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Effect of Some Nanocomposites on Infection Severity with Basil Root Rot

Agha M.K.M., F. M. Salem and Eslam M. Abdullah

Plant Pathology Unit, Plant Protection Dept., Desert Research Center, Cairo, Egypt

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ABSTRACT

Sweet basil (*Ocimum basilicum* L.) is an economically important herb crop in the world. Soil-borne diseases are still the major threat to basil cultivation in Egypt and all over the world. In this study, the most frequent fungi isolated from basil plants were *Alternaria alternate, Fusarium semitectum, Macrophomina phaseolina,* and *Rhizoctonia solani.* Nano-materials have a great potential in plant protection from fungal infection. The results of artificial pathogenicity test found that, *Rhizoctonia* was the highest infection followed by *Macrophomina.* Chitosan was the most effective nanomaterial in inhibiting radial growth of tested fungi in *vitro,* while CuNPs gave the highly effect in control of *in vivo* artificial infection by *A. alternata, F. semitectum,* and *M. phaseolina* on the basil plants - as pre and post emergence damping off.

Keywords: *Ocimum basilicum*, soil-borne diseases, fungi, Nano-materials

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an economically important herb crop worldwide and in several Mediterranean countries, popularized by demand in the fragrance, flavor, fresh culinary and dried herb industries (Simon *et al.,* 1990; Simon *et al.,* 1999). While *O. basilicum* is the most popular basil grown, it is just one species of *Ocimum* which comprises more than 50 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America (Paton *et al.,* 1999), As with most agricultural commodities, fungal diseases are significant production constraints, affecting both yield and overall quality of basil (Garibaldi *et al.,* 1997). The essential oils obtained from aerial parts are enriched with volatile organic compounds with high market demand for food and pharmaceutical industries. The volatile organic compounds have been shown to exhibit biological activities (Tangpao, *et al.,* 2022), Based on these morphological and molecular data, the isolates were confirmed as *M. phaseolina* the-causal agent of a disease of basil (Koike *et al.,* 2021). Soil-borne diseases are still a major threat to basil cultivation in Egypt (Mona, *et al.,* 2009; Al-Sohaibani *et al.,* 2011). Many soil-borne fungi, including *Rhizoctonia solani, Fusarium solani*, *Fusarium oxysporum* and *Macrophomina phaseolina*, infect basil plants causing damping-off and wilt diseases (Garibaldi *et al.,* 1997; Toussaint *et al.,* 2008; Al-Sohaibani *et al.,* 2011). Charcoal rot, caused by the fungus Macrophomina phaseolina (Tassi) is a root and stems pathogen of many plants (Gupta *et al.,* 2012). *M. phaseolina* is widespread soil-borne pathogen infects a wide host range, great longevity with high competitive saprophytic potency (Su *et al.,* 2001). Commercial basil (*Ocimum basilicum*) in field showed symptoms of a soilborne disease, affected plants were stunted, foliage wilted and dried, and plants eventually died. Internal crown tissue was light to dark brown in color *Macrophomina phaseolina* as one of the causal agents of Charcoal rot disease, fungal isolates had identical, where dark gray colonies were consistently recovered, isolates formed numerous sclerotia that were dark brown to black, irregularly shaped, and 65 to 180 µm long and 45 to 100 µm wide. Based on morphology, the isolates were identified as (Mihail 1992). In the last decade, there have been several reports of an increasing occurrence of *Fusarium* wilt, which causes serious damage to basil production (Moya *et al.,* 2004; Summerell *et al.,* 2006; Toussaint *et al.,* 2008). *Fusarium* wilt of basil, caused by *F. oxysporum f. sp. basilici* (FOB)-, is a destructive disease, represents a major problem and

Corresponding Author: Agha M.K.M., Pathology Unit, Plant Protection Dept., Desert Research Center, Cairo, Egypt.

limits the production on this crop (Gamliel *et al.,* 1996; Chiocchetti *et al.,* 2001). The symptoms associated with FOB include chlorosis, necrosis, wilt of the stems and leaves, crown and root rot, dark lesions and vascular discoloration, all of which often lead to plant death. (Li *et al.,* 2018) reported that, *Fusarium semitectum* causing root rot on greenhouse pepper in China. The plants showed root rot symptoms, with brownish roots and varying degrees of vascular discoloration. Abdel-Wahed, (2019) found that, damping off and root rot of basil were widespread in Beni-Suef and Fayoum governorates during 2016 and 2017. The isolated fungi from naturally infected basil plants were *Fusarium subglutinans, F. oxysporum, F. moniliforme, F. semitectum, F. nival ,F. roseum, Rhizoctonia solani* and *Macrophomina phaseolina .Fusarium moniliforme* and *R. solani* had the highest occurrence percentage in isolation from Beni-Suef and Fayoum governorates. *Rhizoctonia solani* was more virulent than the other fungi in pathogenicity tests. El- Sheshtawi *et al.,* (2016) reported that *Rhizoctonia solani* is the causal agent of basil root rot. Abdel-Wahed, (2018) revealed that, *Rhizoctonia solani, Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina* and *Pythium debaryanum* caused root rot and /or wilt diseases to Verbscum (*Verbascum thapsus* L.) plants in Beni-Suef governorate, Egypt. *Rhizoctonia solani* and *Fusarium solani* were the most frequent fungal species in isolation also they were the most virulent fungi with the highest percentages of root rot and /or wilt in pathogenicity tests under greenhouse conditions.

Nanotechnology has recently become one of the most interesting research field in Biology, Chemistry, Physics, Mathematics, Technology, and Engineering which are integrated to explore benefits of the nano-world towards the betterment of society (Koopmans *et al.,* 2010). Nanomaterial may contribute in plant disease management in two broad ways; pathogen suppression and disease detection (Khan *et al.,* 2019). The dimension of matter important in nanoscience and nanotechnology is typically on the 0.2 nm to 100 nm scale (nanoscale). The properties of materials change as their size approaches the Nanoscale (Eustis S. *et al.,* 2006).

Research in bio-nanotechnology has been shown to provide reliable, eco-friendly processes for the synthesis of noble nanomaterial. Biosynthesis of nanoparticles using fungal species has been targeted by many research projects (Ahmad *et al.,* 2003). Pooja *et al.* (2019) illustrated that, effects of nanoparticles can be either positive or negative it depending on the plant species and type of NPs used and its concentrations. Modern nano-biotechnological tools have a great potential to increase food quality, global food production, plant protection, detection of plant and animal diseases, monitoring of plant growth nano-fertilizers, nano-pesticides, nano-herbicides and nano-fungicides. Many types of nanoparticles have been shown to be effective in inhibiting pathogenic fungi and thus had an effective role in combating plant diseases. Copper (CuNPs) nanoparticles proved to be the highest effective treatment, inhibiting the growth of the tested fungi, and reduced fungal infection under greenhouse conditions. Chitosan were derived from a variety of marine species, insects, and fungi. It described as a biodegradable and biocompatible substance with no toxicity or negative effects. Currently, the use of chitosan in sustainable agricultural initiatives is technologically justifiable because it poses no risk to public health or safety. Chitosan chloride was the first member of a basic ingredient list of plant protection compounds in the fresh produce business (Romanazzi and Feliziani, 2016).

Copper has been used as an antimicrobial agent for over a century and is now being added to commercial fungicides. Nanomaterials have attracted much attention due to the special properties they have over their bulk form. .

Silver (Ag) was used to control bacterial and fungal growth in a variety of applications because Ag ions and Ag-based compounds were highly toxic to microorganisms (Burchette *et al.,* 2003). The reduction of the Ag^{+} ions occur due to reductases released by the fungus into the solution, thus opening a novel fungal/enzyme-based approach of synthesis of nanomaterials (Schuller *et al.,* 2004). The long-term stability of the nanoparticles in the solution may be due to the stability of proteins like cystine (Gole *et al.,* 2001). Khan *et al.* (2019) pointed out that there are many studies confirmed that AgNPs are capable to inhibiting pathogenic fungi viz.; *A. alternate*, *A. brassicicola*, *Botrytis cinerea, Fusarium oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *Lycopersici*, *F. oxysporum*, *F. solani*, *Pythium aphanidermatum*, *Pythium spinosum* and *Stemphylium lycopersici in vitro*.

The current study aims to evaluate the effectiveness of some types of nanoparticles in combating some pathogens in the basil plants, by studying their effectiveness under laboratory and greenhouse conditions to reach the best treatments and concentrations that lead to reducing the rates of infection with root rot fungi in basil.

2. Material and Methods

2.1. Seeds and growth of plants

Sweet basil seeds (*Ocimum basilicum* L.) cultivar Balady (local cultivar) was used in this study. The experiment was carried out in 30 cm Pots in diameter containing 5 kg of loamy sand soil. Soil and pots were sterilized with a 5% formalin solution, formalin solution was added to soil and covered with plastic sheet for 10 days, then the soil was stirred well several times for two weeks to ensure that all traces of formaldehyde disappeared. The pots were watered as required and each pot received about 100 ml of N.P.K. fertilizer suspension (5 g /1L of water) per week.

2.2. Isolation and identification of the causal pathogens

Samples of basil plants showing root rot symptoms were collected from different locations of Beni-Swayf governorate (Al-Fashn, Biba, Nassir, and Sumusta). Stems and roots of diseased plants were thoroughly washed with tap water several times, cut in small pieces and surface sterilized for 2 min in 2% sodium hypochlorite solution, then rinsed several times in sterilized distilled water and dried between sterilized filter papers. The surface-sterilized samples were plated onto potato dextrose agar (PDA) medium and incubated for seven days after incubation at 28 ± 1 °C. The developed fungal colonies were purified by hyphal tip and single spore isolation techniques. The obtained fungal isolates were identified according to their cultural and microscopically characters as described by Nelson *et al.,* (1983). Subcultures of the obtained isolates were kept on PDA slants and stored at 5 °C for further studies.

2.3. Frequency of isolated fungi

To calculate the percentage of fungi presence in each of the isolation areas, 10 plants showing signs of infection are collected along Z-transect in each surveyed field and the affected parts of these plants are divided into small parts 2-5 ml and placed in 25 Petri dishes containing Potato Dextrose Agar (PDA) as 4 plant pieces per plate, the frequency for each fungi present calculated using the following formula:

$$\text{Frequency for isolated fungi} = \frac{\text{Times the fungus appears in isolation dishes}}{\text{Total pieces in all dishes}} \times 100$$

2.4. Pathogenicity test

Artificial infection of basil plants by *Alternaria alternata*, *Fusarium semitectum, Macrophomina phaseolina and Rhizoctonia solani* was carried out on Balady cultivars of basil. Inoculums were prepared on autoclaved barley medium (75 g washed dried barley grains, 100 washed dried coarse sand and 75 mL tap water) in 500 mL glass bottles. Each bottle was inoculated with five discs (0.7 cm in diameter) of seven-day-old cultures of the pathogen, then the bottles were incubated at 28 ± 1 °C for 15 days. For each fungus, the contents of five bottles were thoroughly mixed in a plastic container and used as a source of inoculum. Inocula of each fungus were added to sterile soil in pots (30 cm diameter) at the rate of 3% of soil weight two weeks before planting. Pots containing non-infested soil and barley medium were used as control. Each pot was seeded with five seeds of basil and three replicates were used for each fungus. All treated pots and inoculated plants were under greenhouse conditions. The pots were irrigated periodically, Pre- and post-emergence damping off was determined as percentage (15 and 30 days after planting), the surviving plants at the end of the experiment were calculated according to Mona, *et al.* (2009) as follow:

$$\text{Percentage of pre emergence damping off after 15 days} = \frac{\text{Total No. of ungerminated seeds}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Percentage of post emergence damping off after 30 days} = \frac{\text{Total No. of rotted seedlings}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Percentage of survived seedlings} = \frac{\text{Total No. of survived seedlings}}{\text{Total No. of planted seeds}} \times 100$$

2.5. Preparation and Characterization of Nano materials

Chitosan, Copper, and Silver nanoparticles were prepared and characterized in Nano-Phytopathology Lap., Desert Research Center (Matarya-Cairo) according to methods described by Tang *et al.,* (2007), for chitosan nanoparticles; Shantkriti and Rani (2014) for Copper nanoparticles; and according to Abu-Elsaoud *et al.,* (2015) for Silver nanoparticles.

Optical measurements, by using a UV-Vis spectrophotometer (LW-200 Series) and scanning the spectra between 200 and 800 nm.

Particle size distribution by Dynamic Light Scattering (DLS) (NICOMP N3000, particle size analyzer).

2.6. Transmission Electron Microscopy

Chitosan nanoparticles were provided further analyzed for their morphology and particle size with Transmission Electron Microscopy (TEM).

2.7. Effect of Ag NPs, Ch NPs and Cu NPs on liner growth of fungi on petri dishes

Four concentrations (10 ppm, 25ppm, 50ppm and 100pm) from each of Ag, Ch, and Cu NPs were tested separately to inhabit the liner growth of *A. alternata*, *F. semetectium*, *M. phasiolina* and *R. solani*.

Each of NPs composites was incorporated into sterilized PDA medium after cooling and before solidification to prepare final concentrations of 10, 25, 50 and 100 ppm. The medium was then poured into petri dishes, (with three replicates for each treatment), while untreated PDA medium was used as control. After solidification of the medium, each plate was inoculated centrally with a mycelial disc (5mm diameter) taken from the outer margins of seven days old PDA cultures of each isolate by sterile corkborer, then plates were incubated at 28±20˚C and colony diameters were measured when the untreated control had just covered the plate. Percentage of inhibition was calculated using the following formula according to Perveen and bokhari (2012).

$$\text{Percentage of growth inhibition} = (C - T) / C \times 100$$

Where, C = average of three replicates of hyphal growth (cm) of test fungus in control plate and T = average of three replicates of hyphal growth (cm) of the same test fungus in plates treated with the tested material.

2.8. Effect of Nanoparticles on pre, post and survival plants caused by pathogenic fungi

The fungal inoculums were prepared and added to the soil, as explained previously, for each fungus separately. Basil seeds were treated with nanoparticles at concentrations of 25, 50, and 100ppm for each substance. The treated seeds were planted in the infected pots with fungi, five seeds in each pot and three replications for each treatment, in addition to three pots infected with the fungus as a control.

3. Results

3.1. Survey of basil root rot fungi

Data presented in Table (1) showed that the survey was carried out in Beni Suef governorate in the centers of Al-Fashn - Beba - Nasser – Sumasta, among the eightth isolated fungi *Alternaria alternate*, *Fusarium semitectum, Macrophomina phaseolina and Rhizoctonia solani* were the most frequent rate of isolation and that *Rhizoctonia solani* was the most frequent in all centers, with an average of 38.75, followed by *Macrophomina phaseolina*, then *Fusarium semitectum*, while *Alternaria alternate* was the least frequent,, and Sumasta center was the highest in the proportion of isolated fungi, with an average of 23.13, while Nasser center, was the lowest with an average of 19.37, on the other hand, *Pythium spp* was the least frequent in isolation in all locations.

3.2. Pathogenicity test

The results in Table (2) showed significant differences in the ability of the isolated fungi to cause artificial infection of basil plants compared to the non-infectious control, and Rhizoctonia fungus was the most capable of causing seedling as pre and post emergence damping off (46.67-20.0%). The percentage of surviving plants in case of infection with Rhizoctonia was the lowest (33.3%), followed

by *M. phaseolina*, then *Fusarium semitectum* and *A.alternata,* while *Pythium spp.* the least fungus was able to cause infection.

Table 1: Frequency of isolated fungi from different locations

Fungi \ Area	Al-Fashn	Beba	Nasser	Sumusta
Alternaria alternata	25%	35%	25%	35%
Fusarium oxysporum	20%	20%	15%	15%
Fusarium equiseti	10%	-	5%	-
Fusarium semitectum	30%	30%	30%	35%
Fusarium solani	5%	10%	5%	10%
Macrophomina phaseolina	35%	30%	45%	35%
Pythium spp.	5%	-	-	10%
Rhizoctonia solani	35%	45%	30%	45%

Table 2: Effect of isolated fungi in causing pre, post emergence damping off as well as survival plants.

Control	Pre-emergence damping off	Post- emergence damping off	Survival Plants
	0.0	**0.0**	**100.0**
A.alternata	40.0	13.3	46.7
F.oxysporum	6.7	20.0	73.3
F.equiseti	6.7	6.7	86.7
F. semitectum	33.3	20.0	46.7
F. solani	26.7	6.7	66.7
M. phaseolina	40.0	20.0	40.0
Pythium spp.	6.7	6.7	86.7
R. solani	46.7	20.0	33.3
LSD 1%	19.30	16.24	15.67

3.3. Effect of Ag NPs, Ch NPs and Cu NPs on liner growth of fungi

with studying the ability of three Nano particle types (silver, chitosan and cupper) at four concentrations (10 ppm, 25 ppm,50 ppm and 100 ppm) to inhibit the growth of *A. alternata, Fusarium semitectum,R. solani,* and *M. phaseolina*. The results in table (3) (3a, 3b, 3c and 3d) indicated that there is a significant difference between The ability of the three substances, with their different concentrations, to inhibit radial growth of fungi, chitosan was the most effective (average 46%), followed by silver and copper (average 82%), *F. semitectum* was the most affected by the use of different substances with their concentrations (average 60%), followed by *R. solani* (average 63%), then *M. phaseolina*, and *A. alternata* was the least affected (average 72%). The concentration of (100ppm) (average 43%) for all substances gave the highest effect, while (10ppm) (average 74%) had the lowest effect. The concentration of chitosan 100ppm was the most effective in inhibiting the growth of all fungi compared to the untreated control dishes. *R. solani* was the most affected followed by *A. alternata*, *F. semitectum* and *M. phaseolina* (6-8-15-16) gradually, while *R. solani*was least affected by copper in all its concentration (average 89%).

Table 3a: Effect of interaction between NPs × F on linear growth

NPs	Fungi				Mean
	A. alternata	*F. semitectum*	*M. phaseolina*	*R. solani*	
AgNPs	73	57	80	70	70
ChNPs	64	43	46	30	46
CuNPs	78	82	81	89	82
Mean	72	60	69	63	66
LSD	NPs	F.	NPs X F		
	0.12	0.14	0.25		

Table 3b: Effect of interaction between NPs × Con. on linear growth

NPs	Concentrations					Mean
	Control	**10**	**25**	**50**	**100**	
AgNPs	100	72	68	60	50	70
ChNPs	100	50	46	22	11	46
CuNPs	100	99	74	70	69	82
Mean	100	74	63	51	43	66
LSD	**Conc.**		**NP x Conc.**			
	0.16		0.28			

Table 3C: Effect of interaction between F × Con. on linear growth

Fungus	Concentrations					Mean
	Control	**10**	**25**	**50**	**100**	
A. alternata	100	92	80	48	41	72
F. semitectum	100	65	54	47	36	60
M. phaseolina	100	73	62	58	51	69
R. solani	100	65	55	51	45	63
Mean	100	74	63	51	43	66
LSD	**F x Conc**	**0.32**				

Table 3d: Effect of interaction between NPs × F. × Con. on linear growth

NPs	Fungus	Concentrations				
		Control	**10**	**25**	**50**	**100**
Ag NPs	***A. alternata***	100	75	73	72	48
	F. semitectum	100	58	55	41	31
	M. phaseolina	100	78	78	72	71
	R. solani	100	78	66	57	50
Ch NPs	***A. alternata***	100	100	100	14	8
	F. semitectum	100	39	32	28	15
	M. phaseolina	100	44	39	33	16
	R. solani	100	17	16	13	6
Cu NPs	***A. alternata***	100	100	67	58	66
	F. semitectum	100	98	76	72	64
	M. phaseolina	100	99	71	68	66
	R. solani	100	100	84	83	80
LSD	**NP x F x Conc. 0.55**					

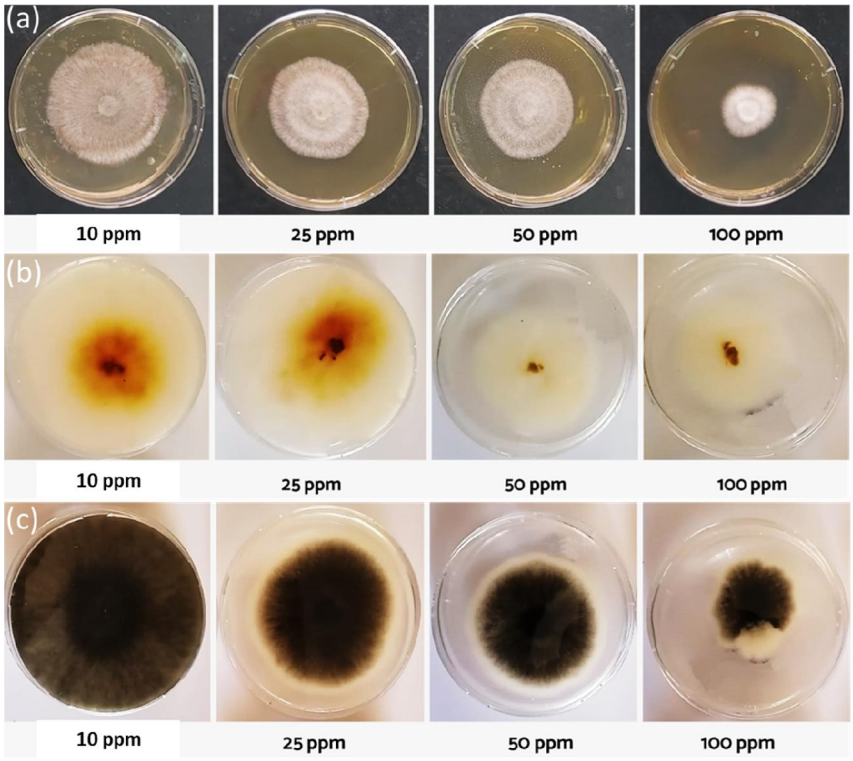

Fig 1: Effect of Chitosan Nano-material in its concentration on inhabiting fungal linear growth: (a *R. solani*), (b *F. semitectum*), and (c *A. alternata*).

3.4. Greenhouse experiment

3.4.1. Effect of Nanoparticles on basil seedlings infected with *A. alternata*

The results in Table (4) showed significant differences between nanomaterials in reducing seedlings pre - post emergence damping off and increasing surviving plants, on otherwise no significant effect of the concentrations in reducing the rate of seedling death compared with the control. Chitosan and copper had the highest effects in reducing the percentage of seedlings pre and post emergence damping off, followed by silver nanoparticles.

Table 4: Effect of Nano materials on pre and post damping-off and survival basil plants infected with *A.alternata*

	Pre-emergence damping off				**Post-emergence damping off**				**Survival plants**			
	25	**50**	**100**	**mean**	**25**	**50**	**100**	**Mean**	**25**	**50**	**100**	**Mean**
AgNPs	0	26. 7	26. 7	17.8	0	20	20	13. 3	60	66.7	66.7	64.4
ChNPs	26.7	6.7	0	11.1	33.3	0	0	11.1	46.7	93.3	100	80
CuNPs	20	13.3	0	11.1	26.7	20	6.7	17.8	53.3	66.7	93.3	71.1
Con. infected	40	40	40	40	13.3	13.3	13.3	13.3	46.7	46.7	46.7	46.7
Con. Uninfected	0	0	0	0	0	0	0	0	100	100	100	100
Mean	17.3	17.3	13.3	16	14.7	10.7	8	11.1	61.3	74.7	81.3	72.4
LSD												
Nano	0.59				0.59				0.62			
Concentrations	n.s.				n.s.				0.48			
Interaction	1.02				1.02				1.07			

On the other hand, the use of nanomaterials concentrations led to significant increase in the percentage of surviving plants compared with control. Chitosan NPs was the most significant in increasing the percentage of surviving plants in relative to control (80% and 46.67%) respectively,

followed by Copper NPs (71.11%) then silver NPs (64.44%), the concentration 100 ppm was the most efficient in raising the percentage of surviving plants, followed by 50 ppm and then 25 ppm, concentration 100 ppm of chitosan NPs was the most effective in increasing percentage of survival plants, followed by copper NPs and then silver NPs (100%, 93.33%, and 66.67%) respectively.

3.4.2. Effect of Nano-materials on basil seedlings infected with *F. semitectum*

The results in Table (5) showed that, the ability of the Fusarium fungus to cause seedlings pre - post emergence damping off was significantly affected by the use of nano materials, while there was no significant effect of the concentrations used. CuNPs was the most efficient in reducing the proportion of both as well as the increase in the percentage of surviving plants (17.78%, 11.11% and 71.11%), respectively, for the control (40%, 26.67%, and 33.3%). The use of copper at a concentration of 100 ppm was the highest efficiency in reducing the infection rate and increasing the percentage of surviving plants.

Table 5: Effect of treated plants by CNP, CU NP, and Ag NP Nano materials on pre, post damping off and survival basil plants infested with *F. semitectum*

	pre-emergence damping off				**post-emergence damping off**				**Survival plants**			
	25	**50**	**100**	**Mean**	**25**	**50**	**100**	**Mean**	**25**	**50**	**100**	**Mean**
AgNPs	33.3	26.7	33.3	31.1	26.7	26.7	20	24.4	40	46.7	46.6	44.4
ChNPs	46.7	33.3	33.3	37.8	20	26.7	13.3	20	33.3	40	53.3	42.2
CuNPs	26.7	20	6.7	17.8	13.3	13.3	6.7	11.1	60	66.7	86.7	71.1
Con. infected	40	40	40	40	26.7	26.7	26.7	26.7	33.3	33.3	33.3	33.3
Con. Uninfected	0	0	0	0	0	0	0	0	100	100	100	100
Mean	29.3	24	22.7	25.3	17.3	18.7	13.3	16.4	53.5	57.3	64	58.2
LSD												
Nano	0.66				0.74				0.56			
Concentrations	n.s.				n.s.				0.43			
Interaction	n.s				n.s.				n.s			

3.4.3. Effect of Nano-materials on basil seedlings infested with *M. phaseolina*

The results in Table (6) showed that, the ability of the *M. phaseolinato* cause the seedlings pre - post emergence damping off was significantly reduced by using Nano-materials at the tested concentrations in comparison with control.

Table 6: Effect of treated plants by CNP, CU NP, and Ag NP Nano materials on pre, postdamping off and survival basil plants infested with *M. phaseolina*

	pre-emergence damping off				**post-emergence damping off**				**Survival plants**			
	25	**50**	**100**	**Mean**	**25**	**50**	**100**	**mean**	**25**	**50**	**100**	**Mean**
AgNPs	26.7	26.7	20	24.4	20	20	6.7	15.6	53.3	53.3	73.3	60
ChNPs	40	40	26.7	35.6	20	13.3	20	17.8	40	46.7	53.3	46.7
CuNPs	20	20	13.3	17.8	20	13.3	6.7	13.3	60	66. 7	80	68.9
Con. infected	40	40	40	40	26.7	26.7	26.7	26.7	33.3	33.3	33.3	33. 3
Con. Uninfect	0	0	0	0	0	0	0	0	100	100	100	100
Mean	25.3	25.3	20	23.6	17.3	14.7	12	14.7	57.3	60	68	61. 8
LSD												
Nano	0.40				0.48				0.54			
Concentrations	0.23				n.s				0.42			
Interaction	n.s.				n.s.				n.s.			

CuNPs gave the highly reduction of pre and post emergence damping off (17.78 % for pre and 13.33% for post), followed by the AgNPs, while ChNPs were the least effective (35.56% for pre and17.78 for post), control also recorded (40.00% for pre and 26.67% for post). The use of NPs at concentrations (25,

50 and 100 ppm) led to a significant increase in the percentage of surviving plants compared to control, and the CuNPs at 100 ppm had the highest effect, followed by AgNPs, then ChNPs at the same concentration (80%, 73.33% and 53.33%) respectively.

3.4.5. Effect of Nano-materials on basil seedlings infested with *R. solani*

The results in Table (7) indicated that, the use of nanoparticles led to a significant decreasing in seedlings pre and post emergence damping off caused by *R. solani*, CuNPs 100 ppm had the highest effect (6.67%) in relation to the control (53.3%) and this was reflected in an increased percentage of surviving plants where reaching (86.67%), AgNPs and ChNPs followed the CuNPs in reduction of seedling pre and post emergence damping off, as well as in induction of plant survival percentage.

Table 7: Effect of treated plants by ChNPs, CuNPs, and AgNPs on pre post damping off and survival basil plants infected with *R. solani*

	Pre-emergence damping off				**Post- emergence damping off**				**Survival plants**			
	25	**50**	**100**	**mean**	**25**	**50**	**100**	**Mean**	**25**	**50**	**100**	**Mean**
AgNPs	33.3	33.3	20	28.9	6.7	0	6.7	4.4	60	66.7	73.3	66.7
ChNPs	40	40	33.3	37.8	13.3	13.3	13.3	13.3	46.7	46.7	53.3	48.9
CuNPs	26.7	20	6.7	11.7	6.7	0	6.7	4.44	66.7	80	86.7	77.8
Con. infected	53.3	53.3	53.3	53.3	20	20	20	20	26.7	26.7	26.7	26.7
Con. Uninfected	0	0	0	0	0	0	0	0	100	100	100	100
Mean	30.7	29.3	22.7	27.6	9.3	6.7	9.3	8.4	60	64	68	64
LSD												
Nano	0.59				0.51				0.61			
Concentrations	0.34				n.s.				n.s.			
Interaction	n.s.				n.s.				n.s			

5. Discussion

Basil is one of the most important medicinal and aromatic plants due to its many uses, Where it is used in the food fields in many forms, either as fresh leaves or using the oil extracted from it is also used in the medical and cosmetic fields. However, in its cultivation areas, basil is affected by many diseases, the most important of which is a group of fungi that cause root rot and wilt., whose problem is exacerbated and gradually increasing, due to, the repetition of basil cultivation in the same place consecutive times, that's lead to increase of pathogens percentage and accumulation in soil, also the danger of root rot is due to the multiplicity of its causes, which increases the difficulty to resist.

The conducted survey in Beni-Suef Governorate revealed a diversity of isolated fungi and difference in their prevalence rates from one location to other. where *A. alternate - F. semitectum, M. phaseolina* and *R. solani* were the most frequent isolates in all centers, and the fungi *R. solani* was the most frequent and Repeated in isolatation through all centers and these results were consistent with (Mona *et al.,* 2009; Al-Sohaibani *et al.,* 2011, El- Sheshtawi *et al.,* 2016, Abdel-Wahed 2018 and 2019). Pathogenicity test on basil plants with isolated fungi showed that, all fungi had the ability to cause pre and post emergence damping off and therefore affect the percentage of surviving plants. *R. solani* was the most capable of causing the highest rate of seedling death (46.7% pre and 20.0% post) then *F. semitectum* and *A. alternate*, and *Pythium* spp. was the lowest capable of causing seedling death (6.7%pre and 6.7% post).

To overcome the problems that result from management seedling death and root rot by using fungicide, such as soil contamination and the emergence of pathogens strains resistant to fungicides, it was necessary to find safe, economical and effective alternatives that contribute to reducing the risk of disease and environmental hazards. Among these alternatives is the use of some compounds in the form of nano-particles that are bio-synthesized to resemble what is done in nature. In this study, chitosan, copper, and silver nanoparticles were used at concentrations of 25,50 and100 ppm to test their efficacy in reducing the rate of seedling death caused by *A. alternate – F. semitectum*, *M. phaseolina* and *R. solani* and raising the percentage of surviving plants.

AgNPs have extremely large relative surface areas which increases their contact with fungi, vastly improving its fungicidal effectiveness. The larger surface area-to-volume ratio of AgNPsincreased their contact with microbes and their ability to permeate cells in contact with fungus, they will adversely affect

cellular metabolism and inhibit cell growth. AgNPs suppresses respiration, basal metabolism of electron transfer systems, and transport of substrates in the microbial cell membrane. AgNPs are highly reactive because they generate Ag^+ ions, while metallic silver is relatively unreactive Nanoparticles penetrate into fungal cells, which imply lower concentrations of nano-sized silvers are sufficient for microbial control (Nel *et al.,* 2003, Morones, *et al.,* 2005, Samuel *et al.,* 2004).

Chitosan nanoparticles (ChNPs) improved plant's innate immune response causing increased activity of the defense enzymes, peroxidase (PO) polyphenol oxidase (PPO) phenylalanine ammonia lyase (PAL), β-1, 3-glucanase, and the activity of antioxidant enzyme catalase (CAT) (Chandra *et al.,* 2015).The positive charge of chitosan interacts with negatively charged phospholipid components of fungi membrane, which in turn alter cell permeability of plasma membrane and causes the leakage of cellular contents, which consequently leads to death of the cell (Palma-Guerrero *et al.,* 2010). Chitosan chelates with metal ions, which has been implicated as a possible mode of antimicrobial action on binding to trace elements, it interrupts normal growth of fungi by making the essential nutrients unavailable for its development. ChNPs could penetrate fungal cell wall and bind to its DNA then inhibit the synthesis of mRNA therefore affect the production of essential proteins and enzymes (Henics and Wheatley 1999; Ren *et al.,* 2012).

Cu nanoparticles (Cu NPs) can penetrate the cell directly through the pores present in the cell membrane due to their small size, or they enter through ion channels and transporter proteins present in the plasma membrane. Nanoparticles that are introduced into the cell can have direct contact with oxidative organelles such as mitochondria. Furthermore, redox-active proteins can stimulate reactive oxygen species (ROS) production in cells, (Chang *et al.,* 2012).

In this present study ChNPs was the most effective nanomaterial as an inhibitor of the radial growth of the fungi *in vitro* (46%), followed by AgNPs and CuNPs. 100 ppm concentration for all nanomaterials was the most effective in decreasing fungi growth and this results were in line with Kanawi, (2021) who found that ChNPs have shown varying efficacy in inhibition of pathogenic fungi and the highest Concentration of ChNPs gave the highest inhibition percentages with all tested fungi.

Basil plants infected *by A. alternata* under greenhouse conditions, ChNPs were the most significant in increasing the percentage of surviving plants in relative to control (80% and 46.67%) respectively, followed by CuNPs (71.11%) then AgNPs (64.44%), and the concentration 100 ppm was the most efficient in raising the percentage of surviving plants.

Basil plants infected *by F. semitectum in vitro,* the use of CuNPs at a concentration of 100 ppm were the highest efficiency in reducing the infection rate and increasing the percentage of surviving plants.

Basil plants infected *by M. phaseolina in vitro,* CuNPs gave the highly effect in reducing pre and post emergence damping off (17.78 % for pre and 13.33% for post), followed by the AgNPs, and the ChNPs compound were the least effective (35.56% for preand 17.78 for post).

Decreasing in seedlings pre and post emergence damping off caused by the fungus *R. solani*, CuNPs at 100 ppm had the highest effect (6.67%) followed by AgNPs and ChNPs.

These results were in the line with Abdel-Wahed (2018) who found Cu-NPs proved to be the highest effective treatment against tested fungi. In the applied field experiments, all treatments induced a highly significant protection.

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