

# Applications of Nickel Nanoparticles for Control of Fusarium Wilt on Lettuce and Tomato

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**ABSTRACT:** Fusarium wilt diseases of vegetable crops are the major challenges for vegetable production. To explore alternative control measure of Fusarium wilt, antifungal activities of nickel nanoparticles were investigated for controlling two forma specials of *Fusarium oxysporum*, *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *lycopersici*, on lettuce and tomato under in vitro and greenhouse conditions. Under *in vitro* condition, nickel nanoparticles at the concentration of 100 ppm significantly inhibited the mycelial proliferation and sporulation activities of fungal pathogens. The inhibition of mycelial growth of *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *lycopersici* was 60.23 and 59.77%, respectively. While under greenhouse condition, nickel nanoparticles at the concentration of 50 ppm caused the highest reduction in disease severity and the reduction was 58.4% and 57.0% on lettuce and tomato, respectively. However, when the plants were inoculated with fungal pathogens before application of nickel nanoparticles, the concentration of 50 ppm was found more effective than 100 ppm of NiNPs in relation to plant health. The application of nickel nanoparticles at 100 ppm significantly retarded the plant growth as compared to application of nickel nanoparticles at 50 ppm in all combinations of treatments. So, we conclude that the application of NiNPs as antifungal agents against *Fusarium* will be better with concentration of 50 ppm. This study suggests that nickel nanoparticles could be used to control Fusarium wilt. However, further field studies are needed to verify their disease control efficiency and effects on soil physicochemical properties and other beneficial soil microorganisms.

**KEYWORDS:** Antifungal activity, disease incidence, Fusarium wilt, nickel nanoparticles.

## I. INTRODUCTION

Control of plant diseases is the most challenging aspect of crop production. In recent years, resistance to commercially available fungicides by phytopathogenic fungi has been increasing and has become a serious problem (Goffeau, 2008). Among the plant disease causing microorganisms, fungi cause a significantly higher percentage of diseases in agricultural systems (Omer and Ahmed, 2014). Wilt caused by *Fusarium oxysporum* is a deadly vascular wilting syndrome in plants belonging solanaceae family (Shishido et al., 2005). The fungus *F. oxysporum* comprises over 120 known strains or formae speciales, each of which is specific to a unique plant host in which it causes disease. *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *lactucae* cause Fusarium wilt in tomato and lettuce. Fusarium wilt is a destructive disease of tomato and lettuce in several countries of the globe and has a significant concern to growers due to its severe production loss, prolonged survival of fungus in soil and generation of resistant races (Jarvis, 1988). The disease can be reduced to some extent with the use of resistant cultivars and chemicals. However, the occurrence and development of new pathogenic races is a continuing problem and the use of chemicals are expensive and not always effective (Jones et al., 1991; Lewis et al., 1996). Thus, the control mechanism of this kind of diseases in a way that does not have harmful effects to the crops and no adverse environmental effects have been a great concern for researchers working in the field of disease management. So, it is of immediate necessity to seek alternative disease control measures which are safe to environment. In recent years, use of nanomaterials is expanding and has been considered as an alternative solution to control plant pathogens (Kanhed, 2014; Sekhon, 2014; Ahmed

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and Lee, 2015). Some metal nanoparticles have been studied and proved for their antifungal properties (Lemire et al., 2013; Singh et al., 2013; Xu et al., 2013). However, few studies are available on the effects of nickel nanoparticles on fungal pathogens especially Fusarium wilt causing pathogens. Thus, the antifungal effects of nickel nanoparticles on Fusarium wilt causing pathogens were investigated in the present study under *in vitro* and green house conditions.

## II. MATERIALS AND METHODS

### Nickel nanoparticles

Tested nickel nanoparticles were provided 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 nanoparticles used in this study were <100 nm in size.

### Antifungal activity test through solid agar plate assay

In our previous work four concentrations, 10, 20, 50, and 100 ppm of nickel nanoparticles were evaluated against 68 fungal isolates belonged to various species of *Fusarium* (Ahmed et al., 2016). Significant inhibitions of mycelial growth of 42 isolates were recorded at 50 and 100 ppm concentrations of nickel nanoparticles (Data not shown). On the basis of this preliminary screening, two *Fusarium* wilt causing pathogens, *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *lactucae* were studied for *in vitro* antifungal activity of nickel nanoparticles. The antifungal activity was determined by measuring the growth inhibition of tested pathogens through solid malt extract agar (MEA) media assay. The fungal pathogens were obtained from Korean Agricultural Culture Collection (KACC), Suwon, Korea. Nickel nanoparticles were prepared as suspensions for tested concentrations of nickel nanoparticles by adding 0.05 g/L and 0.1 g/L to growth media before sterilization to get the concentrations of 50 and 100 ppm of nickel nanoparticles, respectively. The MEA media was poured into a 9-cm petri dish. Media without nanomaterial was used as a control. Media containing nickel nanoparticles was 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 fungal pathogens grown on PDA, and were placed at the center of each petri dish containing nickel nanoparticles, then incubated at 28°C for 14 days. For each pathogen, plates in triplicate were arranged in completely randomized design. Radial growth of fungal mycelium was recorded after 10 days of incubation at 28°C. The growth inhibition rate was calculated when growth of mycelia in control plates reached the edge of the petri dish (Kim et al. 2012). Inhibition rate was calculated by using the following formula:

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

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 nickel nanoparticles.

### Antifungal test through broth culture method

To study the effect of nickel nanoparticles on mycelium weight of tested fungi, discs (5 mm in diameter) of fungal pathogens were inoculated into 100 mL of potato dextrose broth (PDB) amended with two concentrations (50 and 100 ppm) of nickel nanoparticles in 250 mL conical flasks at 28°C on a rotary shaker at 150 rpm. For each pathogen, flasks in triplicate were kept. Broth without nickel nanoparticles served as control. The differences in fresh mycelia weights between treated and the control cultures were recorded by passing 10 days grown fungal cultures through pre-weighed filter paper (Navale et al., 2015). The percent reduction in mycelial weight of the fungal pathogens was calculated using the following formula:

$$\text{Reduction in weight (\%)} = (W_1 - W_2) / W_1 \times 100$$

Where,  $W_1$  represents the weight of the test fungus in control flasks and  $W_2$  with the treated flasks.

### Conidial germination test

Conidia of tested fungal pathogens were adjusted using haemocytometer to a concentration of  $10^5$  conidia/ml. Ninety microliter of the conidial suspension were mixed with 10  $\mu$ L solution of 50 and 100 ppm nickel nanoparticles concentrations and the mixtures were added to the surface of dried depression slides. The slides were then placed on a

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glass rod in petri dish under moistened conditions and incubated at 28°C for 24 h. Control conidia received an equivalent amount of the distilled water. After incubation, slides were fixed and observed microscopically for spore germination. The experiment was laid out in completely randomized design with five replications. The number of spore was scored to calculate the percentage inhibition of spore development (Palma-Guerrero et al., 2008).

## Evaluation of nickel nanoparticles on wilt incidence under greenhouse conditions

The effects of nickel nanoparticles on growth parameters of lettuce and tomato and wilt severity were evaluated under greenhouse conditions. Plastic pots were filled with autoclaved commercial soil (Baroker®, Seoul Bio Co., Ltd., Korea). Two seeds of each crop were seeded in each pot and the pots were kept at 25-28°C, watered every other day until seedling emergence and daily thereafter for 25 days. After 7 days of seedling emergence, one seedling per pot was maintained. Inocula of fungal pathogens (*F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *lactucae*) were prepared by inoculating autoclaved potato dextrose broth (121°C for 30 min) in 250 ml flasks with four agar discs (5-mm diameter) of 7-day-old culture of fungal pathogens. Flasks were incubated at 28°C in a rotary shaker at 150 rpm and after incubation of 14 days; the conidial suspension was diluted in sterile distilled water to obtain a final concentration of 10<sup>6</sup> ml<sup>-1</sup>. For each pot, 50 mL inocula of tested *Fusarium* pathogens were added to the root zone of 10-day-old seedlings of lettuce and tomato. The experiment was arranged in RCB design with five replications. The details of treatments are presented in Table 1. Three weeks after fungal inoculation, plant canopies were rated for disease severity on the scale 1-5; where 1 = no disease, 2 = chlorosis in basal leaves, 3 = sign of wilt and necrosis, 4 = plant showing severe of wilt, and 5 = dead. Root and shoot dry matter production was recorded after drying the samples in an oven (MOV-212F, Sanyo Electric Co., Ltd., Osaka, Japan) at 80°C for 72 h.

**Table 1.** Treatments of wilt disease assessment under greenhouse conditions

Treatment*	Description
T1	Control (plants inoculated with fungal pathogen)
T2	Seed coated with 50 ppm NiNPs solution + fungal pathogen
T3	Seed coated with 100 ppm NiNPs solution + fungal pathogen
T4	Soil drenching with 50 ppm NiNPs solution before inoculation with fungal pathogen
T5	Soil drenching with 100 ppm NiNPs solution before inoculation fungal pathogen
T6	Soil drenching with 50 ppm NiNPs solution after inoculation with fungal pathogen
T7	Soil drenching with 100 ppm NiNPs solution after inoculation with fungal pathogen

\* The same set of treatments was applied both in tomato and lettuce. *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *lactucae* were used as wilt pathogens for tomato and lettuce, respectively.

## Statistical analysis

*In vitro* and greenhouse data were analyzed by analysis of variance (ANOVA) to determine the significance of treatment effects. The percent data and data set having value zero (0) were transferred into arcsine square root transformation before further statistical analysis to improve the homogeneity of the variance of the data. Mean separation test was done using the Duncan's multiple range test (DMRT) at the 0.05 probability level. The analysis was done using CROPSTAT version 7.2.3 (IRRI, 2007).

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## III. RESULTS AND DISCUSSION

### Effect of nickel nanoparticles on fungal mycelial growth

Both the concentrations of nickel nanoparticles (50 and 100 ppm) inhibited the fungal mycelial growth on solid media and the inhibitions were significant ( $p \leq 0.05$ ) over control (Table 2 and Fig. 1). Nickel nanoparticles at 100 ppm concentration inhibited the mycelial growth of *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *lycopersici* by 60.23 and 59.77%, respectively over control.

The inhibitory effects of nickel nanoparticles were also assessed in liquid medium and the results were similar with solid media. In the liquid media, the fresh mycelial weight of the tested fungal pathogens decreased significantly and more than 50% reduction was recorded with the use of nickel nanoparticles at the concentration of 100 ppm in fresh mycelial weight of both the tested fungal pathogens. The results revealed that mycelial growth of tested pathogens was inhibited in a concentration dependent manner. These results suggest that using Ni nanoparticle solution can dramatically increase the surface areas acting on the mycelia of Fusarium and mycelial growth.

**Table 2.** Effect of nickel nanoparticles on mycelial growth inhibition of Fusarium wilt pathogens

Nanoparticles conc. (ppm)	Percent inhibition of mycelial growth	
	<i>F. oxysporum</i> f. sp. <i>lactucae</i>	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>
50	39.53 (39.21) <sup>b</sup>	42.59 (41.00) <sup>b</sup>
100	60.23 (51.19) <sup>a</sup>	59.77 (50.91) <sup>a</sup>
Control	0.00 (4.05) <sup>c</sup>	0.00 (4.05) <sup>c</sup>

Values are the average of five replications; values in a column followed with different alphabetic superscripts are significantly different according to Duncan's test at 5% probability; and figures in the parentheses are arc sine square root transformed values.

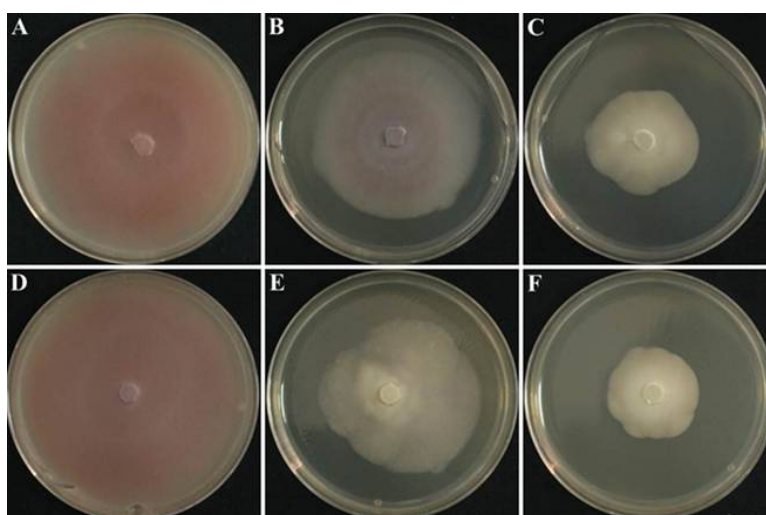


Fig. 1. Petri dishes showing inhibition of Fusarium wilt causing pathogens. 1st row, *Fusarium oxysporum* f. sp. *lycopersici* (A, Control; B, 50 ppm nickel nanoparticles; C, 100 ppm nickel nanoparticles); 2nd row, *Fusarium oxysporum* f. sp. *lactucae* (D, Control; E, 50 ppm nickel nanoparticles; F, 100 ppm nickel nanoparticles).

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### Effect of nickel nanoparticles on spore development

The number of spores was decreased with the use of nickel nanoparticles in both tested pathogens and the decrease was significant ( $p \leq 0.05$ ) over control (Table 3). Nickel nanoparticles at the concentration of 100 ppm decreased the number of spore development by 81.40 and 74.60% in *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *lycopersici*, respectively. The conidial germination was negatively affected by nickel nanoparticles (Fig. 2). It is clear from the results that different concentrations of nickel nanoparticles caused significant inhibition in the spore germination as compared to control. The highest inhibition was observed at the highest concentration of nanoparticles followed by lower concentrations of Ni nanoparticles, respectively. The significant inhibition in the spore germination of both tested fungi was observed at 100 ppm concentration followed by 50 ppm. The inhibitory effect of Ni nanoparticles on spore germination may be due to their fungicidal effect. These results agreed with the results obtained from research works on antifungal effects of various metal nanoparticles against some pathogenic fungi such as silver nanoparticles (Pulit et al., 2013, Kim et al., 2012, Lamsal et al., 2011) and zinc nanoparticles against (Jones et al., 2008; Aneesh et al., 2007; Tarasenko et al., 2010), copper nanoparticles (Gates 1995; Chandra et al., 2014; Kanhed et al., 2014). Inhibitory effect of Ni nanoparticles may be due to production of extracellular enzymes from fungi as survival agents caused by stress of toxic materials (Pere-de-Luque and Diego, 2009; Bhainsa, and D'Souza, 2006; Vahabi et al., 2011) or it may be due to large surface areas and small sizes, enough to penetrate into the cell membrane of pathogen and work in the cytosols (Seo, et al 2010; Cho et al. 2013).

**Table 3.** Effect of nickel nanoparticles on sporulation ability of Fusarium wilt pathogens

Nanoparticles concentration (ppm)	Reduction in number of spores (%)	
	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	<i>F. oxysporum</i> f. sp. <i>lactucae</i>
50	55.40 (48.39) <sup>b</sup>	58.00 (49.91) <sup>b</sup>
100	74.60 (60.24) <sup>a</sup>	81.40 (64.99) <sup>a</sup>
Control	0.00 (4.05) <sup>c</sup>	0.00 (4.05) <sup>c</sup>

Values are the average of five replications; values in a column followed with different alphabetic superscripts are significantly different according to Duncan's test at 5% probability; and figures in the parentheses are arc sine square root transformed values.

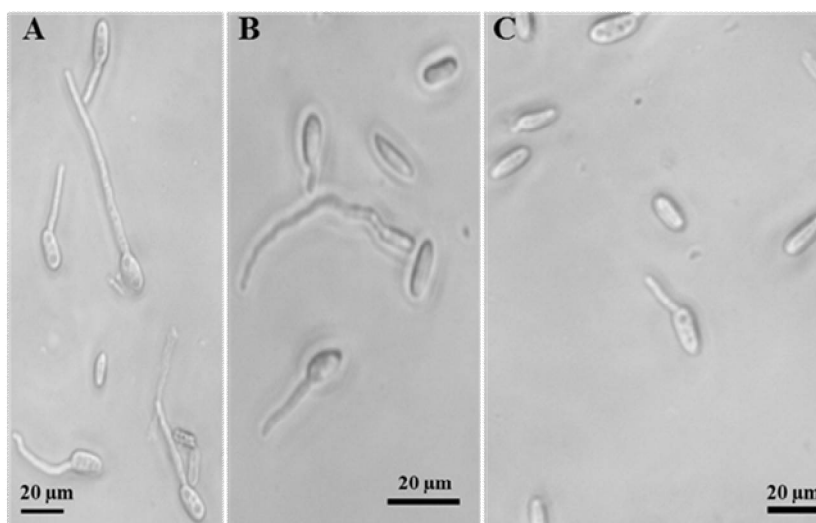


Fig. 2. Germination of *Fusarium oxysporum* f. sp. *lycopersici* conidia. A, Control; B, 50 ppm nickel nanoparticles; C, 100 ppm nickel nanoparticles.

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### Greenhouse assessment of nickel nanoparticles on wilt incidence

Nickel nanoparticles significantly reduced Fusarium wilt of lettuce and tomato by 37.5-58.4% and 37.2-57.0%, respectively over control (Table 4). Among the application methods, soil drenching before pathogen inoculation was found the most effective to reduce the wilt incidence in both the crops. The highest reduction of wilt incidence was obtained with the application of nickel nanoparticles at the concentration of 50 ppm as soil drenching before pathogen inoculation and the reductions were 58.4 and 57.0% in lettuce and tomato, respectively.

**Table 4.** Development of Fusarium wilt on lettuce and tomato as affected by nickel nanoparticles treatments

Treatments	Lettuce		Tomato	
	% wilt	% reduction	% wilt	% reduction
T1	72.6 <sup>a</sup>	-	74.5 <sup>a</sup>	-
T2	45.4 <sup>b</sup>	37.5	46.8 <sup>b</sup>	37.2
T3	42.8 <sup>c</sup>	41.0	43.7 <sup>b</sup>	41.3
T4	30.2 <sup>e</sup>	58.4	32.0 <sup>d</sup>	57.0
T5	36.8 <sup>d</sup>	49.3	38.3 <sup>c</sup>	48.6
T6	35.4 <sup>d</sup>	51.2	37.4 <sup>c</sup>	49.8
T7	41.8 <sup>c</sup>	42.4	44.2 <sup>b</sup>	40.7

Values in a column followed with different alphabetic superscripts are significantly different according to Duncan's test at 5% probability.

### Effect of nickel nanoparticles on growth of tomato and lettuce under greenhouse conditions

The aim of this experiment was to assess the effect of nickel nanoparticles on growth of lettuce and tomato under greenhouse conditions. Table 5 shows that root and dry weights of tomato and lettuce were affected with the different treatments of nickel nanoparticles and the effects were significant ( $p \leq 0.05$ ). The lowest root and shoot dry weights of tomato and lettuce were obtained in control treatment. The lower values of root and shoot dry weights were found in the treatments where seeds were coated with 100 ppm concentration of nickel nanoparticles. The highest values of plant growth parameters were obtained when the soil was drenched with nickel nanoparticles at concentration of 50 ppm. The inoculation of plants with fungal pathogens before application of nickel nanoparticles significantly decreased the root and shoot dry weights of both the crops. The application of nickel nanoparticles at 100 ppm significantly retarded the plant growths as compared to application of nickel nanoparticles at 50 ppm in all combinations of treatments.

**Table 5.** Effect of different concentrations of nickel nanoparticles on growth parameters of tomato and lettuce

Treatments	Tomato		Lettuce	
	Root dry weight (mg/plant)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)	Shoot dry weight (mg/plant)
T1	27.00 <sup>e</sup>	191.26 <sup>g</sup>	178.46 <sup>f</sup>	321.48 <sup>g</sup>
T2	89.68 <sup>b</sup>	382.62 <sup>d</sup>	262.14 <sup>c</sup>	529.94 <sup>e</sup>
T3	53.26 <sup>d</sup>	224.24 <sup>f</sup>	238.84 <sup>e</sup>	336.14 <sup>f</sup>
T4	143.96 <sup>a</sup>	1049.58 <sup>a</sup>	377.60 <sup>a</sup>	803.80 <sup>a</sup>
T5	84.78 <sup>c</sup>	673.08 <sup>c</sup>	254.12 <sup>d</sup>	687.96 <sup>c</sup>
T6	91.94 <sup>b</sup>	823.74 <sup>b</sup>	278.22 <sup>b</sup>	755.80 <sup>b</sup>
T7	81.48 <sup>c</sup>	325.50 <sup>e</sup>	241.42 <sup>e</sup>	571.48 <sup>d</sup>

Values in a column followed with different alphabetic superscripts are significantly different according to Duncan's test at 5% probability.

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## IV. CONCLUSION

In this work, we have demonstrated the antifungal effects of nickel nanoparticles against causal agents of wilt in tomato and lettuce under *in vitro* and greenhouse conditions. The findings of the study demonstrated the potentiality of nanoparticles as inhibitor agent of fungal growth. Nickel nanoparticles at 100 ppm concentration inhibited the mycelial growth of *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *lycopersici* by 60.23 and 59.77%, respectively compared with control. In the liquid media, the fresh mycelial weight of pathogens decreased significantly and the reduction was more than 50% with the use of nickel nanoparticles at the concentration of 100 ppm. Although the best effect of nanoparticles was observed at the concentration of 100 ppm in *in vitro* concentration, but in greenhouse NiNPs at 50 ppm was better than 100 ppm of NiNPs in relation to plant health measured in terms of growth parameters. The application of nickel nanoparticles at 100 ppm significantly retarded the plant growths as compared to application of nickel nanoparticles at 50 ppm in all combinations of treatments. So we suggest that the application of NiNPs as antifungal agents against *Fusarium* will be better with 50 ppm concentration. Moreover, Nickel nanoparticles to control *Fusarium* wilt of tomato and lettuce are the most promising to inhibit the pathogenicity of fungi and reduction of *Fusarium* wilt disease. Thus, it can be concluded that nickel nanoparticles can be used as fungicide after further confirmatory and biosafety studies.

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