at the rate of 1 liter of concentrated solution (36-40% formaldehyde) to 20 liter of water. The soil was covered with plastic sheets for 7 days to retain the gas. The soil was not planted until the odour of formaldehyde had disappeared.

2.7.1. Inoculum preparation, growing medium and soil infestation technique

The tested fungus, *R. solani* was allowed to grow in 500 ml milk bottles, each containing 75 gm washed dried barley, 100 gm washed dried coarse sand and 65 ml potato decoction [19] (Attia, 1966). After sterilization, a disc (5-mm-diameter) was taken from the margin of 7-days-old culture of the tested fungus and was manipulated to the autoclaved medium in the bottle and incubated at 30^{0}c for 15 days. After the sufficient growth of the fungus was achieved, the inoculum of the fungus was mixed alone with the soil at the rate of 30 gm/kg soil one week before planting. In check experiments, equal amounts of the uninoculated substrate were added to the soil. Five seeds of bean cv. Nebraska were planted in each pot and each treatment consisted of 3 replicate pots, plants were irrigated as necessary. A randomized block design was followed, having fungi treatments and 3 replicates. Emergence counts were recorded 15 and 30 days after sowing and percentages of pre- and post-emergence damping-off were calculated. Meanwhile, the survived plants were also examined periodically and the number of dead plants due to infection by root rot was counted 60 days after sowing.

2.7.2. Effect of cyanobacteria cultural filtrates on the infection with the tested fungus and on some crop parameters

This experiment was conducted under greenhouse conditions at the Plant Pathol. Res. Inst., Agric. Res. Cent, Giza.

The tested fungus, was grown on barley and sand medium for 15 days at 30 °C. Pots (30 cm-diameters) were sterilized by dipping in 5%formaline. Soil also was sterilized with 5%formaline, Soil infestation was carried out by adding the inoculum at the rate of 3% of soil weight, irrigated twice within 7 days before sowing to enhance fungal growth. Seeds of bean cv. Nebraska were soaked for 10 min. in the tested cultural filtrate concentrations of the three cyanobacteria before planting, seeds were then sown in the pots at the rate of 5 seeds / pot. Three pots were used as replicates for each particular treatment. Percentages of pre- and post-emergence damping - off were recorded 15 and 30 days after sowing, respectively. Survived plants were counted 60 days after sowing. By the end of the experiment, (65- days after sowing) the plants were uprooted to study some plant parameters, *i.e.* plant height (cm); fresh and dry weight (g); No. of leaves/ plant and No. of pods/ plant.

2.7.3. Effect of nanomaterials on the infection with the tested fungus and some crop parameters

Seeds of bean cv. Nebraska were soaked for 10 min. in the tested nanomaterials suspension's concentration before planting, seeds were then sown in the pots, prepared as mentioned before, at the rate of 5 seeds / pot, three pots were used as replicates for each particular treatment. Percentages of pre- and post-emergence damping -off were recorded 15 and 30 days after sowing, respectively. Survived plants were counted 60 days after sowing. By the end of the experiment, (80- days after sowing) the plants were uprooted to study some plant parameters, *i.e.* plant height (cm); fresh and dry weight (g); No. of leaves/ plant and No. of pods/ plant.

2.8. Determination of oxidative-reductive enzymes activity

These analyses were carried out to shed light on the effect of cyanobacteria and nanomaterials on the activity of peroxidase and polyphenoloxidase in the tissues of bean plants grown from bean seeds treated with cultural filtrates of the aforementioned cyanobacteria at (75%) concentration and from bean seeds treated with the tested nanomaterials suspensions at two different concentrations (20 and 250 µg/ml) . Leaf samples representing the second true leaf of the desired treatment were collected to determine the activity of peroxidase and polyphenoloxidase according to the method described by [20] Lisker *et al.* (1983).

Procedure started by cutting off 1 gm from leaves of treated replicates and then crushed them well in 2 ml sodium phosphate buffer at pH 7.1. The homogenate was filtrated through Whatman No.1 filter paper. The suspension was centrifuged at 6000 rpm for 20 min and stored at -18 °C until use. One tenth extracted enzyme sample was added to 0.5 ml sodium phosphate buffer at pH 7.1, 0.1ml H_2O_2 1% and 0.3 ml pyrogallol. The mixture was completed to 3 ml using distilled water and color density was read in absorbance spectrophotometer Miltonroy Spectronic 601 at 425 nm every 30 second for 10 reads [21] (Kochba *et al.*, 1977). Peroxidase activity was calculated as mg/gm fwt.

Polyphenoloxidase activity: Enzyme samples were extracted as mentioned before in peroxidase activity extraction. One tenth extracted sample was added to 0.5 ml sodium phosphate buffer at pH 7.1 and 0.5 ml catechol 0.001 N. The mixture was completed to 3 ml using distilled water and color density was read in spectrophotometer Miltonroy Spectronic 601 at 495 nm every 30 second for 10 reads [20](Lisker *et al.*, 1983).

2.9. Disease assessment

For damping-off of seedlings, the numbers of un-emerged seedlings (pre-emergence damping-off) as well as the number of dead seedlings (post-emergence damping-off) at 15 days and 30 days after planting, respectively were recorded and the percentages of damping-off were calculated. Plants showing root rot symptoms were recorded at 60 days after planting. The number of collapsed or wilted plants was recorded and percentage of disease incidence was calculated. Root rot severity was calculated according to [22] Liu *et al.*, (1995). Also, the survived plants were counted after 60, 80 days from planting for seeds treated with cyanobacteria and nanomaterials, respectively, uprooted and used for determining the values of some plant parameters, *i.e.* plant height, fresh and dry weight, number of leaves / plant, number of pods / plant.

2.10. Statistical analysis

Data obtained were statistically analyzed using the complete design block or split design block suggested by [23] Snedecor and Cochran (1989). Averages were compared at 0.05 level of probability using the least significant difference (L.S.D.) as mentioned by [24] Fisher (1948).

3. RESULTS

3.1. Characterization of the as-synthesized nanomaterials

Characterization of ZnO and $ZnFe_2O_4$ NPs was done using two methods; TEM and XRD analysis. Fe_3O_4 NPs was purchased from Sigma Chemical Company USA. They were characterized and identified by the company and their particle size is ≤ 50 nm.

(a) Characterization of ZnO NPs

The morphology of the prepared ZnO NPs was investigated by TEM measurement (**Fig. 1**). The micrograph revealed that the produced particles have spherical shape displaying a relatively uniform morphology with particles scale was less than 50 nm.

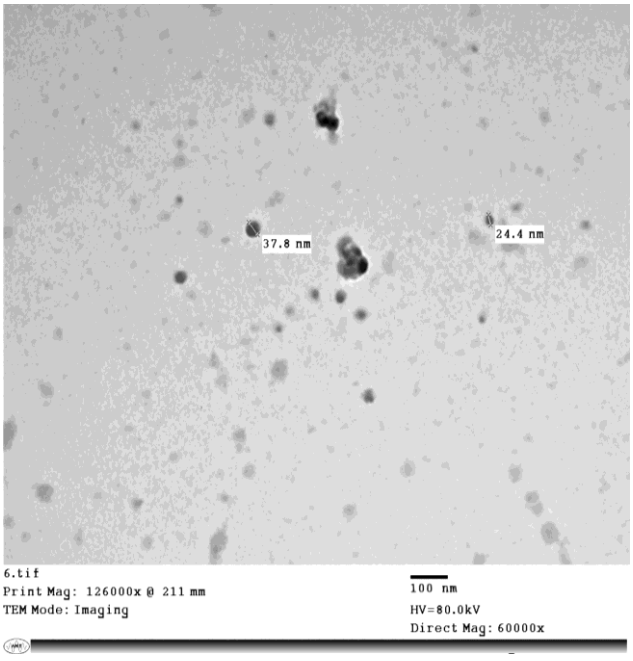

Fig. 1. TEM image of ZnO nanoparticles prepared using Zn acetate and NaOH, spherical in shape with approximately size 40 nm (Scale bar = 100 nm).

The XRD pattern of ZnO nanoparticles (Fig, 2) shows sharper peaks observed at 2θ of 31.3670, 34.0270, 35.8596, 47.1635, 56.2572, 62.5384, 67.6356, and 68.7978 correspond to the reflection from: 100, 002, 101,102, 110, 103, 200, and 112 crystal planes, respectively [25] (Gu *et al*, 2004) The sharper diffraction peaks indicate the nanocrystalline nature (JCPDS card no.0-3-0888) and is identical to the hexagonal phase with Wurtzite structure with space group (C6V=P63mc) and unit cell parameters a = b = 3.248 Å and c = 5.2 Å.

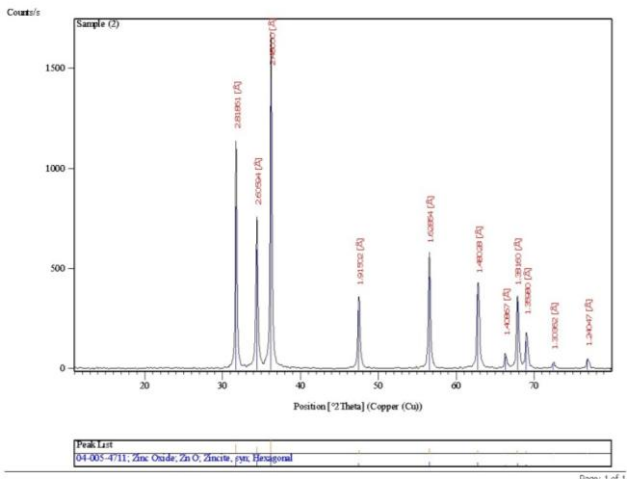

Fig. 2. XRD pattern of ZnO nanoparticles produced using Zn acetate and NaOH.

(b) Characterization of $ZnFe_2O_4$ NPs

The zinc ferrite nanoparticles were prepared by co-precipitation of Zn and Fe precursors in a highly basic medium. The size and morphology of the as-prepared $ZnFe_2O_4$ NPs analyzed by TEM are represented in Fig.(3). This image reveals that the product consists of aggregated spherical particles with the average size of 80-120 nm.

The XRD pattern of the as-synthesized $ZnFe_2O_4$ NPs is shown in Fig.(4). The pattern shows that the as-prepared material consisted entirely of nano-crystalline $ZnFe_2O_4$ NPs and coincides with the standard data of the cubic Zn ferrite (Franklinite) phase with the Fd3m space group (JCPDS card No. 74-2397) [26] (Girshick and Chiu, 1990) . The pattern reveals peaks of low intensities and observed at 2θ values corresponding to the crystal plane of cubic $ZnFe_2O_4$ (220), (311), (222), (400), (422), (511), (440), (620), (533) and (622).

The low intensities of the reflection peaks of the XRD pattern indicate the small size of the crystallite particles. This clearly explains that the large particles of ferrite shown in TEM image are aggregations of particles of small sizes.

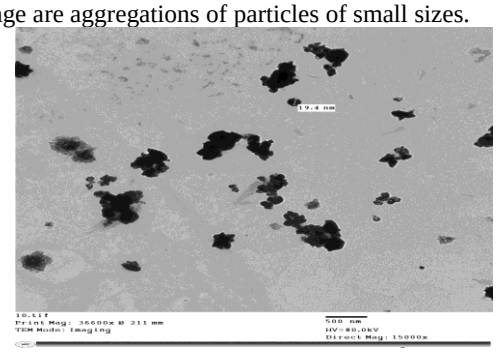

Fig. 3 . TEM image of $ZnFe_2O_4$ nanoparticles prepared by co-precipitation of Zn and Fe precursors in high basic medium, aggregated spherical in shape with approximately size 100 nm (Scale bar = 500 nm).

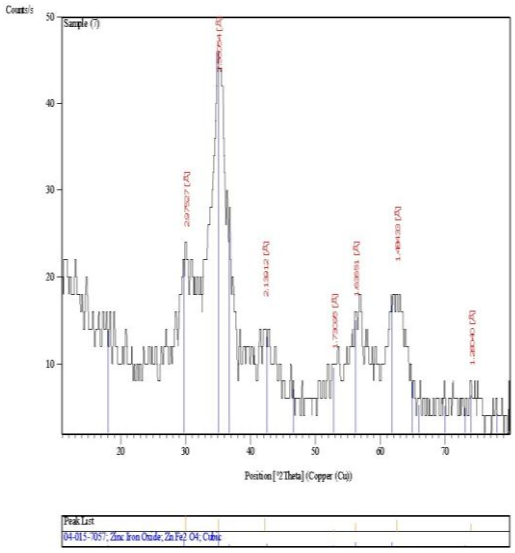

Fig. 4. XRD pattern of cubic $ZnFe_2O_4$ nanoparticles prepared by coprecipitation of Zn and Fe precursors in high basic medium.

3.2. Effect of the tested three types of nanomaterials on the linear growth of the tested fungus

Most concentrations of ZnO and $ZnFe_2O_4$ NPs resulted in significant inhibitory effect to the growth of the tested fungus (Table,1). The reduction in the linear growth was increased by increasing the concentration of the nanomaterials in the media till reaching the critical concentration of each as shown in Figure(5). On the other hand, Fe_3O_4 NPs resulted in increasing and accelerating the growth.

The growth of the fungus was entirely inhibited due to using ZnO NPs (concentration 250 µg/ml) until the end of experiment (6 days incubation). It was of great interest to notice that the fungus began to grow after the elapse of the experimental incubation period (6 days) and occupied a great area on the medium during 5 days after the end of the experiment (Figure 5,c).

The effect of the tested nanomaterials on the mycelium of *R. solani* was of great interest. The examination of the Compound microscopic preparations showed clear malformation in the hyphae of *R. solani* treated with ZnO NPs at concentration 250 µg/ml (Figure, 6). Electron microscopic preparations showed plasmolysis in treated mycelium with ZnO NPs at the same concentration (Fig. 7).

Table 1. Effect of 3 Nanomaterials on the linear growth of *R.solani* , 6 days after incubation at 30 ± 1ºC.

The tested nanoparticles	Average linear growth (mm) at conc.				Mean
	20	100	250	350	
ZnO	73	72	00	42	46.8
Fe_3O_4	90	90	86	90	89.0
$ZnFe_2O_4$	83	82	84	90	84.8
Control	90	90	90	90	90.0
Mean	84	83.5	65	78	----
L.S.D. at 5 % for :					
The tested nanoparticles (N)=					**2.6**
Concentrations (C)=					**1.9**
N x C =					**3.9**

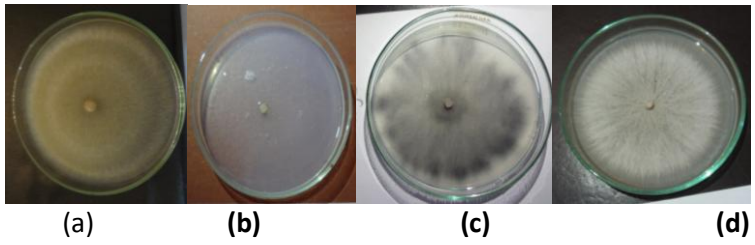

Fig. 5. Effect of the tested nanomaterials on the linear growth of *R. solani* (a) control (At the end of the experiment) , (b) ZnO NPs treatment (250 µg) (At the end of the experiment) (c) ZnO NPs treatment (250 µg) (5 days after the end of the experiment) ,(d) treated with $ZnFe_2O_4$ NPs (250 µg) (at the end of the experiment).

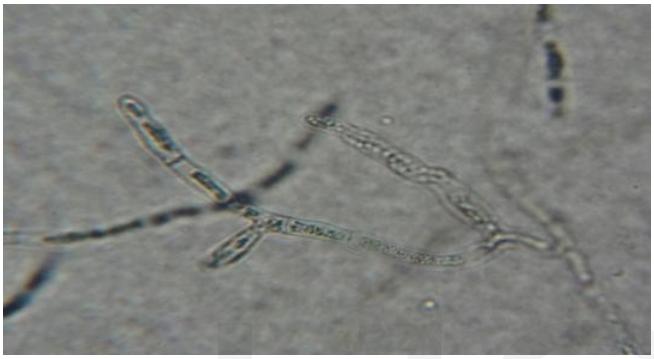

Fig.6. Microscopic preparation showing malformation of the hyphae and plasmolysis of *R. solani*, due to the effect of ZnO NPs (250 µg/ml) treatment (400X)

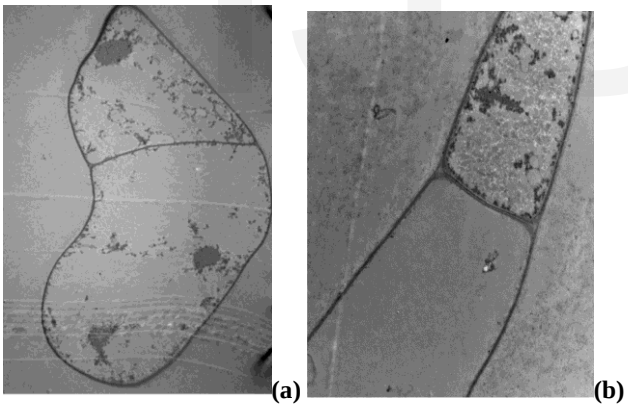

Fig.7. Transmission electron micrograph showing the effect of ZnO NPs (250 µg/ml) on the cells of *R. solani* (a) Control (b) Treated, showing complete plasmolysis in one cell of the hypha.

3.3. Effect of Cyanobacterial filtrates on the linear growth of the tested fungus

Most of the concentrations of the three Cyanobacterial filtrates resulted in significant inhibitory effect on the growth of the tested fungus. The reduction in the linear growth was increased by increasing the concentration of the used filtrate as shown in Table (2).

The lowest linear growth of *R. solani* on the average , was recorded using *S. platensis*, being 80.5 mm, followed by *A. Flos-aquae*, being 84.6 mm and *N. muscorum* being 86.5 mm as shown in Table (2). Even when no inhibition was noticed ; the growth was poor with pale colour and no zonation.

Table 2. Effect of three Cyanobacteria cultural filtrates on the linear growth of *R. solani* , 6 days after incubation at 30 ± 1 ºC.

The tested Cyanobacteria	Average linear growth (mm) at conc. (%)			Mean
	25	50	75	
S. platensis	90.0	84.0	67.6	80.5
N. muscorum	88.0	86.0	85.6	86.5
A. flos-aquae	87.0	84.0	83.0	84.6
Control	90.0	90.0	90.0	90.0
Mean	88.75	86.0	81.5	----
L.S.D. at 5 % for :				
Cyanobacteria (CB) =				**2.7**
Conc. (C)=				**3.1**
CB x C=				**4.3**

Data in (Table 3) show that the dry weight of *R. solani* was significantly affected by using the culture filtrates of the tested Cyanobacteria in comparison with the control treatment . It was found that the highest reduction in the dry weight of *R.solani* was obtained by using *N. muscorum* cultural filtrate at 75% conc., since the dry weight was 11.7 mg. . However no significant differences were found due to the effect of the culture filtrates on the tested fungus among the tested cyanobacteria.

Table 3 . Effect of three Cyanobacteria cultural filtrates on the dry weight of *R. solani* , 15 days after incubation at 30 ± 1 ºC.

The tested Cyanobacteria	Average dry weight (mg) at conc. (%)			Mean
	25	50	75	
S. platensis	12.6	12.2	12.1	12.3
N. muscorum	12.3	12.1	11.7	12.0
A. flos-aquae	12.3	12.2	11.9	12.1
Control	18.0	18.0	18.0	18.0
Mean	13.8	13.6	13.4	-----
L.S.D. at 5 % for :				
Cyanobacteria (CB) =				**1.9**
Conc. (C) =				**n.s.**
CB x C =				**2.8**

3.4.Greenhouse experiments

3.4.1. The effect of treating bean seeds c.v. Nebraska with the tested nanomaterials

The effect of treating bean seeds c.v. Nebraska with the tested nanomaterials against infection by *R. solani* was studied under greenhouse conditions.

In general, seed treatment with the tested nanomaterials caused significant reduction in pre- and post- emergence damping-off and increased the number of survived plants in the presence of *R. solani*. Meanwhile, using the tested nanomaterials against this fungus greatly reduced the infection by Rhizoctonia root rot of beans and also the severity of the disease (Tables, 4, 5).

Table 4 . Effect of treatment bean seeds with the tested nanomaterials on the infection by *R. solani* , greenhouse experiment.

Tested Nanoparticle materials	Conc.	No. of damping-off seedlings		No. of the survived plants
		Pre-emergence	Post-Emergence	
ZnO	20	9	0	6
	250	8	0	7
Fe_3O_4	20	3	0	12
	250	6	0	9
$ZnFe_2O_4$	20	4	1	10
	250	7	0	8
-ve check	==	0	0	15
+ve check	----	7	0	8
L.S.D. at 5 %	----	2	----	3

Where -ve check is untreated seeds with nanomaterials and planted in soil un-infested with *R. solani* , and +ve check, untreated seeds planted in soil infested with *R. solani.*

Table 5. Effect of the tested nanomaterials on the incidence of bean damping- off and root-rot severity caused by *R. solani,* greenhouse experiment.

The tested nanoparticles	% Damping-off at conc.**		Mean	Root-rot severity at conc.***		Mean
	20	250		20	250	
ZnO	60.0	53.4	56.7	3	4	3.5
Fe_3O_4	20.0	40.0	30.0	1	1	1
$ZnFe_2O_4$	33.4	46.4	40.0	2	3	2.5
***Control**	46.7	46.7	46.7	4	4	4.0
Mean	40.0	46.7	---	2.5	3.0	---
L.S.D. at 5 % for :						
The tested Nanoparticles (N) =			**3.0**			**0.9**
Conc.(C) =			**2.1**			**n.s.**
N x C =			**3.4**			**1.0**

***Untreated bean seeds planted in soil infested with *R.solani.* ** 30 days after sowing . *** 80 days after sowing.**

3.4.2.Effect of seed treatment with the Cyanobacteria cultural filtrates on disease incidence and root-rot severity

The effect of bean seeds treatment with the different Cyanobacteria cultural filtrates against *R. solani* was significant. Data presented in Tables (6) show that the number of survived plants in the +ve check treatment was 4, while this figure was 11 for *A. Flos-aquae* treatment and 10 for *N. muscorum* treatment at 75% concentration.

In all cases, seed treatment with *A. flosaquae* at 75% conc. was the best (Table, 7), where damping-off percentage reached 26.7%, followed by *N. muscorum* (75%), being 33.4 % in comparison with the +ve check (73.4 %). The same trend was also true for their effect on root rot severity.

Table 6. Effect of treatment bean seeds with the tested Cyanobacteria cultural filtrates on the infection by *R.solani,* greenhouse experiment

The tested Cyanobacteria	Conc. %	No. of damping-off		No. of survived Plants
		Pre-emergence	Post-emergence	
S. platensis	25	6	3	6
	50	4	4	7
	75	4	4	7
N. muscorum	25	5	3	7
	50	4	2	9
	75	3	2	10
A. flos-aquae	25	5	4	6
	50	5	3	7
	75	3	1	11
-ve check	---	0	0	15
+ve check	---	6	5	4
L.S.D. at 5 %	--	1	1	2

Where -ve check is untreated seeds with Cyanobacteria and planted in soil un-infested with *R. solani* , and +ve check, untreated seeds planted in soil infested with *R. solani.*

Table 7 . Effect of three Cyanobacteria on the incidence of bean damping- off and root-rot severity caused by *R. solani,* under greenhouse conditions.

The tested Cyano-bacteria	% Damping-off at conc.(%)**			Mean	Root-rot severity at conc.***			Mean
	25	50	75		25	50	75	
S. platensis	60.0	53.4	53.4	55.6	4	4	4	4.0
N. muscorum	53.4	40.0	33.4	42.2	3	3	4	3.3
A. flos-aquae	60.0	53.4	26.7	46.7	2	3	1	1.7
***Control**	73.4	73.4	73.4	73.4	5	5	5	5.0
Mean	61.7	36.8	46.7	-----	3.5	3.8	3.5	----
L.S.D. at 5 % for :								
Cyanobacteria (CB) =				**3.0**				**0.9**
Conc. (C) =				**2.7**				**n.s.**
CB x C =				**3.9**				**1.0**

***Untreated bean seeds planted in soil infested with *R.solani.* ** 30 days after sowing . *** 80 days after sowing.**

3.6. Effect of nanomaterials on the activity of oxidative reductive enzymes

The effect of treating bean seeds c.v. Nebraska with the tested nanomaterials on the activity of both peroxidase and polyphenoloxidase was studied. In general, it was found that the infection by the tested fungus decreased the activity of both enzymes (Table, 8). ZnO NPs at the low concentration treatment increased the activity of polyphenoloxidase, while Fe_3O_4 NPs increased the activity of peroxidase.

Table 8. Effect of seed treatment with the tested nanomaterials on the activity of two oxidative reductive enzymes in the tissues of bean plants grown in soil infested with *R. solani*

Treatment	Concentration (µg/ml)	Peroxidase activity (mg/min)	Polyphenoloxidase activity (mg/min)
-ve check	*	0.46	0.09
+ve check	**	0.28	0.09
ZnO	**20**	0.39	0.11
Fe_3O_4	**20**	0.56	0.08
$ZnFe_2O_4$	**20**	0.51	0.07
ZnO	**250**	0.42	0.09
Fe_3O_4	**250**	0.46	0.07
$ZnFe_2O_4$	**250**	0.41	0.06

*** Untreated seeds planted in un-infested soil with *R. soalni***
**** Untreated seeds planted in infested soil with *R. soalni***

3.7. Effect of seed treatment with the tested Cyanobacteria on the activity of two oxidative-reductive enzymes

The obtained results (Table, 9) show that soaking bean seeds in the cultural filtrate of any of the three Cyanobacteria increased the activity of the assessed oxidative reductive enzymes .

In all cases, data presented in Table (9) show that infection by *R. solani* decreased the activity of peroxidase . Meanwhile, treatment of bean seeds with the filtrate of Cyanobacteria increased the activity of peroxidase. The highest value of peroxidase activity was recorded when *A. Flos-aquae* was used against *R. solani.* followed by treatment with *N. muscorum* and *S. platensis.* The corresponding values were 0.70, 0.66 and 0.60 (mg/min.), respectively. Meanwhile, the activity of peroxidase in the tissues of healthy control plants (-ve check) was 0.46(mg/min) and in the tissues of plants grown from untreated seeds in soil infested with *R.solani* recorded 0.28(mg/min).

On the other hand , using the filtrate of *S. platensis* improved the activity of polyphenoloxidase in the presence of the tested fungus.

Table 9. Effect of Cyanobacterial filtrates on the activity of two oxidative reductive enzymes in tissues of bean plants c.v. Nebraska grown in soil infested with *R. solani.*

Treatment	Peroxidase activity (mg/min)	Polyphenoloxidase activity (mg/min)
-ve check	0.46	0.09
+ve check	0.28	0.09
S. platensis	0.60	0.11
N. muscorum	0.66	0.03
A. flos-aquae	0.70	0.095

Where -ve check : untreated seeds with Cyanobacteria planted in un-infested soil . +ve check : untreated seeds planted in soil infested with *R. solani*

3.8. Effect of the tested nanomaterials on some crop parameters

The effect of treating bean seeds c.v. Nebraska with the tested nanomaterials , *i.e.* ZnO, Fe_3O_4 and the $ZnFe_2O_4$ NPs of them on some crop parameters , *i.e.* plant height, No. of leaves/plant, No. of pods/ plant, fresh and dry weight was investigated under greenhouse conditions in pots infested with the tested fungus (Table , 10).

The effect of the tested nanomaterials on both fresh and dry weight was significantly increased in comparison with the control plants (Table 11). In all cases, seed treatment with ZnO NPs resulted in the highest values of both fresh and dry weight, followed by Fe_3O_4 NPs and the $ZnFe_2O_4$ NPs compared to their respective control plants.

Plants grown in soil infested with *R. solani* exhibited significant differences due to the treatment with cyanobacteria filtrates (Table, 12). The highest value of plant height, being 37 cm was obtained using *A. flosaquae* at 50% concentration and 35cm using *N. muscorum* at 75% concentration . Meanwhile, control plants grown in soil infested with *R. solani* only were significantly the shortest, being 22cm.

Table 10. Effect of treatment bean seeds with three nanomaterials on plant height , number of leaves and pods/ plant , grown in the greenhouse in soil infested with *R.solani.*

The tested Nanoparticles	Plant height (cm) at conc. (µg/ml)		Mean	No. of leaves / plant at conc. (µg/ml)		Mean	No. of pods/ plant at conc. (µg/ml)		Mean
	20	250		20	250		20	250	
ZnO	25.0	30	27.5	9	8	8.5	4	5.0	4.5
Fe_3O_4	31.0	34	32.5	5	9	7.0	4	5.0	4.5
$ZnFe_2O_4$	32.0	30	31.0	5	8	6.5	4	5.0	4.5
Control*	26.0	26	26.0	5	5	5.0	4	4.0	4.0
Mean	28.5	30	---	6	7.5	----	4	4.8	----
L.S.D. at 5 % for :									
Nanoparticles (N) =			**2.7**			**1.2**			**n.s.**
Conc.(C) =			**n.s.**			**0.9**			**n.s.**
N x C =			**3.2**			**2.0**			**n.s.**

Control : plants grown from untreated seeds in soil infested with *R. solani.

Table 11. Effect of seed treatment with the tested nanomaterials on fresh and dry weight of bean plants grown in soil infested with *R. solani* , under greenhouse conditions.

The tested Nanoparticles	Fresh weight (g) / plant at conc. (µg/ml)		Mean	Dry weight (g) / plant at conc. (µg/ml)		Mean
	20	250		20	250	
ZnO	34	29	31.5	5.4	4.9	5.2
Fe_3O_4	19	27	23.0	3.3	4.5	3.9
$ZnFe_2O_4$	16	25	20.5	2.9	4.1	3.5
Control*	19	19	19.0	3.2	3.2	3.2
Mean	22	25	-----	3.7	4.2	---=
L.S.D. at 5 % for :						
Nanoparticles (N) =			**3.0**			**0. 7**
Conc.(C) =			**2.0**			**n.s.**
N x C =			**3.3**			**n.s.**

Control : plants grown from untreated seeds in soil infested with *R. solani

3.9. Effect of the tested Cyanobacteria on some crop parameters.

Table 12. Effect of three Cyanobacteria filtrates on some crop parameters, plant height , number of leaves and pods/ plant, greenhouse experiment in the presence of *R. solani*

The tested Cyanobacteria	Plant height (cm) at conc.(%)			Mean	No. of leaves / plant at conc.(%)			Mean	No. of pods/ plant at conc.(%)			Mean
	25	50	75		25	50	75		25	50	75	
S. platensis	22	26	34	27.3	4	6	8	6.0	1	2	4	2.3
N.muscorum	33	32	35	33.3	7	6	9	7.3	3	3	4	3.3
A. flos-aquae	32	37	33	34	7	9	8	8.0	3	5	3	3.7
Control *	22	22	22	22	6	6	6	6.0	2	2	2	2.0
Mean	27.3	29.3	31.0	----	6.0	6.8	7.8	----	2.3	3	3.3	----
L.S.D. at 5 % for :												
Cyanobacteria (CB) =				**2.6**				**1.4**				**0.8**
Conc. (C) =				**2.0**				**1.0**				**0.7**
CB x C =				**3.0**				**2.0**				**1.0**

*** Control =plants grown from untreated seeds in soil infested with *R. solani***

Table 13. Effect of three Cyanobacteria filtrates on fresh and dry weight g /plant of bean plants grown in soil infested with *R. solani* , greenhouse experiment.

The tested cyanobacteria	Fresh weight (g) / plant at conc.(%)			Mean	Dry weight (g) / plant at conc.(%)			Mean
	25	50	75		25	50	75	
S. platensis	15.0	30	26.2	23.7	2.9	5	6.2	4.7
N.muscorum	29.5	23.3	18.0	23.6	5.4	5.2	3.2	4.6
A. flos-aquae	28.0	19.5	18.5	22.0	5.1	3.4	3.2	3.9
Control*	20.0	20.0	20.0	20.0	3.9	3.9	3.9	3.9
Mean	23.1	23.2	20.7	-----	4.3	4.4	4.1	----
L.S.D. at 5 % for :								
Cyanobacteria (CB) =				**2.3**				**0.9**
Conc. (C) =				**2.1**				**n.s.**
CB x C =				**2.6**				**1.7**

Control: plants grown from untreated seeds in soil infested with *R. solani.

4. DISCUSSION

During the last few years, bean growers, in most areas, have complained of the noticeable increase in the number of plant mortality at any growth stage as a result of infection with damping-off, wilt and root rot diseases, causing great reduction in plant stand and consequently, substantial losses in bean crop and its quality.

Therefore, this work was planned to study this firmness to throw light on the trials of disease management. The present work was designed to look for active substances that could be used as antimicrobial agents in an sufficient and safe manner.

It is obvious that there is a growing need to find new alternative methods to fungicides. Biological control using Cyanobacteria is one of these promising alternatives. The obtained results showed that the culture filtrates of the three Cyanobacteria; *N. muscorum* , S. *platensis*, *A. flos-aquae* exhibited inhibitory effect on the growth of the tested fungus, *i.e. R. solani* using P.D.A. in Petri dishes. These data are in accordance with [27]Carmichael (1992) and [28] Kulik (1995) who proved that the Cyanobacteria cultural filtrates contained a wide variety of biologically active compounds such as antibiotics and toxins

Also, [29] De Cano *et al.* (1990) found that phenolic compounds in extracts from cells of *N. muscorum* significantly inhibited the growth of *Candida albicans* . Also, [30] FrankmÖlle *et al.* (1992a) reported that crude ethanolic extracts from *A. flos-aquaea* inhibited the growth of *Aspergillus oryzae, Penicillium notatum, Saccharomyces cerevisiae*. These fungicidal compounds were isolated and purified and given the name laxaphycinsA, B, C, D and E and their structures were determined by[31] FrankmÖlle *et al.* (1992b).

[32] Morsy (2011) indicated that cyanobacterial filtrates reduced the infection of faba bean with fungi causing root rots and improved plant growth parameters and found that the cultural filtrates of *N. muscorum, A. flos-aquae, S. platensis* contain indol acetic acid, protease enzymes .

Also, [33] Abdel-Hafez *et al.* (2015) proved *in vitro* and under greenhouse conditions that extracellular metabolites of *N. muscorum* and *Oscillatoria* sp. reduced the linear growth of *Alternaria porri*, which causes onion purple blotch disease.

Data of the present investigation indicated that the filtrates of the tested Cyanobacteria were able to reduce pre- and post-emergence damping-off caused by *R. solani* and increased the number of survived plants compared to the check under greenhouse conditions.

These results are in accordance with those reported by [34] Menamo and Wolde (2013) who recorded that the dried Cyanobacteria bio-fertilizer significantly increased leaf length of lettuce over the control and this might be due to the release of enough nitrogenous compounds for lettuce growth, mostly nitrates and ammonium, which can be readily taken up by vascular plants .Absorbed nitrogen in turn increases leaf length through stem elongation brought about by cell division and expansion, also the highest leaf fresh weight of the lettuce under dried Cyanobacteria application could be linked to cyanopith as a biofertilizer improving the growth of lettuce by providing essential nutrients, which result in maximum cell growth, a phenomenon that influenced the growth of plant.

In recent years, numerous researchers have shown that the reduction in disease incidence is due to increasing the activity of defence related enzymes such as peroxidase and polyphenoloxidase [35,36,37,38](Zdor and Anderson, 1992; Inbar *et al.*, 1994; Nandakumar *et al.*, 2001; Sivakumar and Shorma, 2003).

During the progress of the present study, the activity of defence related enzymes was determined to shed light on the effect of the tested Cyanobacteria, *i.e. Nostoc muscorum*, *Spirulina platensis*, *Anabaena folsaquae* and nanoparticles materials, *i.e.* zinc oxide, iron oxide and alloy of them on the activity of peroxidase and polyphenoloxidase in the tissues of bean plants c.v. Nebraska.

The results of the present study revealed that in most cases, there was a pronounced increase in the activity of defence related enzymes, *i.e.* peroxidase and polyphenoloxidase in the tissues of bean plants grown from seeds treated with any concentration of the tested Cyanobacteria and/ or nanomaterials and grown in soil infested with *R. solani*, in comparison with the untreated control plants grown from untreated seeds in soil infested with the tested fungus, as judged by the markedly improved seedling survival, plant growth parameters and the noticeable decrease in the amount of the damping-off and root- rot diseases.

The results achieved in this study concerning the use of nanomaterials and their effect on the tested fungus were proved by many studies.[39] Sirelkhatim *et al.* (2015) mentioned that the antimicrobial effect of ZnO NPs was reported to occur by 2 ways. The first is the formation of H_2O_2 on the surface of ZnO NPs due to the possible formation of hydrogen bond between hydroxyl group of cellulose molecules of fungi with oxygen atom of ZnO NPs leading to inhibition of the microbial growth, while the second is the release of Zn^{2+} which causes damages of cell membrane and interacts with intraocular contents**.** Several natural and engineered nanomaterials have demonstrated strong antimicrobial properties through diverse mechanisms including photocatalytic production of reactive oxygen species that damage cell components and viruses (as ZnO), compromising the cell envelope (peptides, chitosan carboxyfullerene, carbon nanotubes and ZnO) and interruption of energy transduction.

The effect on the functional properties of soil microbes will depend on the composition and morphology of the nanoparticle materials under consideration and its local concentration. [40] Priester *et al*. (2012) found that ZnO NPs had relatively little effect on nitrogen-fixation in a soybean crop system, but CeO_2 had significant negative effects on nitrogen fixation at medium and high concentrations**.** These studies suggest that toxic effects on soil microbes could be selective, and inhibition of certain microbial groups could alter soil microbial communities within the plant rhizosphere with negative consequences for plant nutrient uptake and soil fertility. Although comparative studies on the effects of NPs have been performed, most studies are in culture or conducted under artificial conditions, and their applicability to plant-microbial responses in agro-ecosystems is uncertain.

Chemical mechanisms of Fe_3O_4 NPs include the production of reactive oxygen species (ROS), termination and release of toxic ions, oxidative damage through catalysis, disturbance of the ion cell membrane transport activity , However, free radical formation can also have direct impacts on cell stability [41,42] (Singh *et al*, 2010; Li *et al*, 2008). These results are in accordance with the results obtained in this study as Fe_3O_4 NPs affected the cell stability and increased the growth rate but caused malformation in the growth appeared clearly in the color and texture of mycelium.

$ZnFe_2O_4$ NPs, as characterized in this study, is an alloy of mixture between zinc oxide and iron oxide nanoparticles, so its effect on the tested fungus was variable according to the consumption of the tested fungus to the alloy components, *R.solani* consumed the iron oxide in alloy more than it's consumption to zinc oxide and as a result, the effect of alloy on the linear growth and greenhouse experiments was much more like the effect of Fe_3O_4 NPs.

5.CONCLUSION

Bean crop is sensitive to various soil borne fungi, among which *Rhizoctonia solani* is widespread, the most important and the most aggressive causing damping-off of seedlings and root rot diseases, consequently causes great losses in bean yield and its quality. The present work was designed to look for active substances that can be used as antimicrobial agents, in an sufficient and safe manner, as alternatives to the most commercially recommended fungicides.

Therefore three types of Nanomaterials , ZnO NPs, Fe_3O_4 NPs, $ZnFe_2O_4$ NPs and the cultural filtrate of three species of cyanobacteria, *Nostoc muscorum* , Spirulina *platensis*, *Anabaena flos-aquae* were tested for their antifungal activity against *R. solani*.

The obtained results showed that the cultural filtrates of the three cyanobacteria exhibited inhibitory effect on the growth of *R. solani* using P.D.A. in Petri dishes. The inhibitory effect of *S. platensis* filtrate was significantly remarkable as the linear growth values were the lowest , however the three species of cyanobacteria decreased the dry weight of the tested fungus greatly.

In respect to the nanomaterials, ZnO NPs showed significantly remarkable antifungal effect followed by $ZnFe_2O_4$ NPs. Meanwhile Fe_3O_4 NPs showed no effect on the linear growth of the tested fungus.

There was a clear influence of treatment bean seeds with either Nanomaterials or cyanobacteria on seed germination, incidence of pre- and post-emergence damping-off and root rot severity.

In addition, most of these treatments improved some plant parameters and increased the activity of defence related enzymes, peroxidase and polyphenol oxidase.

Therefore the authors, in summing up, concluded that the tested Nanomaterials and cyanobacteria have considerable antifungal activity. At any rate, further studies are needed to throw light on the mechanism of inhibitory effect, the proper concentration to be used under field conditions and their residues in plant tissues.

6. REFERENCES

1. El-Mougy Nehal S. , El- Gamal Nadia G. and Abd-El-Kader M.M. Control of wilt and root rot incidence in *Phaseolus vulgaris* L. by some plant volatile compounds . J. of Plant Protection Res. 2007; 47: 255- 265.

2. Abeysinghe S. Biological control of *Fusarium solani* f.sp *phaseoli,* the causal agent of root rot of bean using *Bacillus subtilis* CA32 and *Trichoderma harzianum* Ruol. Ruhuna J. Sci. 2007 ; 2: 82-88.

3. Mukankusi C., Melis R., Derera J., Laing M.D. and Buruchara R.A. Identification of sources of resistance to Fusarium root rot among selected common bean lines in Uganda. J. Animal and Plant Sci. 2010; 7 : 876-891.

4. Baker K.F. Types of Rhizoctonia diseases and their occurrence. In: Parmeter JR.Jr. editor : *Rhizoctonia solani*, Biology and Pathology.Berkeley. CA: California Univ. Press 1970; 124- 148.

5. Anderson N.A. The genetics and pathology of *Rhizoctonia solani* .Ann. Rev. Phytopathol. 1982; 20: 329-347.

6. Abd-El-Khair H, Khalifa R.K.M. and Haggag Karima H.E. Effect of *Trichoderma* species on damping-off diseases incidence, some plant enzymes activity and nutritional status of bean plants. J. of American Sci. 2011; 7: 156-167.

7. Dekker J. and Georgopoulos S.G. Fungicide Resistance In Crop Protection. Wageningen, Centre for Agricultural Publishing and Documentation. 1982; 265 p.p.

8. Brent K.J. and Hollomon D.W. Fungicide resistance in crop pathogens: How can it be managed. FRAC Monograph No. 1 (second, revised edition). Fungicide Resistance Action Committee. Monogr. 1 GCPF, FRAC, Brussels 1995; p 1–48.

8. Goffeau A. Drug resistance: The fight against fungi. Nature 2008; 452: 541-542.

9. Kanhed P., Birla S., Gaikwad S., Gade A., Seabra A. B., Rubilar O., Duran N. and Rai M. *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. Materials Letters 2014; 115: 13-17.

10. Burja A.M., Banaigs B., Abou-Mansour E., Burgess J.G. and Wright P.C. Marine cyanobacteria a prolific source of natural products. Tetrahedron 2001; 57:9347–9377.

11. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J. Microbiol. Methods 2003; 54:177-182.

12. Abada K.A. , Ashour A.M.A. , Morsy K. M.M. , Attia Amany M.F. Role of blue-green algae in managing damping-off and charcoal rot diseases of bean, Inter. J. of Sci. & Eng. Res. 2016; 7(5): 109-116.

13. Attia Amany M.F. Improvement of Bean Resistance to Damping-off and Root-rot Diseases. Ph.D. Thesis, Fac. Agric. Cairo Univ. 2016; 130 pp.

14. Watanabe A. Production in cultural solution of some amino acids by the atmospheric nitrogen-fixing blue-green algae . Arch. Biochem. Biophys.1951; 43 : 50-55.

15. Zarrouk C. Contribution a l'etude d'une cyanobacterie: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. Ph.D. Thesis, Univ. of Paris, France 1966

16. Cobb F.W. , Krstic Jr.M. , Zavarin E. and Barber H.W. Jr. Inhibitory effects of volatile oleoresin components on *Fomes annosus* and four *Ceratocystis* species. Phytopathology 1968; 58: 1327:1335.

17. Bozzola J.J. and Russell L.D. Electron Microscopy, Second Edition. Sudbury, MA: Jones and Bartlett Publishers 1999; 670 p.

18. Attia M.F. Pathological and Physiological Studies on *Sclerotium bataticola* Taub., The Incitent of Charcoal Rot Disease of Sweetpotato in U.A.R. M.Sc Thesis., Fac. of Agric., Cairo Univ. 1966; pp.154.

20. Lisker N., Cohen L., Chalutz E. and Fuchs Y. Fungal infections suppress ethylene-induced phenylalanine ammonia- lyase activity in Grapefruits. Physiol. Plant Pathol. 1983; 22, 331-338.

21. Kochba J., Lavee S. and Spiegel-Roy P. Differences in peroxidas activity and isoenzymes in embryogenic and non- embryogenic 'Shamouti' orange ovular callus lines. Plant Cell Physiol. 1977; 18:463– 467.

22. Liu L., Kloepper J.W. and Tuzun S. Introduction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 1995; 85: 695-698.

23. Snedecor G.W. and Cochran W.G. Statistical Methods.8th Ed., Iowa State Univ. Press , Ames, Iowa , U.S.A. 1989

24. Fisher R.A Statistical Methods For Research Workers. Oliver and Boyd. London. 1948

25. Gu F., Wang S.F., Lu M.K., Zhou G.J., Xu D. and Yuan D.R. Structure Evaluation and Highly Enhanced Luminescence of Dy3+-Doped ZnO Nanocrystals by Li+ Doping via Combustion Method, Langmuir. 2004; 20: 3528.

26. Girshick L., Chiu P. Kinetic nucleation theory: a new expression for the rate of homogeneous nucleation from an ideal supersaturated vapour The Journal of Chemical Physics 1990; 93(2): 1273-1277.

27. Carmichael W.W. Cyanobacteria secondary metabolites-the cyanotoxins. J. Applied Bacteriol. 1992; 72: 445-459.

28. Kulik M.M. The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Plant Pathol. 1995; 101(6) : 585-599.

29. De Cano M.S., De Mulé M.C.Z, De Caire G.Z, De Halperin D.R. Inhibition of *Candida albicans* and *Staphylococcus aureus* by phenolic compounds from the terrestrial cyanobacterium *Nostoc muscorum* . J. of Appl. Phyco. 1990; 2:79–81.

30. FrankmÖlle W.P., Larsen L.K., Caplan F.R., Patterson G.M.I. , Knuabal G., Levine I.A. and Moore R.E. Antifungal cyclic peptides from the terrestrial blue-green algae *Anabaena laxa*. Isolations and biological properties . J. Antibiotics 1992a; 45: 1451-1457.

31. FrankmÖlle W.P., Knubal G., Moorc R.I. and Patterson G.M.I. Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. Structures of laxaphycins A.B. C. D. and E. J. Antibiotics 1992b; 45 : 1458:1466.

32. Morsy K.M.M. Biological control of damping-off, root rot and wilt diseases of faba bean by cyanobacteria (blue-green algal) culture filtrate. Egypt.J. Phytopathol. 2011; 39 (2):159-171.

33. Abdel-Hafez S.I.I., Abo-El yousr K.A. M. and Abdel-Rahim I.R. Fungicidal activity of extracellular products of cyanobacteria against *Alternaria porri* , Euro. J. of Phyco. 2015; 50: 239-245.

34. Menamo M. and Wolde Z. Effect of cyanobacteria application as biofertilizer on growth, Yield and yield components of Romaine Lettuce (*Lactuca sativa* L.) on soils of Ethiopia. American Scientific Research Journal for Engineering, Technology, and Sciences 2013; 4 (1): 50-58.

35. Zdor R.E. and Anderson A.J. Influence of root colonizing bacteria on the defense responses of bean. Plant and Soil 1992; 140:99-107.

36. Inbar J., Abramsky M., Cohen D. and Chet I. Plant growth enhancement and disease control by *Tricoderma harzianum* in vegetable seedlings grown under commercial conditions. Eur. J. Plant Pathol. 1994; 100:337-346.

37. Nandakumar R., Babu S., Viswanathan R., Raghuchander T. and Samiyappan R. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol. and Biochem. 2001; 33:603-612.

38. Sivakumar G. and Sharma R.C. Induced biochemical changes due to seed bacterization by *Pseudomona fluorescens* in maize plants. Indian Phytopathol. 2003; 56:134-137.

39. Sirelkhatim Amna, Shahrom M., Azman S., Noor K., Ling C., Siti B., Habsah H. and Dasmawati M. Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. Nano-Micro Letters 2015; 7(3): 219-242.

40. Priester J.H., Ge Y., Mielke R.E., Horst A.M., Moritz S.C., Espinosa K., Gelb J., Walker S.L., Nisbet R.M and An Y.J. Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. Proc. Natl. Acad. Sci. USA 2012; 109: E2451–E2456.

41. Singh N., Jenkins G. J. S., Asadi R. and Doak S. H. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). *Nano Reviews, 1,* 2010; 10.3402/nano.v1i0.5358.

42. Li G.Y., Jiang Y.R., Huang K.L., Ding P. and Chen J. Preparation and properties of magnetic Fe_3O_4–chitosan Nanoparticles. Journal of Alloys and Compounds 2008; 466(1): p.451- 456.