Physiological Studies on the Toxicity of Nano and Bulk Nickel Oxide in Germinating Seedlings of *Fennel* (*Foeniculum vulgare* Mill.)

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Abstract

The effect of nanoparticles on living organisms is not well understood yet. The physicochemical properties of nanoparticles enhance the use of nanotechnology in various fields. In this study, the effect of different concentrations of nano and bulk nickel oxide (0, 10, 20, 50, 100, 200, 400, 600, 800, and 1000 ppm) on fennel seed germination and growth was tested in a randomized complete design with four replications. After 14 days of seed treatment, germination factors (germination percent, relative germination percent, mean germination time, germination rate, germination index, and weight germination index) were measured. In general, low nano concentrations and medium bulk concentrations on fennel seed growth showed stimulating effects, while high nano and bulk concentrations showed toxic effects. Therefore, exposure of fennel seeds to low concentrations of NiO nanoparticles and medium concentrations of bulk NiO stimulated seed germination. These concentrations can be used as a new approach to overcome seed germination problems of some plant species, especially medicinal plants such as fennel.

Keywords: *Foeniculum vulgare*, NiO, nanoparticle, bulk NiO, germination factors, seedling growth

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Introduction

Nanotechnology is one of the most advanced sciences in the world. The use of nanoparticles is increasing due to their special physicochemical properties in various sciences (Joudeh and Linke, 2022). Nanoparticles have numerous uses in agriculture, industry, medical, and pharmaceutical sciences (Mohamad et al., 2018). Regarding the widespread application of these nanoparticles in consumer products, their release into the environment is unavoidable(Alfei et al., 2020). Plant communities play vital roles in ecosystem function; hence, they are exposed to these nanoparticles and are important routes for the transfer of nanoparticles to the environment as well as their accumulation in the food chain (Rai et

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al., 2018). The use of nanoparticles has significant effects on plants' growth (Siddiqui et al., 2015; Thul and Sarangi, 2015). The rapid growth of nanotechnology and nanoparticles has recently led to a greater focus on their toxicity potential on living organisms exposed to these particles (Ruttkay-Nedecky et al., 2017).

Nickel is a rare element that is important for the proper functioning of living organisms and most plants, affecting the activity of enzymes (Mielcarz and Smolińska, 2016). Its main role is related to the function of enzymes such as ureases, acetylo-S-CoA synthase, glyoxalase, and hydrogenase. Lack of access to nickel for some plants, such as beans, can even lead to their death. Nickel affects the process of air molecular nitrogen uptake and is involved in the movement of plants' shoots (Hayyat et al., 2020). Nickel is critical to some plants, and its salts have a beneficial effect on their development .Excess nickel is very dangerous; it can even cause cell death(Begum et al., 2022).

Toxic effects of bulk and nano nickel oxide on *Lemna gibba* L. were investigated(Oukarroum et al., 2015). Both NiO-NPs and NiO-Bulk resulted in a strong increase in the formation of reactive oxygen species (ROS), especially at high concentrations (1000 mg L^{-1}). These results clearly showed that oxidative stress toxicity was not directly related to the dissolution of NiO-NPs (Oukarroum et al., 2015). Researchers observed that NiO-NPs formed sediments in the algal cells, which caused growth inhibition and ultrastructural changes in the cells after 72 hours of exposure to 10-50 mg L^{-1} of NiO-NPs (Tiwari et al., 2024). In a study, nanoparticles of nickel reduced water relative content, root and shoot length, photosynthetic pigment content, dry weight percentage, and antioxidant activity of coriander(Miri et al., 2017). The effects of NiO nanoparticles on tomato roots showed that NiO-NPs caused displacement, nuclear density changes, increased abundance of peroxisomes, and degeneration of mitochondrial blades(Spormann et al., 2022). The dissolution of Ni ions from NiO-NPs is probably indicative of their potential to induce cell death, with apoptosis pathways dependent on mitochondria (Manna et al., 2023).

In a study, the seeds of lettuce, radishes, and cucumbers were treated with different metal oxide NPs (CuO, NiO, TiO_2, Fe_2O_3, and Co_3O_4), of which only CuO and NiO had harmful effects on the activity of all three seeds(Fasake et al., 2021). Small seeds (i.e., lettuce) were more sensitive to CuO and NiO-NPs in these experiments (Rath et al., 2024). The effects of nano and bulk nickel oxide on *Lemna minor* L. and *Spirodela polyrhiza* showed that negative impacts of nano nickel oxide, especially at high concentrations, were greater than that of bulk nickel oxide (Torbati, 2018). In a study, the effect of various concentrations of nano nickel oxide was investigated on barley, and it was observed that no toxic effect was found at low concentrations, but at high concentrations, growth inhibitory effects increased (Soares et al., 2016). In a study that investigated the effects of nano nickel oxide on germination and root growth in radish, seed treatment for 4 hours resulted in a significant reduction in germination percentage and root length (Abdel-Salam et al., 2018).

Herbs in different countries are used to treat diseases and as dietary supplements. The properties of medicinal plants can be strongly influenced by nanoparticles that affect the environment (Miri et al., 2017). *Fennel* (*Foeniculum vulgare* Mill.) is a very important medicinal plant (Méabed et al., 2018) which belongs to the Apiaceae family. It is also used as a lactating agent for lactating mothers.

Plant chemistry studies have shown numerous valuable compounds such as volatile compounds, flavonoids, phenolic compounds, fatty acids, and amino acids (Pinto et al., 2021). The ability of *fennel* to demonstrate antifungal, anti-bacterial, antioxidant, antithrombotic (anti-clotting), and liver protective properties has been documented (Najem et al., 2024). The extract of this plant contains high phenolic compounds and flavonoids and has antioxidant properties and free radical scavenging activity.

Poor seed germination is a common occurrence in medicinal plants, and studies on the effects of nanoparticles on medicinal plants, especially *fennel*, are very limited, despite *fennel* being one

Table 1
Mean Values (± SE) of Germination Parameters for *Foeniculum vulgare* Seeds Treated with Different Concentrations of Nano and Bulk Nickel Oxide (NiO)

NiO (ppm)		GP	RGP	GR	MGT	GI	WGI
Control	0	82.66±14.0 abcd	--------------	19.94±2.2 bcde	9.9204±0.07 bcd	238.15±28.6 cde	2.42±0.3 bcde
Nano NiO	10	9.3±2.3 a	451.6±11.1a	25.2±2.4 ab	9.5±0.2 bcdef	262.7±19.5 bcde	3.0±0.1ab
	20	85.3±8.3abc	412.9±40.2 abc	29.2±3.6 a	9.1±0.4 fg	333.5±72.1 ab	3.3±0.3 a
	50	72.0±6.9 bcdefg	348.3±33.5 bcdef	16.1±8.2 cdef	10.1±0.8 b	203.3±88.0 ef	1.8±0.9 def
	100	76.0±10.5 abcdef	367.7±51.2 abcd	23.5±2.9 abc	9.2±0.4 defg	302.4±72.6 abcd	2.7±0.2 abcd
	200	57.3±10.0 fghi	277.4±48.7 efgh	14.0±2.9 efg	9.8±0.1 bcde	248.0±13.9 bcde	1.7±0.4 ef
	400	76.0±6.9 abcdef	367.7±33.5 abcd	22.5±4.2 abcd	9.3±0.3 cdefg	280.3±50.9 bcde	2.5±0.3 abcde
	600	69.3±4.6 cdefgh	335.4±22.3 cdefg	16.2±2.5 cdef	9.9±0.1 bcd	233.42±23.9 cde	2.0±0.3 cdef
	800	62.6±15.1 efghi	303.2±73.2 defgh	14.1±5.8 efg	10.0±0.3 bc	216.35±38.9 de	1.7±0.7 ef
	1000	66.6±8.3 cdefghi	322.5±40.2 defg	15.0±3.8 def	10.0±0.4 bc	220.91±43.7 de	1.8±0.4 def
Bulk NiO	10	74.6±18.9abcdef	361.2±91.4 bcde	15.8±2.9 cdef	10.0±0.2 bc	207.5±15.7 e	1.9±0.3 cdef
	20	89.3±2.3 ab	432.2±11.1 abc	18.6±0.7 bcdef	10.0±0.2 bc	209.6±1. e	2.3±0.0 bcde
	50	52.0±4.0 hi	251.6±19. gh	10.8±2.6 fg	10.0±0.2 bc	203.6±45.4 ef	1.3±0.3 fg
	100	65.3±8.3 defghi	316.1±40.2 defgh	21.3±3.1 bcde	9.1±0.1 efg	316.4±3.2 abc	2.4±0.3 bcde
	200	80.0±10.5 abcde	387.1±51.2 abcd	22.9±4.6 abcd	9.4±0.2 bcdefg	282.3±22.8 bcde	2.7±0.5 abc
	400	80.0±8.0abcde	387.1±38.7 abcd	25.8±3.9 ab	9.30±0.2 defg	312.2±27.7 abc	3.0±0.3 ab
	600	54.6±8.3 ghi	264.5±40.2 fgh	21.4±2.8 bcde	8.8±0.3 g	381.8±64.5 a	2.4±0.2 bcde
	800	70.6±8.3 bcdefgh	341.9±40.2 cdef	20.1±7.3 bcde	9.5±0.4 bcdef	278.2±61.2 bcde	2.4±0.7 bcde
	1000	48.0±14.8 i	232.2±69.7h	6.8±2.4g	11.1±0.4 a	125.6±35.7 f	0.8±0.3 g

(Germination Percent (GP, %), Relative Germination Percent (RGP, %), Mean Germination Time (MGT), Germination Rate (GR), Germination Index (GI), and Weight Germination Index (WGI))
The findings demonstrate that similar words do not have statistically significant differences, at p <0.05.

of the most important medicinal plants grown in the world (Tadese et al., 2022). Therefore, this study was conducted to examine plant toxicity or the beneficial effects and stimulation of NiO concentrations at the nanoscale, compared with bulk NiO particles on *fennel* seed growth.

Materials and Methods

Plant Matter

Foeniculum vulgare seeds were purchased from the Pakan Bazr Company, Isfahan Province, Iran. NiO nanoparticle was purchased from US Research Nanomaterials Co. and bulk NiO was prepared from Merck Co. The size of NiO and NiO nanoparticles was measured by atomic force microscopy (AFM); hydrodynamic size was measured by Nanoparticle Sizer (VASCO 3, Cordouan, Pessac, France), and stability of the colloids formed by NiO and NiO nanoparticles was measured by Zeta Sizer (Zeta compact-CAD, France). The nanoparticle phase of bulk NiO and NiO and purity were determined by XRD (X-ray diffraction). These stages were performed at the Central Laboratory of Ferdowsi University of Mashhad.

Plant Cultivation and Treatment

In order to investigate the effect of various concentrations of nano and bulk nickel oxide on seed germination and growth of *fennel*, the experiments were performed in a randomized design with four replications. The treatments included nine concentrations of nano NiO (10, 20, 50, 100, 200, 400, 600, 800, and 1000 ppm), nine bulk NiO concentrations (10, 20, 50, 100, 200, 400, 600, 800, and 1000 ppm), and the control group. Germination experiments were performed at the germinator with a mean temperature of 25 ± 1 °C. The seeds of uniform size were selected and sterilized by sodium hypochlorite (7%) for 3

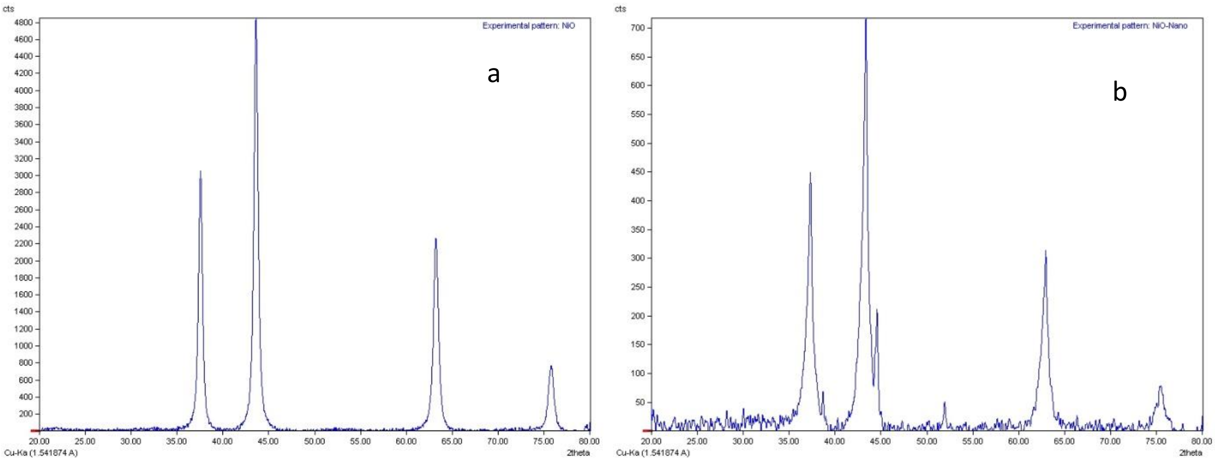

Fig. I. The X-ray diffraction (XRD) pattern of NiO nanoparticle (a) and NiO bulk(b)

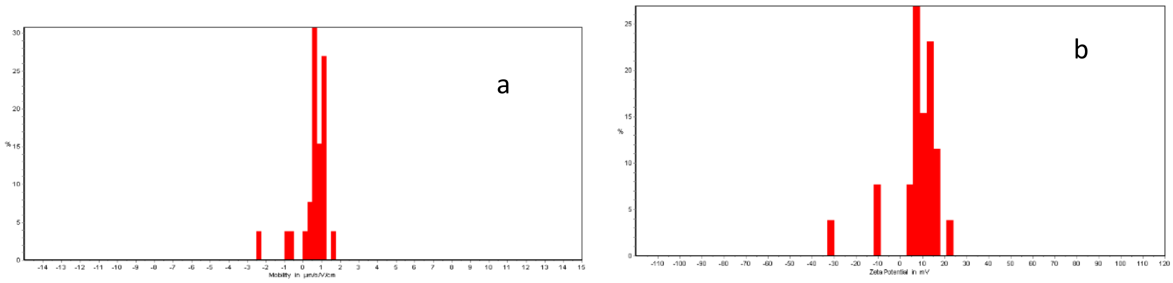

Fig. II. Particles movement (a) and Zeta potential (b) of NiO nanoparticle

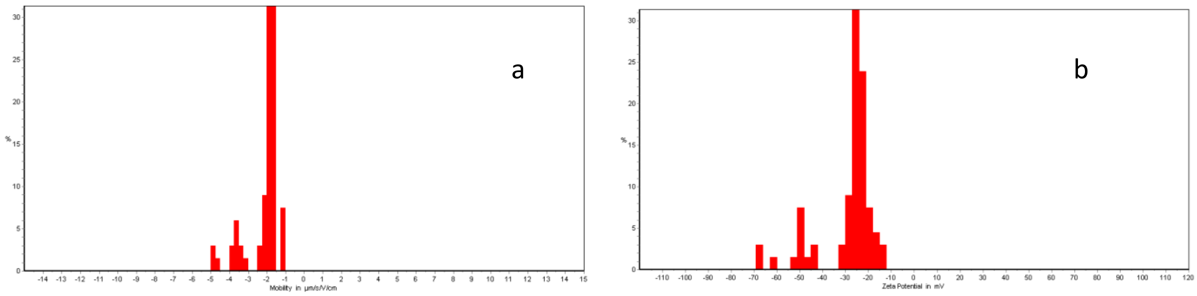

Fig. III. Particles movement (a) and Zeta potential (b) of bulk NiO

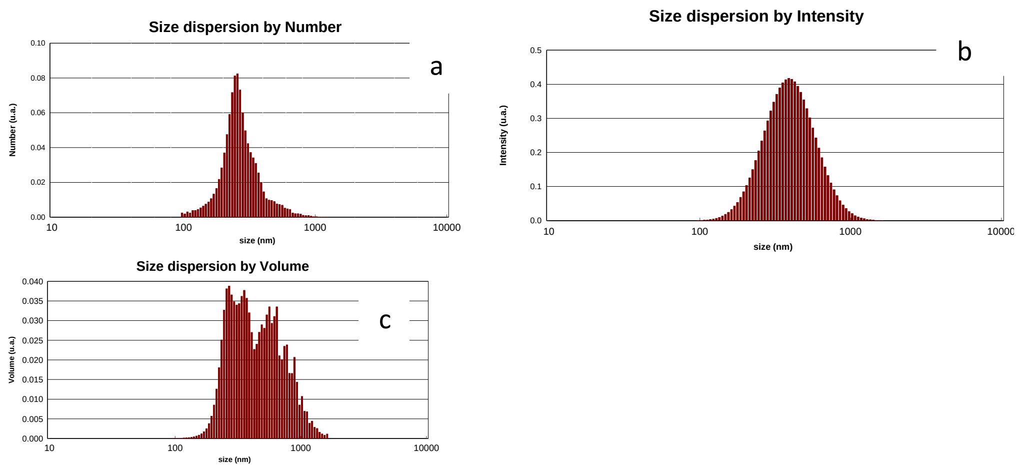

Fig. IV . Hydrodynamic diameter of bulk NiO determined by dynamic light scattering (DLS) and expressed as:
a– intensity percentage, b – volume, c-number

minutes and then washed thrice using distilled water. In order to obtain a homogeneous and

stable suspension of nano and bulk NiO in water, homogenization was done with an ultrasonic device for 15 minutes (GEX 750-5B Ultraccessory Processor VCX 750 Watt, 20 kHz, Cole Parmer, Vernon Hills, IL, USA). Twenty-five seeds in four groups were placed on paper in **a** Petri dish, and 5 ml of each treatment concentration was added. For the control, only distilled water was added to the Petri dish. The number of germinated seeds was counted daily for 14 days. The germinated seeds were considered when the root was at least 2 mm long. Following germination parameters were recorded using the below equations:

GP (Germination Percent) = 100 × GN / SN (1)

GN is the total number of germinated seeds,
SN is the total number of seeds tested

RGP (Relative Germination Percent) = GP treatment / GP control × 100 (2)

MGT (Mean Germination Time) = (3)
GR (Germination Rate) = (4)

Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day i.

GI is a synthetic measure designed to reflect the synthetic germination ability, including germination rate and germination numbers. The number of days since the day of sowing is i, and Gi is the number of seeds germinated on day i.

GI (Germination Index) = (∑ (N-i) × Gi) × 100 / N × GN (5)

A weighted germination index (WGI) as described by Bu et al. (2007)was calculated with the maximum weight given to the seeds germinating early and less to those germinating late.

WGI = [N × n1 + (N-1) × n2 + (N-2) × n3 + … + 1 × n7] / N × N' (6)

Where n1, n2, …, n60 are the number of seeds that germinated on the first, second, and subsequent days until the 14th day, respectively; N is the total number of days of the experiment; N' is the total number of seeds placed in incubation.

Vigor Index I = GP × seedling length (root + shoot) (7)

Response index (RI):
RI = 1 - (C/T) (If T > C) and RI = (T/C) - 1 (If T < C)

RI ranges from -1 to +1, with positive values indicating stimulation by the treatments and negative values indicating inhibition by them, relative to the controls. The absolute value of RI means the degree of inhibition and stimulation of aqueous extracts (Bu et al., 2009; Bu et al., 2008; Figueroa and Armesto, 2001; Kandil et al., 2015; Tang et al., 2008; Wu and Du, 2007).

After an incubation period of 14 days, plumule and radicle length of seedlings were measured using a ruler. In order to weigh dry biomass, the 14-day seedlings were first weighed; then, having been placed in an oven at 80°C for 48 h, they were weighed a second time.

Data Analysis

Significant differences for all statistical tests were evaluated at the level of $P \leq 0.05$ with ANOVA. All data analyses were conducted using SPSS for Windows, Version 18, and graphs were drawn with Excel software.

Results

Fig. I shows the XRD model of nano and bulk nickel oxide. The elements in nickel oxide nanoparticles include nickel oxide and nickel particles. There is also a phase containing nickel oxide in bulk nickel oxide.

Fig. II and Fig. III show the mobility of particles and zeta potential of nano and bulk nickel oxide. For nanoparticles of nickel oxide, dielectric constant = 79.58, mobility means of the particles = 0.58 µm / s / V / cm, and Zeta Mean = 93.7 mV. For bulk

Table 2
Mean value (± SE) of Seedling Vigor Index (SVI), Radicle Length (RL), Plumule Length (PL), Fresh Weight (FW) and Dry Weight (DW) for Seedling of *Foeniculum vulgare* Mill. under nano and bulk NiO different concentrations.

NiO Concentration(ppm)		PL (cm)	RL (cm)	FW (g)	DW (g)	SVI
Control	0	5.5±0.7 abc	5.1±0.2 abcd	0.9±0.1 ab	0.03±0.0 b	875.4±109.1 abc
Nano NiO	10	5.4±0.9 abc	4.8±1.1 abcd	0.9±0.2 ab	0.3±0.0 b	958.2±144.9 ab
	20	4.6±1.2 bc	6.2±2.2 ab	0.7±0.2 abcd	0.02±0.0 b	947.9±372.4 ab
	50	5.5±0.5 abc	4.1±0.9 abcd	0.5±0.3 abcde	0.01±0.0 b	703.7±115.4 bcdef
	100	6.5±0.4 a	5.8±1.7 abc	1.06±0.4 a	0.02±0.0 b	946.9±250.2 ab
	200	4.6±1.7 bc	3.6±1.3 bcd	0.4±0.1 cdef	0.01±0.0 b	476.8±150.1 efg
	400	4.1±0.7cd	5.6±0.6 abcd	0.5±0.0 bcdef	0.01±0.0 b	736.0±57.6bcde
	600	4.2±0.1 cd	5.8±0.5 abc	0.5±0.01 bcdef	0.01±0.0 b	698.9±93.7 bcdef
	800	4.4±0.8 bcd	5.7±1.3 abc	0.4±0.1 ef	0.02±0.0 b	628.4±58.3 cdef
	1000	2.7±0.6 e	3.3±0.6cd	0.4±0.1 ef	0.02±0.0 b	407.7±56.5 fg
Bulk NiO	10	5.1±0.3 abc	5.7±1.1 abc	0.7±0.2 abcde	0.02±0.0 b	804.2±148.3 abcd
	20	6.6±0.1 a	5.2±2.2 abcd	0.8±0.2 abcd	0.02±0.0 b	106.5±210.6 a
	50	3.0±0.4 de	2.9±0.1 d	0.2±0.0 f	0.0±0.0 b	313.9±408.1 g
	100	4.2±0.8cd	6.1±2.8 ab	0.2±0.0 f	0.01±0.01 b	673.8±227.5 bcdef
	200	5.1±0.5 abc	4.9±1.3 abcd	0.8±0.1 abc	0.02±0.0 b	811.7±193.6 abcd
	400	5.6±0.7 abc	6.5±0.4 a	0.7±0.2 abcde	0.08±0.1 a	972.6±45.2 ab
	600	5.6±0.6 abc	4.4±0.5 abcd	0.4±0.5 def	0.01±0.0 b	541.9±45.4 defg
	800	5.8±0.6 ab	5.6±0.8 abcd	0.8±0.3abc	0.02±0.01 b	806.6±8.5 abcd
	1000	5.4±0.9 abc	4.6±1.9 abcd	0.3±0.1 ef	0.01±0.0 b	495.2±208.3 efg

The findings demonstrate that similar words do not have statistically significant differences, at p <0.05.

nickel oxide, these values are 79.44, -1.86, and -25.10, respectively.

Fig. IV and Fig. V illustrate the size of nano and bulk nickel oxide measured by nanoparticle sizer, and Fig. 6 and Fig. 7 present the size of these particles measured by atomic force microscopy (AFM). The mean diameter of the nanoparticles (D mean) was 47.36, the particle hydrodynamic diameter (Z average) = 189.68, and the particle dispersion index (PDI) = 0.39. Fig. V shows these values for bulk nickel oxide. The particle mean diameter (D mean) = 489.61, particle hydrodynamic diameter (Z average) = 386.98, and particle dispersion index (PDI) = 0.155.

The effect of bulk NiO and NiO nanoparticles on germination indicators is shown in Table 1. For germination percentage, the highest germination percentage (93.3) was observed at 10 ppm concentration of nanoparticles with 12.95% increase compared to the control. The lowest germination percentage was observed at 1000 ppm bulk concentration. The nano concentrations of 200, and 800 ppm and bulk concentrations of 50, 600, and 1000 ppm significantly reduced germination percentage. For relative germination percentage, 10 ppm nano concentration (451.6) had the highest percentage of germination. The lowest was observed at 1000 ppm bulk concentration (232.2). The lowest rate of germination (6.8) was observed at 1000 ppm bulk concentration with 65% reduction compared to the control. The highest rate of germination (29.2) was observed at 20 nm nano concentration with an increase of 46% compared to the control. The concentration of 20 ppm of nanoparticles had a significant increase compared to the control, and 50 and 1000 ppm bulk concentrations had a significant reduction compared to the control. The highest mean germination time (11.13) was observed at 1000 ppm bulk concentration with 12.11% increase compared to the control. The lowest mean germination time (8.84) was observed at 600 ppm bulk concentration and 20 ppm nano concentration, which showed 11.1% reduction. The concentration of 20 ppm nanoparticles and 100 ppm and 600 ppm bulk concentrations had a significant reduction compared to the control, and 1000 ppm bulk concentration had a significant increase compared to the control. The highest germination index (381.8) was observed at 600 ppm bulk concentration with a 60% increase. The treatment of 1000 ppm bulk concentration had the lowest germination index (125.6) with a reduction of 47.2%. Nano concentration of 20 ppm had a significant increase compared to the control, and 600 and 1000 ppm bulk concentrations had a

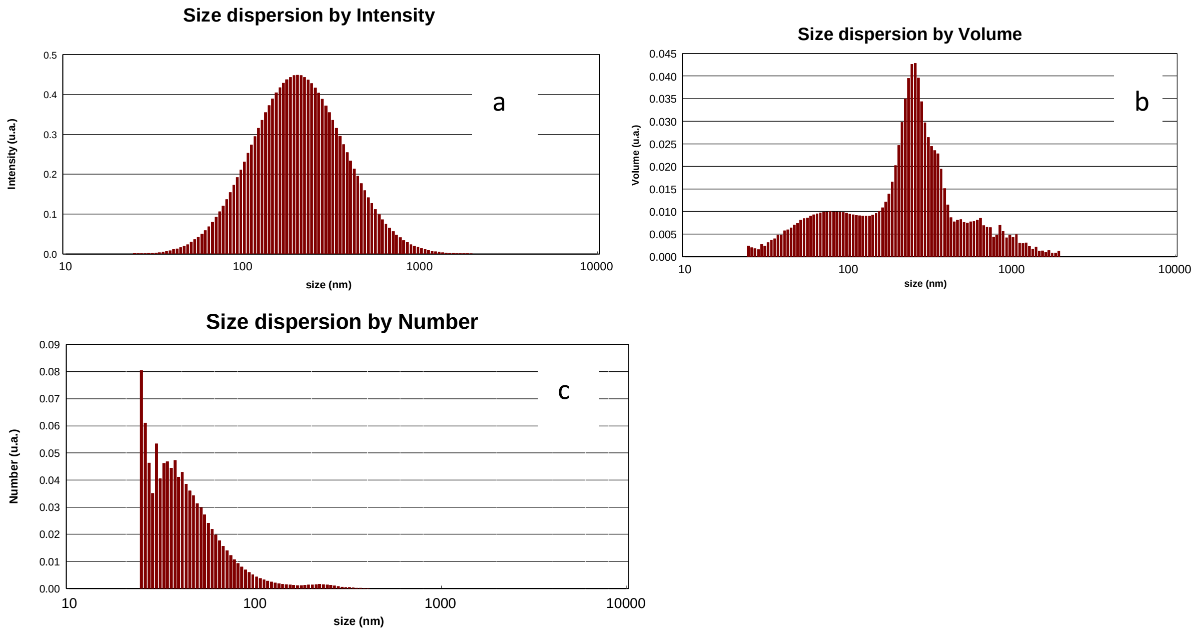

Fig. V. Hydrodynamic diameter of NiO nanoparticle determined by dynamic light scattering (DLS) and expressed as: a– intensity percentage, b – volume, c-number

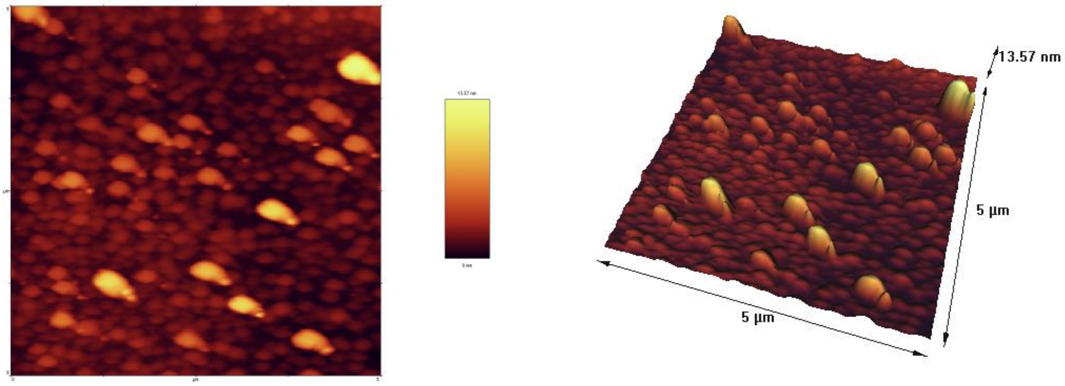

Fig. VI. NiO nanoparticle figures by AFM

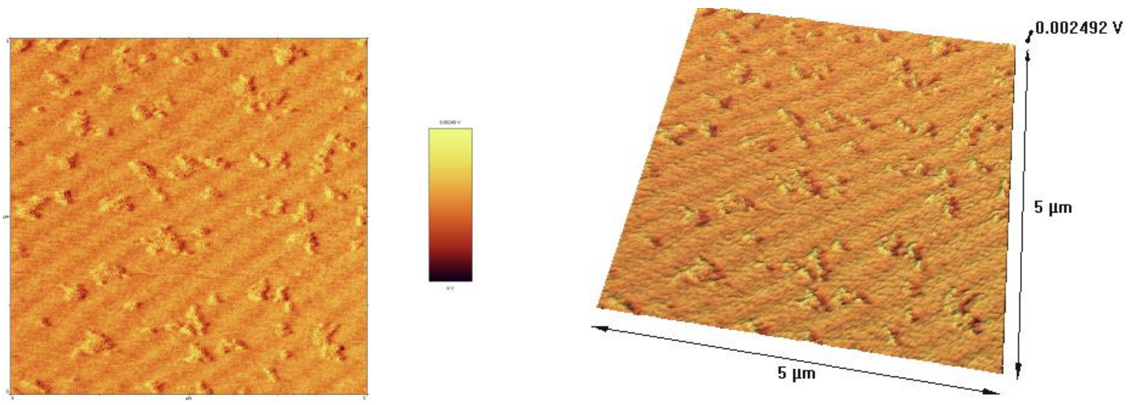

Fig. VII. Bulk NiO figures by AFM

Table 3
Inhibition index value (RI) of of *Foeniculum vulgare* Mill. under nano and bulk NiO different concentrations on seed Germination Percent (GP), Mean Germination Time (MGT), Germination Rate (GR), Germination Index (GI) and Weight Germination Index (WGI) of *Foeniculum vulgare* Mill.

NiO Concentration(ppm)		GP	GR	MGT	GI	WGI
Control	0	0.11	0.20	-0.03	0.09	0.19
Nano NiO	10	0.03	0.31	-0.08	0.15	0.28
	20	-0.12	-0.19	0.20	-0.28	-0.22
	50	-0.08	0.15	-0.06	0.06	0.11
	100	-0.30	-0.29	-0.01	0.39	-0.26
	200	-0.08	0.11	-0.05	0.15	0.06
	400	-0.16	-0.18	-0.001	-0.17	-0.16
	600	-0.24	-0.28	0.009	-0.23	-0.28
	800	-0.19	-0.24	0.009	-0.21	-0.23
	1000	0.11	0.20	-0.03	0.09	0.19
Bulk NiO	10	-0.09	-0.20	0.01	-0.12	-0.20
	20	0.04	-0.06	0.01	-0.11	-0.02
	50	-0.37	-0.45	0.01	-0.14	-0.45
	100	-0.20	0.063	-0.07	0.24	0.02
	200	-0.03	0.12	-0.04	0.15	0.12
	400	-0.03	0.22	-0.06	0.23	0.20
	600	-0.33	0.06	-0.10	0.37	0.01
	800	-0.14	0.008	-0.03	0.14	0.008
	1000	-0.41	-0.65	0.10	-0.47	-0.66

lowest weight germination index (0.8) was observed at 1000 ppm bulk concentration with a 66.9% reduction compared to the control, and the highest value (3.37) was observed at 20 ppm nano concentration with a 39.2% increase compared to the control. The concentration of 20 ppm of nanoparticles had a significant increase compared to the control, and 50 and 1000 ppm bulk concentrations had a significant reduction in comparison with the control.

The effect of bulk NiO and NiO nanoparticles on the growth indicators of *fennel* seed **is** presented in Table 2. The lowest radicle length (2.9) was observed at 50 ppm bulk concentration with a 43% reduction compared to the control, and the highest radicle length (59.6) was observed at 400 ppm bulk concentration with a 28% increase compared to the control. For this factor, no significant difference was observed with the control. The lowest hypocotyl length (2.75) was observed at 1000 ppm nano concentration with a 50% reduction compared to the control. The highest hypocotyl length (6.62) was observed at 20 ppm bulk concentration with a 20% increase compared to the control. Nano concentration of 1000 ppm and 50 ppm bulk concentration had a significant reduction in comparison with the control. The lowest fresh weight (0.2) was observed at 50 ppm bulk concentration with a 77% reduction compared to the control. The highest fresh weight (1.06) was observed at 100 ppm nano concentration with a 17.7% increase compared to the control. Nano concentrations of 200, 800, and 1000 ppm and 50, 100, 600, and 1000 ppm bulk concentrations had a significant reduction compared to the control. The lowest dry weight (0.008) was observed at 50 ppm bulk concentration with a 73.3% reduction compared to the control. The highest dry weight (0.08) was observed at 400 ppm bulk concentration with a 166% increase compared to the control. The lowest seedling vigor index (106.5) was observed at 20 ppm bulk concentration with a reduction in comparison with the control. The highest seedling vigor index (972.6) was observed at 400 ppm bulk concentration with an 11.1% increase compared to the control. Nano concentrations of 200 and 1000 ppm and 50, 600, and 1000 ppm bulk concentrations were significantly different from the control in terms of seedling vigor reduction. Response Index (RI) of various concentrations of nano and bulk nickel oxide on seed germination indicators **is** shown in Tables 3 and 4.

Table 4
Inhibition index value (RI) of nano and bulk NiO different concentrations on Seedling Vigor Index (SVI), Radicle Length (RL), Plumule Length (PL), Fresh Weight (FW) and Dry Weight (DW) on Seedling of *Foeniculum vulgare* Mill.

NiO Concentration(ppm)		PL	RL	FW	DW	SVI
Control	0	-0.009	-0.05	-0.003	-0.003	0.08
Nano NiO	10	-0.16	0.18	-0.16	-0.23	0.07
	20	0.01	-0.18	-0.42	-0.39	-0.19
	50	0.15	0.12	0.14	-0.16	0.07
	100	-0.15	-0.29	-0.48	-0.40	-0.45
	200	-0.25	0.08	-0.40	-0.35	-0.15
	400	-0.23	0.12	-0.36	-0.36	-0.20
	600	-0.19	0.11	-0.53	-0.48	-0.28
	800	-0.50	-0.33	-0.53	-0.28	-0.53
	1000	-0.009	-0.05	-0.003	-0.003	0.08
Bulk NiO	10	-0.05	0.10	-0.12	-0.21	-0.08
	20	0.16	0.02	-0.06	-0.11	-0.87
	50	-0.44	-0.41	-0.73	-0.71	-0.99
	100	-0.22	0.16	-0.68	-0.49	-0.23
	200	-0.06	-0.02	-0.03	-0.031	-0.07
	400	0.01	0.22	-0.20	0.65	0.09
	600	0.01	-0.14	-0.51	-0.53	-0.38
	800	0.06	0.09	-0.04	-0.05	-0.07
	1000	-0.01	-0.09	-0.57	-0.57	-0.43

RI values are in the range of +1 and -1. The positive values indicate stimulation by treatment, and the negative values indicate inhibitory effect by treatment compared to the control group.

Discussion

The results showed that the application of NiO nanoparticles at low concentrations of 10 and 20 ppm increased the germination percentage of fennel seeds, but higher nano and bulk concentrations did not have a stimulating effect on germination percentage. The main reason for this increase in growth percentage can be the optical sterilization and production of active oxygen such as superoxide and hydroxide anion by nanoparticles, which increased seed resistance to stress and stimulated the seed crust to absorb water and oxygen necessary for seed germination (Xi et al., 2022). Wu et al.(2023) showed that TiO2 nanoparticles led to water absorption by spinach seeds and thus accelerated seed germination. In a study on the effects of nano nickel oxide on radish, it was observed that the absorption of nanoparticles to the seed crust increased with increasing concentrations, which may be related to the physical bonding of nanoparticles to the seed's rough surface and electrostatic and hydrophobic attraction between the seed and nanoparticles(Pavitra et al., 2022). The absorption of nanoparticles on the seed crust may stimulate the release of ions from nanoparticles, thus increasing the toxicity of nanoparticles at high concentrations (Rehman et al., 2021). The lowest germination percentage in the present study was observed at the 1000 ppm bulk concentration, which is likely to increase NiO bulk concentration, causing particles to accumulate and congest the root pores, thereby preventing water absorption by the seeds (Bhardwaj et al., 2022).

The highest percentage of relative germination was observed at the 10 ppm nano concentration. In a study on seeds of five cultivars of sunflower exposed to different concentrations of nickel, low concentrations of Ni (10 to 20 ppm) significantly increased germination, while higher concentrations inhibited germination (Baran et al., 2024).

In this study, the rate of germination increased at lower nano concentrations and higher bulk concentrations, so that it increased at nano concentrations of 10, 20, 100, and 400 ppm and bulk concentrations of 100, 200, 400, 600, and 800 ppm. However, the maximum germination rate was obtained at 20 ppm nano concentration, and the lowest was at the 1000 ppm bulk concentration. A number of germination events (gene transcription, energy metabolism, respiration, initial storage mobility, and DNA repair and regeneration) may occur during seed treatment (Ranganathan and Groot, 2023). Although these events are often due to reduced water storage, they are limited compared to conventional germination. The key to increasing the seed germination rate is the penetration of nanoparticles into seeds. Guo et al. (2022)demonstrated that MWCNTs can penetrate tomato seeds and increase the germination rate by increasing water absorption.

The lower mean germination time shows early germination. The results indicated that fennel seeds' exposure to nano concentrations, except for high concentrations such as 800 and 1000 ppm, and high bulk concentrations, except for the concentration of 1000 ppm, resulted in a lower mean time of seed emergence. The lowest mean germination time was observed at the 20 ppm nano concentration and 600 ppm bulk concentration, and the maximum germination time was at the 1000 ppm bulk concentration. DAS et al. (2022)stated that the effect of nano TiO_2 on spinach germination in the experiments was probably due to the small size of the particles that allowed nanoparticles to penetrate the seed during the treatment period, and thus increased growth and improved yield during growth.

The results showed that the germination index and weight germination index were also increased by lower nano concentrations and higher bulk concentrations, except for 1000 ppm, so that the maximum germination index was at the 20 ppm nano concentration and 600 ppm bulk concentration, and the maximum weight germination index was at the 20 ppm nano concentration, with the lowest at the 1000 ppm bulk concentration. According to studies on the effect of nanoparticles on seed germination, it can be argued that nanoparticles may help absorb water by seeds, increase the nitrate reductase enzyme, enhance absorption ability and nutrient use by seeds, expand the seed's antioxidant system, reduce antioxidant stress by decreasing H_2O_2, superoxide radicals, reduce malondialdehyde, and increase some enzymes such as superoxide dismutase, ascorbate peroxidase, and catalase activity, leading to improved seed germination in some plant species (Fujita and Hasanuzzaman, 2022).

The results of this study partially align with the findings of a study on the effect of nickel on sunflower seeds(Ahmad et al., 2009). In both studies, lower concentrations of nickel stimulated hypocotyl length, as observed at 100 ppm nano concentration in the current study. Similarly, the significant reduction in hypocotyl length at the highest bulk concentration (1000 ppm) is consistent with the sunflower study's conclusion that higher concentrations negatively affect growth. However, a divergence is noted as hypocotyl length in this study increased at intermediate bulk concentrations (400, 600, and 800 ppm), whereas the sunflower study reported a general reduction at higher concentrations. These differences may be attributed to variations in plant species, experimental conditions, or the formulation of nickel oxide used.

The radicle length increased under high nano concentration treatments, with the highest radicle length at the 400 ppm bulk concentration and the lowest at the 1000 ppm bulk concentration. Barrena et al. (2009) stated that nano Au increased the radicle length due to the mechanism of protecting the seed from stress by nanoparticles.

The fresh and dry weight were less affected by the treatment than other factors. The highest fresh weight was observed at the 100 ppm nano concentration and 400 ppm bulk concentration. In a study on the effect of nickel on sunflower seeds, the fresh and dry weight of seedlings was higher at lower concentrations, whereas significantly reduced at high nickel concentrations (Ahmad et al., 2009).

The seedling vigor index was maximum at the 10, 20, and 100 ppm nano concentrations and 400 ppm bulk concentration. It seems that the increase in seedling vigor index can improve seed germination and development by treatment, which can be practically used (Feizi et al., 2013).

Conclusion

The study results showed that in most cases, exposure of fennel seeds to low concentrations of NiO nanoparticles and mean concentrations of bulk NiO stimulated seed germination. Since most fennel seeds have poor germination, which may be due to reasons such as climatic conditions (altitude, latitude, precipitation, soil properties, and etc.), native seed conditions, native plant growth conditions, and other factors that can cause seed germination weakening and causing problems for planting, so the use of nano-mass materials can enhance plant germination and improve plant production. Nickel oxide nanoparticles can be stimulating at lower concentrations due to their small size and higher nickel solubility in solutions than bulk nickel, and high concentrations of nanoparticles reduce germination. Bulk nickel oxide showed less stimulation due to its lower solubility compared to nanoscale. In such cases, nano and bulk matter under controlled conditions (for example, plants grown in greenhouse) can be used to increase germination in the given plants. In summary, it can be concluded that nanoparticles are toxic at high concentrations. For Ni ions at high concentrations, a possible way to cause DNA damage has been reported by the production of radical OH through Haber-Weiss reaction. Therefore, NiO-NPs are likely to release Ni ions and contribute to the production of intracellular ROS, which damages biological macromolecules. And non-toxicity at lower concentrations is due to homeostasis in plant cells. However, based on the results, it is recommended that the effect of nano NiO with low concentrations and bulk NiO with mean concentrations should be evaluated to stimulate seed germination and growth of medicinal and aromatic plants, although further research is needed for explanation and development of such probabilities.

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