Study Influence of Silver Nanoparticles on growth rate of plant pathogens : *Rhizoctonia solani, Alternaria alternata, Fusarium solani, Fusarium oxysporum, Aspergillus terreus, Aspergillus parasiticus,* and Bio agent *Trichoderma harzianum*

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Abstract

The study was carried out in (Al-qasim green University / College of Agriculture)on poisons media by Silver Nano particular of levels (0, 10, 15, 20, 25, 30) *µl*/ml to measured First (FGR), second (SGR) and third growth rate (TGR) of seven fungi *Rhizoctonia solani, Alternaria alternata, Fusarium solani, Fusarium oxysporum, Aspergillus Terreus, Aspergillus parasiticus,* and Bio agent *Trichoderma harzianum* which isolated from agricultural field of pepper plant, the result showed a significant effect of Ag Nps at 30, 25, 20, 15, 10 *µl* /ml to inhibition growth rate of all fungi FGR (4.190, 4.904, 5.476, 6.333, 7.190) mm/day, respectively on SGR (9.571, 12.240, 12.810, 14.857, 18.095) mm/day respectively, TGR (12.430, 13.810, 14.100 16.048, 18.095) mm/day respectively compared to 0.0 *µl* /ml . The results also revealed that *T.harzianum* was faster growth rate at FGR, SGR and TGR it was (10.388, 34.722 and 32.333) mm/day, respectively flowed by *R.solani*, which were (13.055, 30.222 and 29.833) mm/day respectively compared to *A.alternata, F.solani, F.oxysporum, A.terreus, A.parasiticus*.

Keywords: Ag Np, Growth rate, *Trichoderma harzianum***,** *Rhizoctonia solani, Alternaria alternata, Fusarium solani, Fusarium oxysporum, Aspergillus Terreus, Aspergillus parasiticus.*

الخلاصة

أجريت الدراسة باستعمال الوسط المسمم بجزيئات الفضة النانوية بمستويات (٠، ١٠، ١٥، ٢٠، ٢٥، ٣٠) مايكرولتر/مل لقياس سرعة النمو الاولى (FGR) والثانية (SGR) والثالثة (TGR) لسبعة فطريات *Rhizoctonia solani* و *Alternaria alternate* و *Fusarium solani* و *Fusarium oxysporum* و *Aspergillus terreus* و *Aspergillus parasiticus* وعامل المقاومة الحيوية *Trichoderma harzianum* التي عزلت عن حقل زراعي بنبات الفلفل، وأظهرت النتائج فروقات معنوية باستعمال التراكيز ٣٠، ٢٥، ٢٠، ١٥،

١٠ مايكرولتر / مل لخفض معدل نمو جميع الفطريات إذ كانت (FGR) (٤.١٩٠، ٤.٩٠٤، ٥.٤٧٦، ٦.٣٣٣، ٧.١٩٠) ملم / يوم على التوالي و لـــ (SGR) كانت (٩.٥٧١، ١٢.٢٤٠، ١٢.٨١٠، ١٤.٨٥٧، ١٨.٠٩٥) ملم / يوم على التوالي و لـــ(TGR) كانت (١٢.٤٣٠، ١٣.٨١٠ ، ١٤.١٠٠ ١٦.٠٤٨، ١٨.٠٩٥) ملم / يوم على التوالي مقارنة بمعاملة السيطرة ٠.٠ مايكرولتر/ مل . كما اظهرت النتائج ان *T.harzianum* كان ذا معدل النمو أسرع في (FGR) و(SGR) و (TGR) إذ كان (١٠.٣٨٨ و ٣٤.٧٢٢ و ٣٢.٣٣٣) ملم / يوم على التوالي تلاه *R.solani*الذي كان (١٣.٠٥٥، ٣٠.٢٢٢ و ٢٩.٨٣٣) ملم / يوم على التوالي بالمقارنة مع *A.alternata* و *F.solani* و *F.oxysporum* و*A.terreus* و *A.parasiticus*.

الكلمات المفتاحية: Ag Nanoparticles و سرعة النمو و *Trichoderma harzianum* و *Rhizoctonia solani* و*Alternaria alternate* و *Fusarium solani* و *Fusarium oxysporum* و *Aspergillus terreus* و *Aspergillus parasiticus*.

1-Introduction

Fungi are one of the most important factors causing plant diseases may be air-borne or soil-borne pathogenic agents for example *Alternaria alternate, Aspergillus terreus, Aspergillus parasiticus, Rhizoctonia solani, Fusarium solani, Fusarium oxysporum* has been subject by many researchers [1] [2] [3].*Rhizoctonia solani* is one of those soil-borne fungus that causes root rot, seeds rot, and stem rot, Whereas leads to pre or post emergence damping off, It causes a number diseases on a wide range of plants, on most of parts of the world, than any other plant pathogenic [4] *alternata* is one of the air-borne which can infected of many plants it was usually most frequently isolated by symptomatic root rot on carrot plant as a result of pathogenic- ity test [5]. *F.solani* cause root and lower stem rots of many Vegetables and *F.oxysporum* consists of more than (120) forma specialis according to the host-pathogen reaction. *Aspergillus* species are found more commonly causing molding of most of grains and legumes [6].

Nanotechnology has great advantage to play some roles in Society life with its different applications. Nanoparticles of many metals like silver Nanoparticles have used in various fields like agriculture, diagnostics and pharmaceuticals. Many researchers enabled to incorporate silver nanoparticles in food, Packing methods, automotive industry, environmental agents, disinfectant, paints, cosmetics etc. Silver nanoparticles have types of applications according to their medicinal properties. different methods were used to synthesized of Silver nanoparticles, chemical or physical or biological methods [7] [8] .

Silver nanoparticles may use as significant applications in agriculture and Horticultural by inhibiting pathogenic fungi and bacteria on seeds and could use as an alternative fertilizer that may enhancement of sustainable agriculture. The seed treatment by nanoparticles can reduce the environmental effect of chemical fungicides and improve the agricultural production [9].

Whereas Ag NPs at lower concentrations indicated of increase Seed germination and

early seedling growth in Fenugreek, while at higher concentration showed slight adverse influence. Additionally A significant positive effects on root, fresh weight and dry weight, root length was observed. The results showed that the influence of AgNPs was increase seeds germination percentage in Fenugreek [10].

2-Materials and methods

3-Fungi Isolation

The fungi were isolated from a plastic house planted with pepper plants from soil and air, it was classified by diagnostic keys: *Fusarium oxysporum* and *Fusarium solani* [11], *Aspergillus terreus* and *Aspergillus parasiticus* [12] [13] *Alternaria alternate* [5] [14].and *Trichodema harzianum* and *Rhizoctonia solani* [15]. Purified, kept in slant agar until use.

4-Pathogenicity test

Pathogenicity were tested of studied fungi on chili pepper seeds which was purchased from local markets by cultured 10 seeds peripheral edge of agar plats, ten days later were calculated healthy seedlings.

5-Preparation of Silver Nanoparticles

Silver nitrate ($AgNo_3$) purity of 99% was purchased , tri sodium citrate and D-distilled deionized water it have been used to prepared silver Nps by using chemical reduction methods , it was dissolved of 0.001 M of $AgNo_3$ in D-distilled water to prepare starting solution and heated to boil ,then 5 ml of 0.01 M tri sodium citrate was added to the solution drop by drop ,the solution was mixed under fixed speed and heated until color changed (pale-yellow) as shown in fig.(1) ,then it removed from heat device and it was stirred until cooled to the room temperature. The mechanism of chemical reaction [16] [17].

$$4\,Ag^{+} + C_6H_5D_7Na + 2\,H_2O \longrightarrow 4\,Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2$$

6- Scanning Electron Microscope (SEM) analysis

Fig.(3) shows the particle size and morphology of Ag Np. From the SEM image, The nanoparticles are spherical shaped and the diameter of the particles being 25-40 nm, it is evident that the little bit of aggregation of nanoparticle .The particles are uniform in shape and well dispersed.

7-UV–Visible spectra

The optical property of the Ag Np. was investigated by the UV–Vis spectroscopy as shown in Fig.(2). It clearly shows that, their absorption edges are located around 430 nm. The band of surface plasmon resonance (SPR) determined the morphology of the nanoparticles, SPR bands are influenced by the, size, morphology ,and shape of the nanoparticles ,many studies have shown that the spherical silver Np contributes to the absorption band around 400 nm [18],absorption band in this study was around (430) as shown in fig.(2) which strongly suggests that silver Np were spherical in the shape and have been confined by SEM .

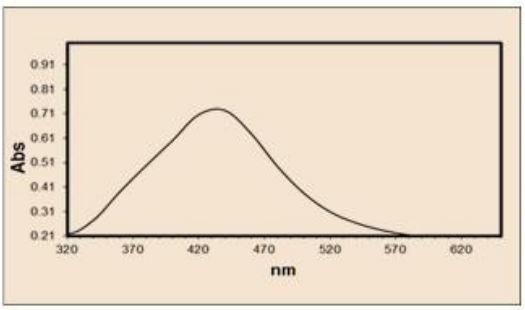

Fig.(2) : Absorbance band of silver Nps

Fig (1):color of silver Np solution

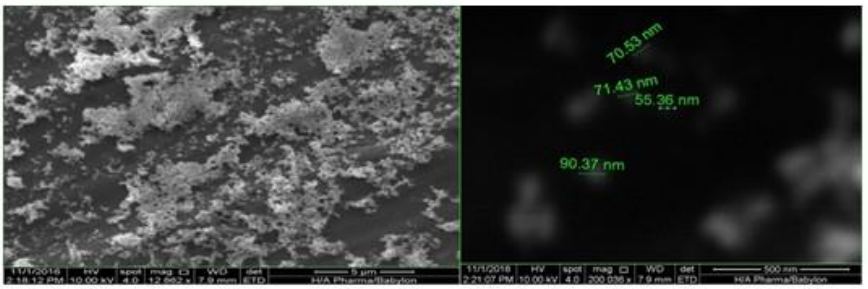

Fig.(3): Scanning Electron Microscope (SEM) of Silver Nps

8- Study effect of Silver Nanoparticles at 0,10,15,20,25, and 30 ul/ml concentrations on growth rate of T.harzianum, A.alternata, R.solani, F.solani, A.terreus, A.parasiticus and F.oxysporum in three times

Potato Dextrose Agar (PDA) Hemidia company was prepared, distributed on six flasks, Autoclaved then concentrations were added when the media were at 45 C^o . 5 mm of each fungus were inoculated on center of petri dishes . Radial growth was measured (mm) after 24 , 48 , 72 hours. The growth rate was calculated according to the following equation:First Growth Rate (FGR) =(Radial growth at 24 h – 5mm)/ 1 day Second Growth rate (SGR) =(Radial growth at 48 h- Radial growth at 24 h)/1day Third Growth rate (TGR) =(Radial growth at 72 h- Radial growth at 48 h)/1day

9-statistical analysis

All experiments were analyzed according to Factorial experiments with Completely Ran- domized Design (CRD) by(GenStat Discovery Edition 3) program, under Least Signifi- cant Difference (L.S.D.)

10-Results and discussion

11-Pathogenicity test

The pathogenicity test showed that only *T.harzianum* has positive effect while other fungi has a negative effect to inhibit seed germination and seedling growth.

12-Silver Nano particles results

Silver Np was prepared by using chemical reduction method by adding Tri Sodium citrate to an aqueous solution of $AgNo_3$,the colorless solution change to pale- yellow color fig
(1) ,this indicates the formation of nano particles. The formation and characterization of silver Np was determine by using UV-Vis absorption spectrometer with a wave length band about 300-600 nm ,the band of surface Plasmon resonance (SPR) determined the morphology of the nanoparticles ,SPR bands are influenced by the morphology, size, and shape of the nanoparticles ,many studies have shown that the spherical silver Np con- tributes to the absorption band around 400 nm (Stamplecoskis , 2010) ,absorption band in this study was around (430) as shown in fig (2) which strongly suggests that silver Np were spherical in the shape.

13-Growth Rate of *T.harzianum*, *A.alternata*, *R.solani*, *F.solani*, *A.terreus*, *A.parasiticus* and *F.oxysporum*at (0,10,15,20,25,30) *µl*/ml concentrate of Ag Nps

The results showed (table 1) Significant differences to decrease of growth rate it was (4.190 , 4.904 , 5.476 , 6.333 , 7.190) mm/day at (30,25,20,15,10) *µl* /ml conc.respectively compared to 7.952 mm/day of control, We observed also a difference in the growth rate of studying fungi It was found that *R.solani* grew faster than other fungi it was (13.055) mm/day followed by *T.harzianum* which was (10.388)mm/day, Compared to the lowest growth rate of *A.terreus* it was (0.444)mm/day.

Table(1) First Growth rate mm/day of *T.harzianum*, *A.alternata*, *R.solani*, *F.solani*, *A.terreus*, *A.parasiticus* and *F.oxysporum* at 0,10,15,20,25 and 30 *µl* /ml concentrates.

Fungi \ Conc. *µl*/ml	Growth rate mm/day						Fungi mean T1
	0	10	15	20	25	30	
T.harzianum	16.333	12.666	12.333	7.333	7.333	6.333	10.388
A.alternata	6.666	6.666	3.333	3.333	3.333	1.666	4.166
R.solani	15.333	13.666	13.333	13.333	11.333	11.333	13.055
F.solani	4.666	4.666	4.333	4.3333	3.333	1.333	3.777
A.terreus	0.666	0.666	0.333	0.333	0.333	0.333	0.444
A.parasiticus	6.666	6.666	5.333	5.333	5.333	5.333	5.777
F.oxysporum	5.333	5.333	5.333	4.333	3.333	3	4.444
conc. mean T2	7.952	7.190476	6.333333	5.47619	4.904762	4.190	
L.S.D T1=0.508 T2=0.470 T1*T2=1.244							

Another results showed lowest growth rate of (9.571, 12.240, 12.810, 14.857,) mm/day at (30, 25,20,15,10) *µl* /ml conc. respectively compared to 19.381 mm/day of 0 *µl* /ml , It was found that *T.harzianum* grew faster than other fungi it was 34.722 mm/day followed by *R.solani* which was 30.222 mm/day Compared to the lowest growth rate of *A.terreus* it was 3.388 mm/day (table 2) . It may be *T.harzianum.*

Table(2) Second Growth rate mm/day of *T.harzianum*, *A.alternata*, *R.solani*, *F.solani*, *A.terreus*, *A.parasiticus* and *F.oxysporum* at 0,10,15,20,25 and 30 *µl* /ml.

Fungi \ Conc. *µl* /ml	Growth rate mm/day						Fungi mean T1
	0	10	15	20	25	30	
T.harzianum	52	47.333	40.333	28.67	27.33	12.67	34.722
A.alternata	12.667	11.333	9.3333	8	7	7	9.2222
R.solani	39.667	39.667	26.333	26	26	23.67	30.222
F.solani	9	8.3333	8.6667	8	8	8	8.3333
A.terreus	5	3.6667	3.6667	3.667	2.667	1.667	3.3889
A.parasiticus	9.6667	8.6667	8	8	7.333	7.333	8.1667
F.oxysporum	7.6667	7.6667	7.6667	7.333	7.333	6.667	7.3889
conc. mean T2	19.381	18.095	14.857	12.81	12.24	9.571	
L.S.D T1=0.919 T2=0.851 T1*T2=2.250							

The results showed a significant difference to add levels of Ag Nps (table 3) to decrease growth rate it was (12.43 , 13.81 , 14.100 , 16.048) mm/day at (30,25,20,15) *µl* /ml conc. respectively compared to 19.524 mm/day of control, it's also a difference in the growth rate of *T.harzianum* It was (32.333) mm/ day, which grow faster than other fungi

followed by *R.solani* which was (29.833) mm/day, Compared to the lowest growth rate of *A.terreus* it was (7.166)mm/day.

Table(3) Third Growth rate mm/day of *T.harzianum*, *A.alternata*, *R.solani*, *F.solani*, *A.terreus*, *A.parasiticus* and *F.oxysporum* at 0,10,15,20,25 and 30 μl /ml.

Fungi \ Conc. µl/ml	Growth rate mm/day						Fungi mean T1
	0	100	150	200	250	300	
T.harzianum	46.667	43	35	23.67	23	22.67	32.333
A.alternata	13.667	13.333	9.333	9.333	9.333	6.667	10.278
R.solani	33	30	30	29.67	29.67	26.67	29.833
F.solani	10	9.6667	8	8	8	7.667	8.5556
A.terreus	8.6667	7.6667	7.333	6.667	6.333	6.333	7.1667
A.parasiticus	13	11.333	11.333	10.33	10	8	10.667
F.oxysporum	11.667	11.667	11.333	11	10.33	9	10.833
conc. Mean T2	19.524	18.095	16.048	14.1	13.81	12.43	
L.S.D T1=1.991 T2=1.844 T1*T2=4.878							

On other hand, the result showed that *T.harzianum* was faster growth rate at FGR, SGR and TGR it was (10.388, 34.722 and 32.333) mm/day respectively flowed by *R.solani* which was (13.055, 30.222 and 29.833) mm/day respectively compared to *A.alternata*, *F.solani*, *F.oxysporum*, *A.terreus*, *A.parasiticus* Figures (4,5,6 and 7). It was Found The SGR faster than other growth rate of *T.harzianum* and *R.solani* as a result of adapter status on SGR which make it able to grow faster because of genetic characters to demesne of enzymes variety capacitate it to analysis and uptake the nutrients compared to FGR. Decreases of TGR has been observed due to lack of culture media and / or accumulation of metabolites.

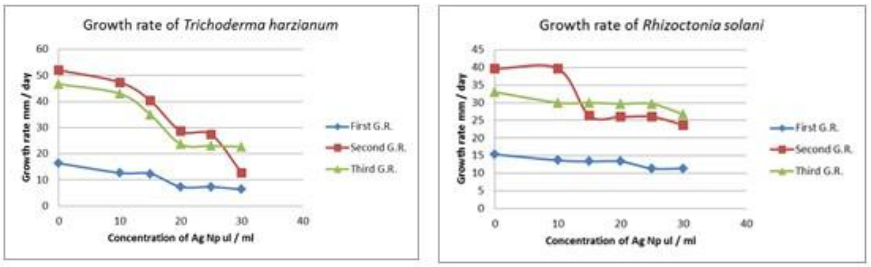

Figure (4) First, second and third Growth rate of *T.harzianum* (left) and *R.solani* (right)

The results, as in figure (5) refer to different growth rate between *F.solani* it was (3.777, 8.333 and 8.555) mm/day respectively and *F.oxysporum* (4.444, 7.388 and 10.833) mm/day respectively it may be as classified key to differentiate between them due to similarity of morphological shape.

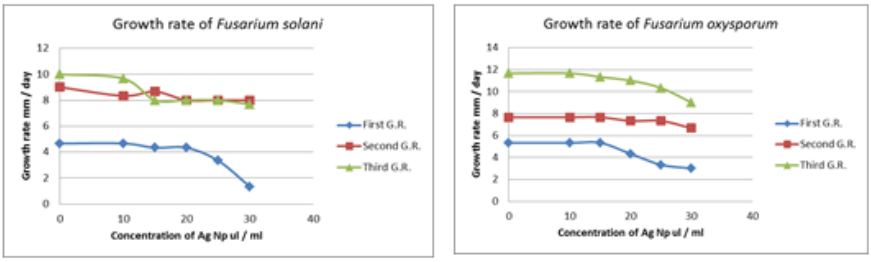

Figure (5) First, second and third Growth rate of *F.solani* (left) and *F.oxysporum* (right)

There were wide range between FGR, SGR and TGR of *A.terreus* (0.444, 3.388 and 7.166) mm/day respectively, might have been to enzymes activity for nutrient utilization *T.harzianum* and *R.solani* can grew faster than other fungi as a genetic reasons of ability to degradation of culture media by many enzymes and grow faster than others , while *A.terreus* lowest grow which couldn't release lot of enzymes .

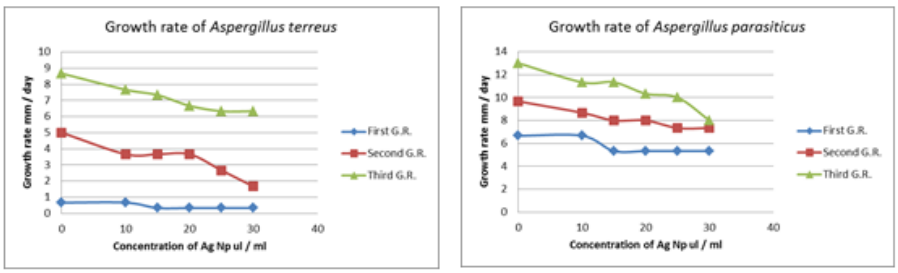

Figure (6) First, second and third Growth rate of *A.terreus* (left) and *A.parasiticus* (right)

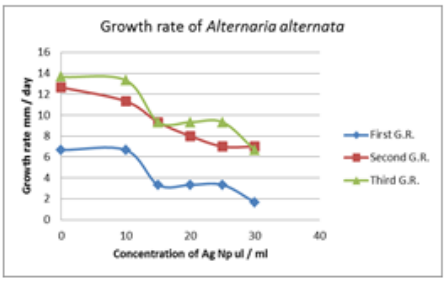

Figure (7) First, second and third Growth rate of *A.alternata*

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