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Efficacy of nanoparticles on seed borne fungi and their pathological potential of cucumber

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Abstract: Several seed borne fungi of cucumber are including pathogenic isolates of *Fusarium oxysporum* , *Tricoderma* spp., *Alternaria alternata* and *Aspergillus niger* were causing seed rot, pre and post emergency damping off of cucumber seedlings. Six nanoparticles of silver and copper were tested at different concentrations *i.e.*, 0,1,2,5, 10, 15 and 20 ppm on mycelial growth of highly pathogenic fungal isolates . Silver nanoparticles NRC4 and NRC3 was completely suppressive mycelia growth 100 % of *A. alternata* and *F. oxysporum* at 10 and 15 ppm respectively ,as well suppressive *Tricoderma* sp. 90 % and 85% at 20 ppm respectively . All nanoparticles tested not effect at different concentrations on mycelial growth of *A. niger*. Soaking cucumber seeds Cv. Beta alfa on silver nanoparticles NRC4 at 20 ppm for different periods *i.e*. 5,10,15,30 and 60 minutes were reduced fungal genera associated with cucumber seeds compare on the untreated seeds. Increasing nano silver concentrations were increasing reduction on fungal of cucumber seeds. Soaking cucumber seeds for 15 mintues ,compeletly suppress fungal genera of *A.flavus*, *Fusarium* spp. and *Tricoderma* spp., No any treatment compeletly inhibition *Alternaria* spp. ,but high reduction of *Alternaria* spp. fungi was recorded at soaking time for 60 minutes . Application of soaking cucumber seeds on silver nanoparticles NRC4 at 20 ppm for 60 minutes before sowing in potted soil artificially infested by each pathogenic fungi were significantly reduced seed rot, pre and post emergency damping-off of cucumber seedlings compare the untreated treatments. Silver nanoparticles NRC4 promising as alternative fungicides for controlling seed borne fungi of cucumber seeds in nurseries for production plantlets free fungal infection.
Key words:cucumber, silver nanoparticles ,seed borne fungi.

Introduction

Cucumber (*Cucumis sativus* L.) is an important vegetable crop worldwide cultivation in greenhouse and field conditions. Seed-borne pathogenic fungi of cucumber *i.e*., *Fusarium* pp. , *Alternaria* sp. and *Macrophomina phaseolina* were causing a serious problem worldwide of cucumber production[1,2,3,4,5].Seed-borne fungi mainly management by synthetic fungicides which causing pollution of soil and water [6]. Little information on fungicides alternatives for controlling damping- off on cucumber such as, biocontrol agents[7], plant extracts and essential oils[4].Nanoparticles has been considered an alternative and effective approach which is eco- friendly, low cost and effective for control plant diseases as well enhance quality and quantity of plant production [8]. Nano silver particles are synthesis by several microorganism [9,10,11].Silver nanoparticles was the best nanoparticles used against white rot disease of green onion caused by *Sclerotium cepivorum*[12] and wilt

disease of oak caused by *Raffaelea uercivorus*[13]. The objectives of this study aimed to study the effects of nanoparticles treatments on seed borne fungi of cucumber and their pathological potential on cucumber.

Materials and Method

Isolation and identification of fungi of cucumber seeds .

Isolation was carried out from cucumber seed of several Cvs. *i.e*.,Jeunco, kespayin, Beta alfa, nechan, Improtor and elama.Seed samples of each cultivars were washed with water, sterilized with 5% sodium hypochlorite for 2 min. then raised in sterilized several times and plated on 2% water agar medium at 28±1 C. Colonization of fungi genera were calculated as %. The growing fungus was purified by the hyphal tip and single spore culture techniques on potato dextrose agar medium (PDA) purified fungus was identified according to [14,15].

Pathogenicity of fungal isolates

Pathological potential of twenty isolates of different fungal genera were tested in plastic pot (5 Cm - diameter) containing sterilized peatmose were infestated by 10 ml (1x 10 8 ml) of each fungal isolates then sowing cucumber seeds (Cv.) Beta alfa after surface sterilization. Four seeds were sowing of each pot and ten pots were used as replicates for each treatment. Percentage of seed rot , pre and post emergency damping off were calculated, 10 days after sowing according to [5].

Nanoparticles

Four silver nanoparticles were prepared using seed borne isolate of cucumber *i.e.,A. niger*, two isolates of *T.harzianum* and one isolate of *Ganoderma* sp. isolated of date palm trunk. In addation two silver and copper nanoparticles were obtained from Prof. Dr.Sabry Younis Mahmoud,Microbiology Department, Faculty of Agriculture, Sohag University, Egypt.

Biosynthesis of silver nanoparticles

Four isolates of fungi as mentioned before were used for preparation silver nanoparticles. Each fungal isolates were cultured on potato dextrose broth (PT). Each culture was incubated at 35 C° on an orbital shaker 180 rpm for 3 days [16]. Cultures were filtered and the resulted biomass was washed extensively by deionized water to get rid of adhered media parts. Mycelial extract was prepared by suspension of each fungal biomass in 100 ml deionized water and incubated as described above for 72 h, after that mycelia suspension was filtered using (Whatman paper No. 1). Filtrate of each isolate was mixed with $AgNO_3$ solution (1 mM $AgNO_3$ final concentration) and incubated on orbital shaker 180 rpm at 35 C° for two days.

TEM measurements

The morphology and size of AgNPs were determined using TEM by Transferring aliquot of each aqueous suspension of AgNPs onto a carbon coated copper grid and allowed to be air dried [17]. The grids were then scanned employing a Phillips EM 208S transmission microscope (Philips, Inc, USA) adjusted at 100 kV., at electron microscope unit,National Research Centre,Egypt.

Effect of nanoparticles on pathogens mycelia growth

Different concentration of silver and copper nanoparticles *i.e*., 0, 1, 2,5,10 ,15 and 20 ppm were tested against isolates of *F. oxysporum, A. alternata, Aspergillus niger* and *Tricoderma* sp. on potato dextrose agar medium. Plates were inoculated by 4 mm disk of each fungal growth 7-days old. Plates were incubated at 27±2 C for 5 days. Ten plates were used as replicates for each treatment and ten plates were served as a control. Linear growth of each plate was measured daily and the average diameters were calculated and percentage of fungal reduction was calculated according formula adopted by [18] as follows:

Reduction %= (A-B/A) x 100.
A=diameter of the control hyphal growth
B=diameter of the treated hyphal growth.

Effect of silver nanoparticles treatments on seed borne fungi of cucumber

In vitro, cucumber seeds Cv. Beta Alfa were soaked for 5,10,15,30 and 60 minutes of silver nanoparticles NRC4 suspension 20 ppm before sowing .Ten plates were used as replicates for each treatment. Five seeds were used for each plate. Ten plates free seed treatment were served as a control. Plates were incubated under 12/12 in light/ dark at 28C for 5 days .Colonization of fungi genera were calculated of each treatments and percentage of cucumber seed rot was estimated [4].

Effect of silvar nanoparticles treatments on damping off incidence of cucumber

Cucumber seeds Cv. Beta Alfa treated by soaking in silver nanoparticles NRC4 at 20ppm for 60 minutes before sowing in soil on pots (10- cm in diameter) artifical infested by each fungi *i.e.F. oxysporum*, *A. alternata*, *Aspergillus niger* and *Tricoderma* sp. as mentioned before in pathogenicity test .Five seeds were sowin in each pot and five pots were used as replicates .Percentage of seed rot ,pre and post emergency damping off were calculated, 13 days after sowing according to [5].

7- Statistical analysis

Data obtained were subject to analysis of variance according to procedures outlined by [19].

Results

Fungal genera associated with seeds of cucumber

Seed samples of several cucumber cultivars jeunco, kespayin, Beta alfa, nechan, Improtor and elama were used for study seed borne fungal flora associated and their pathological potential of cucumber seed rot disease. Data in Table (1) indicated that several fungal genera *i.e*., *Aspergillus* spp., *A.alternata*, *F.oxysporum* ,*Tricoderma* spp. and *Penicillium* spp. were common seed borne fungi causing losses of due to seed rot disease as shown in Fig (1). *Aspergillus niger* was the most fungi associated with cucumber seeds of beta alfa Cv. followed by Kespin Cv. *A. flavus* of Jeunco followed by Importer Cvs. , *A. alternata* of elama followed by nechan and kespayin Cvs. , *F. oxysporum* of Beta alfa and Improtor Cvs. and *Tricoderma* spp . of kespayin followed by Improtor and Beta Alfa cultivars. High percentage of seed rot was observed of Importer followed by beta alfa and nechan cultivars. On the other hand, less seed rot was recorded of jeunco and kespayin cultivars.

Table 1-Fungal flora associated of cucumber seed

Cvs	Seed rot %	Fungal genera colonization %				
		A.niger	*A. flavus*	*A. alternata*	*F. oxysporum*	*Tricoderma* spp.
Jeunco	2.0 f	00.0 f	16.2 a	1.2 d	0.0 c	1.2 f
Kespayin	10.0 e	40.4 b	0.8 e	3.5 c	0.0 c	8.9 a
Beta Alfa	23.7 b	48.1 a	4.6df	1.9 e	2.6 a	6.9 c
Nechan	20.0 c	20.0 c	5.0 d	4.0 b	0.0 c	4.0 e
Improtor	33.3 a	8.9 e	9.3 b	4.2 b	0.8 b	7.9 b
Elama	17.0 d	13.9 d	6.0 c	6.0 a	0.0 c	4.0 a

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Fig 1 - Different fungal genera associated of cucumber seeds, *Aspergillus* spp,.*F. oxysporum* and *A.alternata*

Pathogenicity test of fungal isolates on cucumber

Data in Table (2) indicated that all fungal isolates tested under artificial infestation were varied for causing seed rot, pre and post emergency damping off of cucumber ,*Aspergillus niger* isolate No (8) and *Tricoderma* sp. No (15) were the most pathogenic fungal isolates causing seed rot pre and post emergence loss of cucumber plants followed by *F. oxysporum* No (9 and 11) ,then *A. alternata* isolate No (2).

Table 2- Pathogenicity test of fungal isolates on cucumber

NO	Fungal isolates	Seed rot , pre and post emergencey damping off %
0	Control	0.00 h
1	*Alternaria alternata*	6.25 g
2	*Alternaria alternata*	31.25 d
3	*Alternaria alternata*	25.00 e
4	*Aspergillus niger*	20.00 f
5	*Aspergillus niger*	50.00 b
6	*Aspergillus niger*	62.50 a
9	*Fusarium oxysporum*	43.80 c
10	*Fusarium oxysporum*	18.80 f
11	*Fusarium oxysporum*	43.80 c
13	*Tricoderma* sp.	31.20 d
14	*Tricoderma* sp.	31.30 d
15	*Tricoderma* sp.	62.50 a

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Preparation of silver nanoparticles by fungi

Two particles of nano silver SH1 and copper SH2 were provided by Dr.Sabry Y. Mahmoud, Sohag University, Egypt and used as control preparations. On the other hand, our four silver nanoparticles namely NRC2, NRC3, NRC4 and NRC5 were prepared as following: NRC2 by one isolate of *A. niger*, NRC3 and NRC4 by two isolates of *T. harzianum* and NRC5 by *Ganoderma* sp. as a reducing agent Table (3) depending on color change observation of reaction medium from colorless to brown, green and slight brown as shown in Fig (2).Formation of silver nanoparticles is easily perceptible due to the change of color of the solution while the color of the control run remains unchanged during the course of the reaction TEM observation in Fig (3). All AgNPs have a spherical form, mono dispersed and diameter ranged between 9.6- 20.4 (15.2), 3.9-8.8 (6.4), 11.0-16.1(13.7) and 8.2- 20.2 (14.2) for NRC 2 ,NRC 3,NRC 4 and NRC 5, respectively

Table 3- Types of nanoparticles of silver/ copper used in this study

Nano types	Physical form	Particle size (nm)	Redution source	Solvent
SHU1	Brown	32.0	*Rhizopus stolonifer*	pur water
NRC2	Brown	15.2	*Asperillus niger*	pur water
NRC3	very slight brown	6.4	*T. harzianum*	pur water
NRC4	Green	13.4	*T. harzianum*	pur water
NRC5	slight brown	14.2	*Gandorma* sp.	pur water
SHU6	Brown	18.0	*E. coli*	pur water

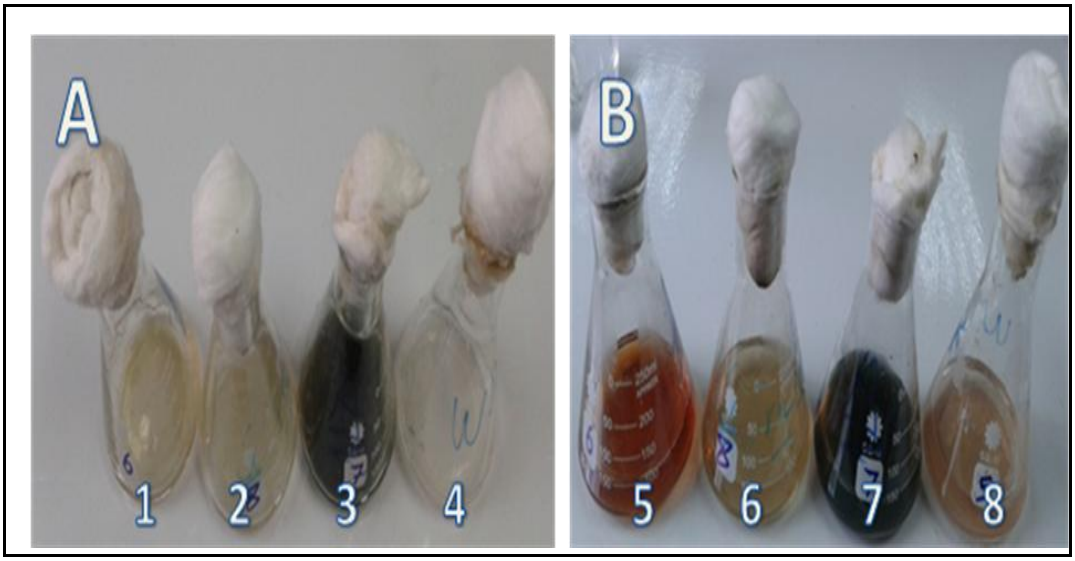

Fig 2- Preparation of silver nanoparticles by fungi, (A) mycelia extracts free of fungal mycelia *i.e*., *A.niger*(1), *T. harzianum* 7, 8 (2&3), and *Ganoderma* sp. (4), respectively (B) mycelial extracts of *A. niger* (5), *T. harzianum* 7, 8 (6 and 7) and (8) after exposure to Ag NO_3 respectively.

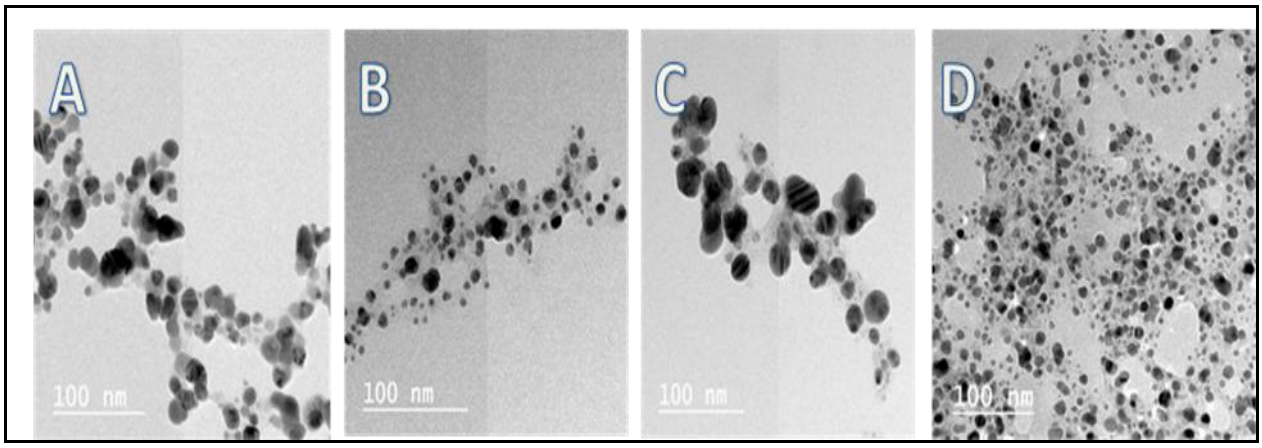

Fig 3 - Transmission electron microscopy (TEM) image exhibiting the biosynthesis of silver nanoparticles using *A. niger* (NRC2) (A), *T. harzianum* 7 (NRC3) (B), *T. harzianum* 8 (NRC4) (C) and *Ganoderma* sp. (NRC5) (D).

Effect of nanoparticles on pathogens mycelia growth

Six silver and copper nanoparticles were tested on mycelial growth of fungi are causing high loss of cucumber seeds during their germination ,seed rot, pre and post emergency damping off. Data in Tables (4,5,6) indicated that all nanoparticles tested were reduced mycelial growth of *F. oxysporum* and *A. alternata* which increased by increasing concentrations. Silver nanoparticles NRC4completely inhibition mycelial growth of *F. oxysporum* and *A. alternata* at 10 and 15 ppm respectively followed by 15 ppm of silver nanoparticles NRC3 Data in Table (6) indicated that 20 ppm of silver nanoparticles NRC4 and NRC3 were reduced mycelial growth of *Tricoderma* sp. by 90% and 85% respectively. On the other hand, copper nanoparticles SHU6 was recorded lowest degree for reducing mycelial growth of fungi tested. Futhermore, all nanoparticles tested had no effect by any degree on mycelial growth of *Aspergillus niger.*

Table 4- Effect of nanoparticles on mycelial growth of *F.oxysporum*

Nanoparticles Types	Reduction of mycelial growth %						
	nanoparticles concentrations (ppm)						
	0	1	2	5	10	15	20
SHU1	00.0	02.0 c	15.0 e	50.0 b	56.0 c	63.0 c	69.0 d
NRC2	00.0	03.0 c	20.0 c	27.0 de	44.0 e	59.0 d	70.0 c
NRC3	00.0	12.0 a	38.0 b	46.0 c	80.0 b	100 .0 a	100.0 a
NRC4	00.0	10.0 b	48.0 a	56.0 a	100.0 a	100.0 a	100.0 a
NRC5	00.0	00.0 c	18.0 d	23.0 e	48.0 d	67.0 b	80.0 b
SHU6	00.0	00.0 c	05.0 f	15.0 f	27.0 f	38.0 e	56.0 e

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Table 5- Effect of nanoparticles on mycelial growth of *A. alternata*

Nanoparticles Types	Reduction of mycelial growth %						
	nanoparticles concentrations (ppm)						
	0	1	2	5	10	15	20
SHU1	00.0	10.0 d	24.0 c	36.0 b	50.0 b	56.0 b	60.0 b
NRC2	00.0	17.0 b	20.0 c	22.0 c	29.0 d	34.0 c	43.0 c
NRC3	00.0	12.0 c	28.0 b	38.0 b	48.0 c	100.0 a	100.0 a
NRC4	00.0	21.0 a	37.0 a	69.0 a	100.0 a	100.0a	100.0 a
NRC5	00.0	00.0 e	00.0 e	00.0 d	03.0 f	30.0 d	40.0 c
SHU6	00.0	00.0 e	00.0 e	02.0 d	14.0 e	20.0 e	34.0 d

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Table 6- Effect of nanoparticle on mycelial growth of *Tricoderma* sp.

Nanoparticles Types	Reduction of mycelial growth %						
	nanoparticles concentrations (ppm)						
	0	1	2	5	10	15	20
SHU1	00.0	00.0	00.0	00.0	00.0	00.0 c	00.0 c
NRC2	00.0	00.0	00.0	00.0	00.0	00.0 c	00.0 c
NRC3	00.0	00.0	00.0	00.0	00.0	70.0 b	85.0 b
NRC4	00.0	00.0	00.0	00.0	00.0	80.0 a	90.0 a
NRC5	00.0	00.0	00.0	00.0	00.0	00.0 c	00.0 c
SHU6	00.0	00.0	00.0	00.0	00.0	00.0 c	00.0 c

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Effect of soaking cucumber seed of silver nanoparticles on seed borne fungi

Data in Table (7) indicated that soaking cucumber seeds of in silver nanoparticles (20 ppm) NRC4 for times *i.e*,5,10,15,30 and 60 minutes before sowing were reduced seed borne fungal genera of cucumber seeds than the control. In general, increasing soaking time of cucumber seeds in silver nanoparticles NRC4 were increasing their effect on seed borne fungi of cucumber. Soaking cucumber seeds for 10 minutes was completely inhibition (100%) of three fungal genera of cucumber seeds *i.e*., *A. flavus*, *Fusarium* spp . and *Tricoderma* spp. Meanwhile,*Alternaria* spp.and *A .niger* were reduced 94% and 17% respectively.

Table 7- Effect of soaking cucumber seed of silver nanoparticles on seed borne fungi

Seed soaking (min) NRC4 (20 ppm)	Seed rot %	Fungi genera of cucumber seeds %				
		A. niger	*A.flavus*	*Alternaria* spp.	*Fusarium* spp.	*Tricoderma* spp.
Control	64.0 a	100.0 a	16.0 a	80.0 a	6.6 a	16.0 a
5	60.0ab	100.0 a	10.0 b	48.0 b	3.2 b	8.0 b
10	56.7 b	100.0 a	0.00 c	43.2bc	0.0 c	0.0 c
15	40.0 c	96.0 b	0.00 c	40.0 c	0.0 c	0.0 c
30	38.0 d	96.0 b	0.00 c	26.0 d	0.0 c	0.0 c
60	33.3 e	83.0 c	0.00 c	6.0 e	0.0 c	0.0 c

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Effect of silver nanoparticles treatments on cucumber diseases incidence

Data in Table (8) indicated that sowing cucumber seeds free nano silver treatment in soil infested with each fungi tested *i.e*., *A. niger, A. alternata, F. oxysporum* and *Tricoderma* sp. were significantly recorded hight percentage of seed rot,pre and post emergency damping off of cucumber compare the control (soil free fungal inoculation) . *F. oxysporum* and *A. niger* were the most pathogenic fungi causing seed rot,pre and post emergency damping off of cucumber followed by *Tricoderma* sp .Meanwhile, *A. alternata* recorded the lowest infection. On the other hand, soaking cucumber seeds in soil infested with each fungi tested 20 ppm of nano silver NRC4 for 60 minutes before sowing significantly reduced seed rot ,pre and post emergency damping off of cucumber. Nano silver treatment was effective against *A.alternata* and *F.oxysporum* then *A. niger*.Meanwhile, the lowest effect was observed with *Tricoderma* sp. for reducing seed rot ,pre and post emergency damping off of cucumber under soil artificial infestation by each pathogenic fungi.

Table 8- Effect of silver nanoparticles treatments on cucumber diseases incidence

Treatments Seed soaking (60 min) NRC4 (20 ppm)		Seed rot , pre and post emergencey damping off %
A. alternate	Non	32.0 g
	Nano	20.0 i
A. niger	Non	48.0 c
	Nano	32.0 f
F.oxysporum	Non	65.0 a
	Nano	28.0 h
Tricoderma sp.	Non	56.0 b
	Nano	44.0 d
Control	Non	36.0 e
	Nano	28.0 h

Values followed by the same letter are not significantly different at *P≥0.05* according to Duncan's multiple test

Discussion

Damping-off as the most series disease of cucumber become the main limiting biotic factor of production cultivation worldwide has reported. In Oman, damping – off causing up to 75% mortality in cucumber seedlings in greenhouse [20,21], in Iran 25% losses of cucumber seedlings under optimal conditions [22].In this study**,**seed-borne pathogenic fungi of cucumber i.e., *F. oxysporum , A. alternata, Aspergillus* spp. and *Tricoderma* spp. are the common fungal genera associated with different cucumber seed cultivars were causing mortality in cucumber seedlings due to seed rot, pre and post emergency damping -off of cucumber seedling production [1,2,3,4,5]. In this study *Aspergillus niger* and *Tricoderma* spp. were firstly recorded as causal pathogens of cucumber .This results are agreement with reported of *A. niger* causing root rot of peanut [23],and [24] reported that isolates of *Tricoderma koningii* and *T.harzianum* were causing necrotic lesions on root mesocotyls associated with maize stunting. Six types of silver and copper nanoparticles were tested against highly pathogenic fungal isolates of cucumber,two silver nanoparticles NRC 3 and NRC 4 were completely inhibited mycelial growth (100%) of *F. oxysporum* and *Alternaria alternata* at 15 and 10 ppm respectively and highly reduced growth of *Tricoderma* sp. by 85 and 90% at 20 ppm. This results are agreement with [12] which found that three types of silver nanoparticles at 50 ppm were completely inhibited mycelial growth on PDA medium of *Sclerotium cepivorum* the causal of white rot disease of onion.Application of silver nanoparticle 20 ppm NRC4 as seed soaking of cucumber seed was suppress fungal seed borne fungi of cucumber and controlling seed rot, pre and post- emergency damping off on cucumber seedlings [12]reported that different nano silver changed populations of bacteria and fungi in onion soil under greenhouse conditions. Application of soaking cucumber seed on silver nanoparticles NRC4 at 20 ppm for 60 minutes before sowing in potted soil artificially infested by each pathogenic fungi were significantly reduced seed rot, pre and post emergency damping-off of cucumber seedlings compare the untreated treatments. This results are agreements with [12] reported that different nano silver at various concentration were inhibited white rot of onion, increased biomass and weight of onion plants in green house conditions, [13] found that application nano silver is effective for control of oak wilt caused by *Raffaelea* sp. in the field. Application of synthesis nano silver at low concentrations not cause any harm to human but control the smoothing metabolism function inside of microbes and well be economic,eco- friendly of agricultural products [25]. So, in this investigation silver nanoparticles NRC4 promising as alternative fungicides for controlling seed borne fungi of cucumber seeds in nurseries for production plantlets free fungal infection.

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