International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.06 pp 174-183, 2016

In Vitro Evaluation of Nickel Nanoparticles against Various Pathogenic *Fusarium* Species

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Abstract : The objective of this study was to evaluate antifungal activity of nickel nanoparticles against *Fusarium* species as an alternative to existing methods. In this study, nickel nanoparticles at concentrations of 10, 20, 50 and 100 ppm, were evaluated for their antifungal activity on 42 isolates of *Fusarium* belonging to different species isolated from crop field soils of different locations in Korea. The fungal isolates were grown on three different media, potato dextrose agar, corn meal agar and malt extract agar, amended with nickel nanoparticles. The results indicate that nickel nanoparticles at concentrations of 50 and 100 ppm inhibited the mycelial growth of *Fusarium* isolates investigated. Nickel nanoparticles at a concentration of 100 ppm caused more than 90% inhibition of mycelial growth of some isolates on malt extract agar media. The range of growth inhibition was 24.7-90.2% and 21.67-85.1% at a concentration of 100 ppm on corn meal agar and potato dextrose media, respectively. The light and scanning electronic microscope examinations revealed that the nickel nanoparticles caused damage of mycelia and spores of tested *Fusarium* species. This study suggests that nickel nanoparticles at high concentration could be used to control *Fusarium* fungi. However, further studies are needed to assess the effect of nickel nanoparticles on the growth of host plant.
Keywords: Antifungal activity, *Fusarium*spp., Nickel nanoparticles.

Introduction

Many approaches are available for the control of fungal plant pathogens but each of them has certain limitations. Generally, fungal plant pathogens are controlled with the applications of chemical fungicides and have been found quite effective for some fungal pathogens, but they cause risks and negative effects on human health and environment by leaving several non-specific effects[1]. Therefore, it has become necessary to adopt alternative strategies to combat plant diseases with reduced dependence on chemicals[2].The genus *Fusarium*, causative agent of various plant diseases, is the most important group of fungal plant pathogens[3,4]. *Fusarium* species are the best known soil borne plant pathogens in terms of loss in agricultural productions all over the world[5,6].

Nickel (Ni) is a component of the enzyme urease, which metabolizes urea nitrogen into useable ammonia within the plant[7,8]. Several researches have reported growth responses of plants to Ni fertilization and indicated that Ni deficiency has a wide range of effects on plant growth and metabolism[9]. The fungicidal effects of Ni compounds were apparent by 1908 and nickel salts have exhibited fungicidal activity against plant pathogens[10,11], and the absorption and movement of nickel in plants has been reported[12].

The discovery and development of fungicides are among the most powerful and successful achievements of modern science and technology for the control of fungal plant pathogens. However, resistance to commercially available fungicides by phytopathogenic fungi has been increasing and has become a serious problem[13]. So, it is of great importance to search for new alternatives to combat newly emerging resistant strains of fungal pathogens. In recent years, nanotechnology has been considered as an alternative solution to control plant pathogens, which enhances antimicrobial activity of materials by converting them into nanoparticles[14,15]. Some metal nanoparticles have been studied and proved for their antifungal properties[16,17,18,19]. However, few studies are available on the effects of nickel nanoparticles on phytopathogenic fungi. Thus, the current study was carried out to evaluate the antifungal activities of nickel nanoparticles on various *Fusarium* isolates belonging to different species under *in vitro* conditions.

Materials and Methods

Nickel nanoparticles

Tested nickel nanoparticles were procured from Cheorwon Plasma Research Institute, Cheorwon-gun, Gangwon-do, Korea, which were produced by RF-thermal plasma system using Ni precursors (Sigma-Aldrich Co., USA). The Ni precursors were vaporized under the condition of very high temperature (10,000 K) by plasma treatment and were crystallized to nano-sized Ni particles by quenching steps. The nanoparticles used in this study were <100 nm in size.

Fungal isolates

To assess inhibitory effects of nickel nanoparticles to *Fusarium* isolates under *in vitro* conditions, the fungal isolates were obtained from Plant Microbiology and Biotechnology Lab (PMBL), Division of Bioresource Sciences, Kangwon National University, Chuncheon, Korea. Sixty eight *Fusarium* isolates were screened to verify their validity and ability to grow into media, while the other 26 isolates did not have the validity to grow well also some of them grew slowly in subcultures on media without nanoparticles (Data not shown) the 42 isolates which showed the significant growth in controls were selected for further investigations. the list of *Fusaium* isolates used in this study is given in Table 1.

Growth and bioassay media

The selected *Fusarium* isolates were grown on potato dextrose agar (PDA) medium for further experiments. Three different types of solid artificial media, potato dextro agar (PDA), corn meal agar (CMA), and malt extract agar (MEA) were used to study the inhibition effect of nickel nanoparticles against selected *Fusarium* isolates. Nickel nanoparticles were prepared as a suspension for four concentrations, 10, 20, 50 and 100 ppm, by adding (0.01 g/L; 0.02 g/L; 0.05 g/L and 0.1 g/L) of nickel nanoparticles, respectively.

Antifungal activity test

The sources of fungal isolates were cultured on PDA. Three different types of growth media, PDA, CMA and MEA, were used for *in vitro* assay and treated with different concentrations, 10, 20, 50 and 100 ppm, of nickel nanoparticles. Media without nanomaterial was used as a control. Media containing nickel nanoparticles were incubated at room temperature to solidify. After 48 h of incubation, mycelial plugs of uniform size (5 mm diameter) were cut from the edge of 7 days old *Fusarium* isolates grown on PDA, and were placed at the center of each petri dish containing nickel nanoparticles, then incubated at 28^{o}C for 14 days. For each isolate, plates in triplicate were arranged in completely randomized design.

Microscopic examination of fungal isolates

Scanning Electron Microscope (SEM) was used to examine morphological changes in hyphae and spores of the tested fungal pathogens with and without treatment of nickel nanoparticles. Pieces of fungal mycelium were cut from 7 days old cultures, inoculated on the PDA containing different concentrations of nickel nanoparticles, then incubated for 14 days and compared with the control. Pieces of mycelium were cut from the edge of the 14 days old fungal cultures and directly subjected to SEM analysis. SEM images were taken by 1450 VP scanning electron microscope (Leo Electron Microscope Ltd., Cambridge, UK).

Table 1. List of *Fusarium* isolates used in this study

Symbol	Isolate code	Location	Year of isolation	Symbol	Isolate code	Location	Year of isolation
Fusarium oxysporum				Fo24	15-118-111	Okcheon, Chungcheongbuk-do	2015
Fo1	*KN1234101	**Gangneung, Gangwon- do	**2012	Fo25	15-97-254	Okcheon, Chungcheongbuk-do	2015
Fo2	JS1226181	Jeongseon, Gangwon- do	2012	Fo26	15-147-50	Okcheon, Chungcheongbuk-do	2015
Fo3	JS123317	Jeongseon, Gangwon- do	2012	Fo27	15-261-99	Okcheon, Chungcheongbuk-do	2015
Fo4	JS12113177	Taebaek, Gangwon-do	2012	Fo28	15-63-181	Okcheon, Chungcheongbuk-do	2015
Fo5	JS12223178	Taebaek, Gangwon-do	2012	*Fusarium solani*			
Fo6	WJ1227305	Wonju, Gangwon- do	2012	Fs1	CW1224	Chorwon, Gangwon-do	2012
Fo7	YY1281171	YangYang, Gangwon-do	2012	Fs2	YW 13-32-497	Yeonngwol, Gangwon-do	2013
Fo8	JS12233179	Taebaek, Gangwon-do	2012	Fs3	13-21-541	Pyeonghung, Gangwon-do	
Fo9	KN12331011	Gangneung, Gangwon-do	2012	Fs4	15-4-191	Jinan, Jeollabuk-do	2015
Fo10	JS12113175	Taebaek, Gangwon-do	2012	Fs5	15-270-200	Okcheon, Chungcheongbuk-do	2015
Fo11	CW12183176	Taebaek, Gangwon-do	2012	Fs6	IJ 12-19-30	Inje, Gangwon-do	
Fo12	JS12183174	Taebaek, Gangwon-do	2012	Fs7	CW124222	Chorwon, Gangwon-do	2012
Fo13	TB12123171	Taebaek, Gangwon-do	2012	*Fusarium equiseti*			
Fo14	YY1210115	YangYang, Gangwon-do	2012	Fe1	1D-SF-5	Ganghwa, Incheon	2013
Fo15	YW 13-4-B	Yeonngwol, Gangwon-do	2013	Fe2	15-114-180	Okcheon, Chungcheongbuk-do	2015
Fo16	14-20-250	Goryeong, Gyeongsangbuk-do	2014	Fe3	15-108-64	Okcheon, Chungcheongbuk-do	2015
Fo17	14-32-44	Yeonngwol, Gangwon-do	2014	*Fusarium merismoides*			
Fo18	14-30-61	Yeonngwol, Gangwon-do	2014	Fm1	HC1320487	Hongheon, Gangwon-do	2013
Fo19	14-24-64	Yeonngwol, Gangwon-do	2014	*Fusarium proliferatum*			
Fo20	14-20-30	Pyeonghung, Gangwon-do	2014	Fp1	PRW 1-16	Goyang, Gyeonggi-do	2013
Fo21	14-38-29	Yeonngwol, Gangwon-do	2014	Fp2	14-1-99	Muju, Jeollabuk-do	2014
Fo22	14-26-42	Yeonngwol, Gangwon-do	2014	*Fusarium fujikuroi*			
Fo23	15-261-143	Okcheon, Chungcheongbuk-do	2015	Ff1	15-261-130	Okcheon, Chungcheongbuk-do	2015

* The accession number of isolates in fungal collection of Plant Microbiology and Biotechnology Lab (PMBL), Kangwon National University.
****** The location of isolates and year of isolation.

Statistical analysis

Radial growth of fungal mycelium was recorded after 14 days of incubation at 28°C. The growth inhibition rate was calculated when growth of mycelia in the control plate reached the edge of the petri dish. Inhibition rate was calculated by using the following formula:

$$\text{Growth inhibition (\%)} = \frac{H - h}{H} \times 100$$

Where (H) is the diameter of fungal mycelial growth in control plate and (h) is the diameter of fungal mycelial growth on the plate treated with Ni NPs concentrations.

Percent data were transformed into arcsine squareroot and then subjected to analysis of variance analysis as described by Gomez and Gomez[20]. However, original means were used for the interpretation of the results. Two-Way ANOVA was used for variance analysis. Treatment means were compared at 5% level of significance by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Inhibition effect of nickel nanoparticles

The observation of mycelial growth inhibition of tested *Fusarium* isolates by Ni nanoparticle revealed that the inhibition effect was observed in each treatment as compared to the control and that the growth inhibition effect increased with increasing of Ni concentration (Table 2 and Fig. 1). The data revealed a significant increase in mycelial growth inhibition of tested *Fusarium* isolates. In most cases, the concentrations of 50 and 100 ppm were most effective against all tested isolates. The efficacy of nickel nanoparticles was varied with type of growth media. The higher inhibition rates were observed on MEA media with all tested Ni concentrations and isolates. There was no inhibition at a concentration of 10 ppm for fungal isolate Fo1 on all growth media and isolate Fo16 on PDA. The inhibition rates against isolates, Fo6, Fo9, Fo10, Fo20, Fo22, and Fe2 were 94.10, 94.10, 96.43, 92.93, 96.83 and 92.53%, respectively on MEA media at a concentration of 100 ppm. The range of inhibition was 52.13-63.53% against Fo13, Fo16, Fo23, and Fo25 isolates on MEA media at a concentration of 100 ppm. On the other hand, the lowest growth inhibition of 47.83% was observed against the isolate Fo2 on MEA media at a concentration of 100 ppm. The inhibition growth rates against isolates, Fo7, Fo22, and Fs2 were 82.73%, 90.20% and 80.00%, respectively on CMA media at a concentration of 100 ppm and the less affected isolates were Fo2 and Fo24 with the growth inhibition of 51.73% and 37.63%, respectively. In case of PDA media, the most affected isolates were Fo1, Fo7, Fo22, Fp1, and Ff1 with inhibition rates of 85.10%, 78.07%, 74.13%, 75.7% and 78.03%, respectively at a concentration of 100 ppm.

The inhibition effect of nickel nanoparticles was comparatively higher for most of the tested isolates on MEA media compared to PDA and CMA. There was no difference in mycelial growth inhibition of isolates Fo3, Fo10, Fs2, Fp1 and, Fm1 between CMA and PDA media. These results indicate the efficacy of nickel nanoparticles varied with the type of growth media as mentioned in Table 2. These results are in agreement with the findings of other authors[21,22,23] who revealed that mycelial growth inhibition rates on various fungal species increased with increasing of nickel nanoparticle concentrations. Similar results were found with the magnesium oxide nanoparticles against various plant pathogens[24,25].

Table 2a. Effect of different concentrations of nickel nanoparticles on inhibition of mycelial growth of various *Fusarium* species on different growth media

Trt †	Media ‡	Fungal isolates																			
		Fo1	**Fo2**	**Fo3**	**Fo4**	**Fo5**	**Fo6**	**Fo7**	**Fo8**	**Fo9**	**Fo10**	**Fo11**	**Fo12**	**Fo13**	**Fo14**	**Fo15**	**Fo16**	**Fo17**	**Fo18**	**Fo19**	**Fo20**	**Fo21**
0	PDA	0.0h	0.0i	0.0j	0.0k	0.0k	0.0l	0.0h	0.0k	0.0k	0.0i	0.0k	0.0k	0.0i	0.0h	0.0j	0.0g	0.0h	0.0h	0.0h	0.0j	0.0k
	MEA	0.0h	0.0i	0.0j	0.0k	0.0k	0.0l	0.0h	0.0k	0.0k	0.0i	0.0k	0.0k	0.0i	0.0h	0.0j	0.0g	0.0h	0.0h	0.0h	0.0j	0.0k
	CMA	0.0h	0.0i	0.0j	0.0k	0.0k	0.0l	0.0h	0.0k	0.0k	0.0i	0.0k	0.0k	0.0i	0.0h	0.0j	0.0g	0.0h	0.0h	0.0h	0.0j	0.0k
10	PDA	0.0h	12.9h	32.9i	26.3g	11.4j	28.6 h	29.4f	23.9j	24.7i	28.6 h	19.6i	26.3j	19.2 h	15.3g	26.6h	0.0g	36.1f	13.7g	22.3f	34.9g h	35.3g h
	MEA	0.0h	37.3d	45.1g	23.5h	50.9e	75.7 d	32.7f	25.5j	73.7c	38.4f	20.8 h	52.9f	24.7f	37.7d	21.2i	7.5f	42.0e	26.7e	28.6f	45.9f	63.2c
	CMA	0.0h	26.7e	38.8h	10.6j	16.8i	13.7 k	27.4g	34.5h	21.2j	27.5 h	15.7j	32.6i	18.4 h	16.8g	34.9fg	9.4f	24.3 g	14.9f g	21.5 g	31.0i	16.8j
20	PDA	67.5 d	15.7g	49.8e	38.4e f	18.8i	37.6 g	29.8f	29.4i	34.5 h	34.1 g	21.5 h	27.4j	20.8 g	27.4f	36.9f	7.5f	40.4e	16.8f	28.6f	37.7g	38.0g
	MEA	0.0h	40.4c	49.0f	23.2h	63.9c	83.7c	39.2e	36.0g h	82.8 b	62.0c	26.7 g	60.0e	39.2c	51.0c	26.3h	22.8e	47.5 d	50.2c	34.1e	51.8e	64.7c
	CMA	15.7 g	28.6e	39.2h	17.7i	26.7h	18.4j	40.4e	46.3g	34.5 h	34.9 g	20.4 h	37.2 h	29.4e	27.4f	52.1d	25.9e	36.1f	37.2d	32.6e	33.7h	22.3i
50	PDA	77.3c	24.3e f	58.4d	41.9e	38.4g	50.9e	56.7d	38.4e	40.4 g	45.5e	47.1e	36.8 h	28.6 d	38.8d	52.1d	34.9 d	46.3 d	25.5e	51.4c	45.5f	45.9f
	MEA	41.2f	47.1b	68.6b	49.4c	68.6b	92.5 b	59.2c d	58.4b	83.6 b	82.0 b	62.7 b	78.0c	52.1 b	55.7b	50.2d e	50.2c	63.9 b	48.6c	57.2 b	67.9c	73.7b
	CMA	37.6f	47.8b	56.7d	40.0e	45.5f	23.9i	62.7c	42.8f	44.7f	45.5e	35.7f	44.7 g	40.4c	33.3e	62.7c	49.0c	52.1c	36.9d	46.3 d	45.1f	34.1h
100	PDA	85.1 b	24.7e f	63.9c	56.1b	60.8d	73.7 d	78.7b	55.3c	49.0e	57.3 d	54.5 d	73.3 d	28.6 d	52.1b c	62.0c	54.9 b	52.1c	49.8c	57.3 b	58.4d	51.4e
	MEA	89.0a	47.8b	71.0a	70.2a	84.3a	94.1a	83.9a	70.6a	94.1a	96.4a	71.8a	90.2a	57.2a	69.8a	84.7a	63.5a	71.0a	74.5a	69.4a	92.9a	87.8a
	CMA	56.1e	51.7a	69.4a b	45.1d	61.9c d	46.3f	82.7a	51.4d	52.9 d	57.7 d	60.0c	79.6 b	51.4 b	53.7b	67.5b	62.4a	66.3 b	58.4b	67.5a	73.3b	59.6d

†Treatments = nickel concentrations at 0, 10, 20, 50 and 100 ppm; values with different alphabet (s) in a column for each isolates are significantly different at 5% level of significance by DMRT; ‡PDA, potato dextrose agar; MEA, malt extract agar; CMA, corn meal agar.

Table 2b. Effect of different concentrations of nickel nanoparticles on inhibition of mycelial growth of various *Fusarium* species on different growth media

Trt †	Media ‡	Fungal isolates																			
		Fo22	**Fo23**	**Fo24**	**Fo25**	**Fo26**	**Fo27**	**Fo28**	**Fs1**	**Fs2**	**Fs3**	**Fs4**	**Fs5**	**Fs6**	**Fs7**	**Fe1**	**Fe2**	**Fe3**	**Fp1**	**Fp2**	**Fm1**	**Ff1**
0	PDA	0.0j	0.0i	0.0j	0.0k	0.0k	0.0l	0.0i	0.0i	0.0l	0.0h	0.0j	0.0m	0.0k	0.0h	0.0l	0.0i	0.0h	0.0i	0.0j	0.0i	0.0i
	MEA	0.0j	0.0i	0.0j	0.0k	0.0k	0.0l	0.0i	0.0i	0.0l	0.0h	0.0j	0.0m	0.0k	0.0h	0.0l	0.0i	0.0h	0.0i	0.0j	0.0i	0.0i
	CMA	0.0j	0.0i	0.0j	0.0k	0.0k	0.0l	0.0i	0.0i	0.0l	0.0h	0.0j	0.0m	0.0k	0.0h	0.0l	0.0i	0.0h	0.0i	0.0j	0.0i	0.0i
10	PDA	29.0i	17.6h	2.7i	12.2j	9.4j	14.9j	9.0h	37.3h	38.4i	60.0e	31.0 h	4.3l	6.2j	17.2 g	33.7i	47.8f	16.8 g	16.8 h	23.9i	39.6 g	33.3 h
	MEA	48.3f	28.6f g	16.8e	26.3h	21.3i	35.7 h	12.5f	39.2g h	34.5j	37.2g	35.3 g	17.6j	8.4i	23.4e	37.3h	43.9 g	39.2 d	35.7f	41.9g	48.6e	54.1f
	CMA	32.2 h	28.2f g	5.1h	14.9i	31.0g	27.1i	12.2g h	37.3h	32.2k	38.0f g	27.4i	20.4i	7.0j	19.2f	21.2k	34.1 h	25.5f	24.3 g	36.9h	33.3 h	33.7 h
20	PDA	40.8 g	28.2f g	13.0 g	27.1h	20.4i	21.6 k	11.0fg	45.1ef	49.8g	62.4e	35.7 g	9.8k	19.9 h	21.7e	40.4g	47.8f	23.9f	38.0e	36.5h	50.2e	46.6 g
	MEA	75.7 d	31.4f	39.2c	48.3d	26.3h	46.3 g	20.8e	47.5e	47.5g h	40.8f	42.8f	25.9 h	22.9 g	28.4 d	38.8g h	87.1c	65.5c	54.1 d	62.0e	55.7 d	83.6c
	CMA	49.4f	39.6e	16.1f	31.0g	52.1d	48.2f	23.6e	43.5f	34.5j	40.4f	34.5 g	28.2 g	24.2f	23.1e	27.5j	45.9 g	31.8e	40.0e	52.1f	43.1f	49.4 g
50	PDA	47.5f	30.2f	26.3 d	37.2f	43.2f	47.9f	44.3cd	47.5e	63.2f	71.8d	47.4e	31.0f	33.8e	30.1 d	47.9e	51.4e	25.9f	63.5c	39.2g h	51.0e	69.8e
	MEA	86.7c	45.9d	52.5 b	52.1c	50.2d e	66.3 b	52.1b	84.3b	81.6b	60.8e	55.7c	35.7e	42.4 b	35.1c	67.8c	90.3 b	80.0 b	63.2c	81.2b	71.8 b	89.8 b
	CMA	69.4e	47.8c	26.3 d	46.3d e	58.8c	56.4 d	39.6d	35.3g	72.9d	61.2e	51.3 d	40.4 d	39.5c	34.1c	62.0d	52.1e	40.4 d	52.1 d	70.6d	54.1 d	70.6e
100	PDA	74.1 d	49.0c	38.4c	43.9e	49.0e	52.1e	50.2bc	54.5d	66.7e	81.6b	52.1 d	48.3c	37.0 d	43.1 b	52.1e	56.9 d	33.7e	75.7 b	52.1f	57.6c	78.0 d
	MEA	96.8a	60.4a	71.4a	71.4a	84.7a	73.7a	62.7a	89.8a	91.0a	84.3a	75.7a	58.4a	62.9a	49.0a	84.3a	92.5a	92.1a	81.6a	87.4a	82.8a	96.6a
	CMA	90.2 b	57.2b	37.6c	55.7b	67.5b	62.4c	51.4b	60.8c	80.0c	75.3c	58.0 b	51.4 b	64.5a	46.9a	71.4b	59.2 d	42.7 d	77.2 b	78.8c	71.8 b	89.4 b

†Treatments = nickel concentrations at 0, 10, 20, 50 and 100 ppm; values with different alphabet (s) in a column for each isolates are significantly different at 5% level of significance by DMRT; ‡PDA, potato dextrose agar; MEA, malt extract agar; CMA, corn meal agar.

Morphological analysis of fungi using SEM and light microscope

Scanning electron and light microscopic analyses demonstrated that hyphae lost their smoothness and formed unusual bulges on the surface of fungal hyphae after treated with nickel nanoparticles (Fig. 2), indicated that nickel nanoparticles inhibited the growth of *Fusarium* spp. by deforming the structure of fungal hyphae. The effect of high concentration of nickel nanoparticles on the treated fungi was observed as damage of mycelial branches, distortion of rough surface of chlamydospores and decreased size of microconidia and phialides (Fig. 3). These results suggest that nickel nanoparticles inhibited the growth of fungi by distorting and damaging their morphological structures. Nickel nanoparticles could penetrate into the spore and mycelial membrane structures of fungi and can work on inhibiting cell functions[26]. However, these observations about the cultures growth show two probable mechanisms of action: (i) mechanical effect through direct contact between the Ni NPs and *Fusarium* spp. on the plate whereby a reduction in the mycelial biomass and growth rate of the pathogen (ii) physiochemical effect through the interaction between cell wall of fungi and surface properties of nanoparticles which produced free radicles and could disturb the membrane lipids then spoil the membrane functions[27,28,29]. Generally, nanoparticles display different modes of inhibitory action to microorganisms[30].

Nickel nanoparticles at concentrations of 100 ppm inhibit > 90% mycelial growth of various *Fusarium* isolates through destruction of membrane integrity. This finding is based on results of *in vitro* experiments. Thus, further investigations are needed for the verification of the results under field conditions.

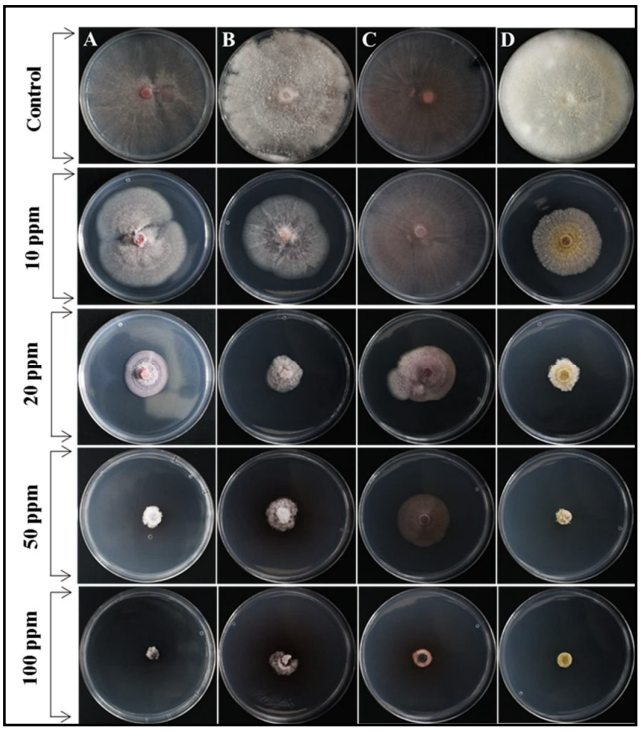

Fig. 1. Effect of nickel nanoparticles on mycelial growth of *Fusarium*spp. on malt extract agar medium. Column A = isolate Ff1, B = isolate Fo9, C = isolate Fo21, and D = isolate Fe3.

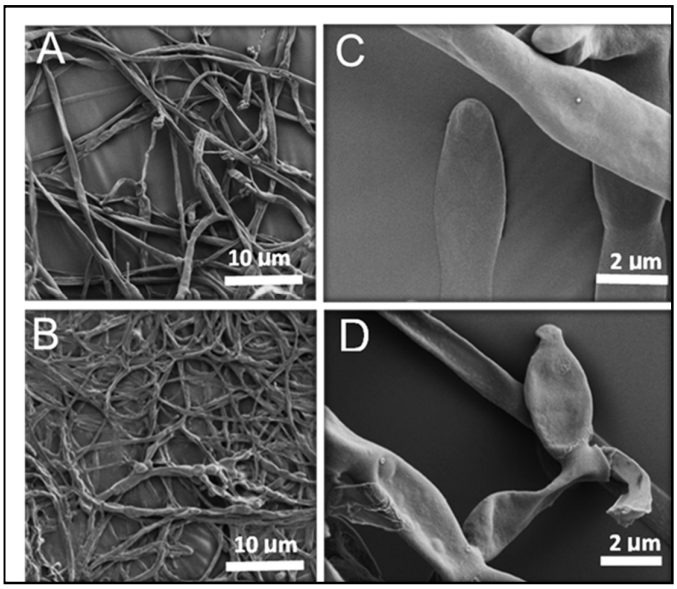

Fig 2. SEM images of *Fusarium oxysporum* untreated (A and C) and treated with nickel nanoparticles (B and D).

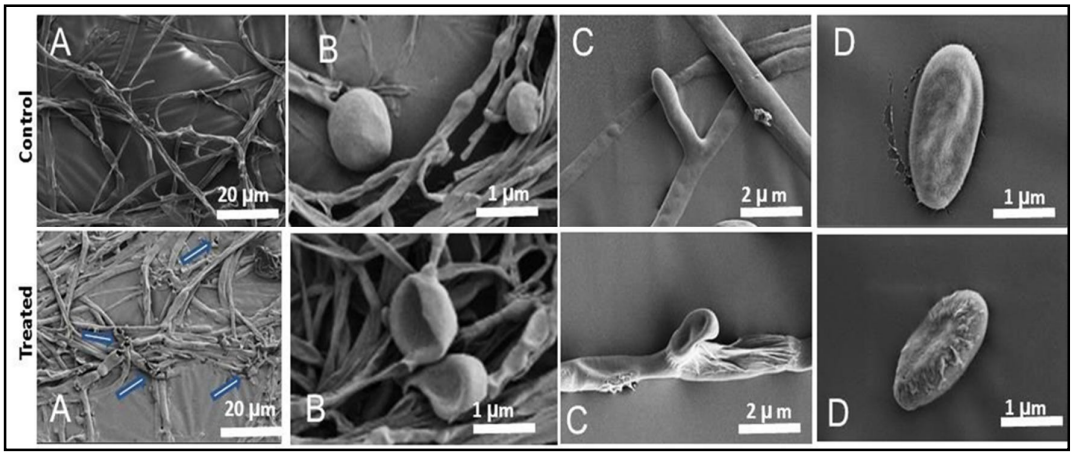

Fig. 3.SEM images of *Fusariumoxysporum*, A (mycelium); B (chlamydosporia); C (phialides) and D (microconidium).

Acknowledgments

The authors would like to acknowledge the University-Industry Cooperation Foundation, Kangwon National University, Korea and Science & Technology Development Fund (STDF), Egypt for their support and fund to execute this study under Short Term Fellowships (STF) program (Project ID: 11982). Authors would also like to thank Mr. Won-Seok Choi, Mr. Joong-Il Kim and Mr. Mi-Ri Park of Nano-Bio Team, Division of Advanced Materials & Strategic Planning, Cheorwon Plasma Research Institute, Cheorwon-gun, Gangwon-do, Korea for providing nanoparticles.

References

1. Manczinger L, Antal Z, Kredics L. Ecophysiology and breeding of mycoparasitic *Trichoderma* strains (a review). Acta Microbiol Immunol Hung 2002;49:1-14.
2. Damos P, Escudero Colomar L A, Ioriatti C. Integrated fruit production and pest management in Europe: The apple case study and how far we are from the original concept? Insects2015;63:626-57.

3. Sunder S, Satyavir. Survival of *Fusarium moniliforme* in soil, grains and stubbles of paddy. Indian Phytopathol 1998;51:47-50.
4. Leslie JF, Summerell BA, Bullock S. The Fusarium laboratory manual. Iowa, USA: Wiley-Blackwell; 2006.
5. Bentley AR, Cromey MG, Farrokhi-Nejad R, Leslie JF, Summerell BA, Burgess LW. *Fusarium* crown and root rot pathogens associated with wheat and grass stem bases on the South Island of New Zeal and .Australas Plant Path 2006;35:495-502.
6. Bockus, WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley, RW. Compendium of wheat diseases and insects. 3rd ed. St. Paul, MN: APS Press; 2007.
7. Dixon NE, Gazzola C, Blakely RL, Zerner B. Jack-Bean urease (E.C.3.5. 1.5.3.). A metalloenzyme.A simple biological role for nickel. J Am ChemSoc 1975;97:4131-33.
8. Liu GD. A new essential mineral element nickel. J Plant NutrFertSci 2001;7:101-3.
9. Brown PH, Welch RM, Cary EE, Checkai RT. Beneficial effect of nickel on plant growth. J Plant Nutr 1987;10:9-16.
10. Forsyth FR, Peterson B. Chemical control of cereal rusts IV. The influence of nickel compounds on wheat, oat and sunflower rusts in the greenhouse. Phytopathology 1959;49:1-3.
11. Venkata Ram CS. Action of nickel salts on the blister blight fungus *in situ*. Phytopathology 1963;53:276-78.
12. Wain RL, Carter GA. Uptake, translocation and transformations by higher plants. In: Torgeson DC, editor. Fungicides: an advanced treatise. New York: Academic Press; 1967. p.561-611.
13. Goffeau A. Drug resistance: The fight against fungi. Nature 2008;452:541-42.
14. Kanhed P, Birla S, Gaikwad S, Gade A, Seabra AB, Rubilar O, Duran N, Rai M. *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi**.** Mater Lett 2014;115:13-17.
15. Sekhon BS. Nanotechnology in agri-food production: an overview. NanotechnolSciAppl 2014;7:31-53.
16. Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Microbiology 2013;11:371-84.
17. Singh M, Kumar M, Kalaivani R, Manikandan S, Kumaraguru A. Metallic silver nanoparticle: a therapeutic agent in combination with antifungal drug against human fungal pathogen. Bioprocess Biosyst Eng 2013;36:407-15.
18. Xu Y, Gao C, Li X, He Y, Zhou L, Pang G, Sun S. *In vitro* antifungal activity of silver nanoparticles against ocular pathogenic filamentous fungi. J Ocul PharmacolTher 2013;29:270-4.
19. Mahdizadeh V, Safaie N, Khelghatiban F. Evaluation of antifungal activity of silver nanoparticles against some phytopathogenic fungi and *Trichoderma harzianum*. J Crop Prot 2015;4:291-300.
20. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. New York, USA: John Wiley and Sons; 1984.
21. Jung JH, Kim SW, Min JS, Kim YJ, Lamsal K, Kim KS, Lee YS.2010. The effect of nano-silver liquid against the white rot of the green onion caused by *sclerotium cepivorum*. Mycobiology 2010;38:39-45.
22. Kim SW, Jung JH, Lamsal K, Kim YS, Min JS, Lee YS.2012. Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology 2012;40:53-8.
23. Yousef N, Nafady N.Combining of biological silver nanoparticles with antiseptic agent and their antimicrobial activity. Int J Pure App Biosci 2014;2:39-47.
24. Wani AH, Shah MA. A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. J App Pharm Sci 2012;2:40-44.
25. Imada K, Sakai S, Kajihara H, Tanaka S, Ito S. Magnesium oxide nanoparticles induce systemic resistance in tomato against bacterial wilt disease. Plant Pathol 2015 Sep 18 [Epub]. http://doi/10.1111/ppa.12443.
26. Cho JS, Seo YC, Yim TB, Lee HY.Effect of nano encapsulated vitamin B1 derivative on inhibition of both mycelial growth and spore germination of *Fusarium oxysporum* f. sp. *Raphani*.Int J MolSci 2013;14:4283-97.
27. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir 2002;18:6679-86.
28. Sondi I, Salopek-Sondi B.Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. J Colloid InterfaceSci 2004;275:177-82.
29. Navarro EA, Baun A, Behra R, Harmann NB, Filser J, Miao AJ, Santschi PH, Sigg L. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology, 2008;17:372-86.

30. Kim SW, Kim KS, Lamsal K, Kim YJ, Kim SB, Jung M, Sim SJ, Kim HS, Chang SJ, Kim JK, Lee YS. An *in vitro* study of the antifungal effect of silver nanoparticles on oak wilt pathogen *Raffaelea* sp. J MicrobiolBiotechnol 2009;19:760-64.

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