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Bryophyllum pinnatum Growth Response to Nickle Nanoparticles Foliar Spray

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Abstract. Nickel is an important and fundamental element for the growth and development of the plant. This study conducted to investigate the effect of different sized Ni nanoparticles (Ni NPs) with different concentrations on the physiological and anatomical characters of Brophyllum pinnatum seedlings. Four treatments used; 20 and 40 nm each with two concentrations; 0.20 and 0.40 ppm in four replications. Two doses of foliar application applied each after 30 days. The results showed that 40 nm/ 0.2 ppm spray had a promoter effect on plant height, shoot dry weight and chlorophyll content. While 20 nm/ 0.2 ppm act an inhibitor for all but stimulator for leaf area. Thus, adding an appropriate level of Ni NPs could improve general health, physiological growth and anatomical characters of Brophyllum pinnatum.

Keywords. Ni NPs, Chlorophyll, Biomass, Epidermis, Vascular bundles.

1. Introduction

Kurz or Bryophyllum pinnatum (Lam) belongs to the family of Crassulaceae. It is a widely cultivated, perennial plant adapted to live moist and warm a climate. It is a pant with a wonder or divine Leaf originated to Madagascar and become adapted to the tropical and subtropical life [1]. The challenges around the world to increase plant yield greatly rely on the type and dosage of fertilizer addition either by soil or by foliar spray. Water drainage in different types of soils due to gravity leached to the water body and cause eutrophication. That leads to low amount of nutrients in the soil. Thus, excess addition of nutrients to the plant will be necessary through leaves [2]. Foliar NPs have a promoter role in plants; increases the pest and diseases resistance, improves the quality of crops via increasing nutrient content and final increase of dry matter content and productivity [3]. Ni is an important micronutrient for plant, it is very crucial cellular redox state, biochemical and physiological growth responses [4]. As well as has an essential role in enzyme activities, because it's a partial active site of urease enzyme that hydrolyze urea in plant tissues. Urease act to metabolize the excess toxic amounts of urea into ammonia and N. Ammonia may be recycled to other metabolic synthesis pathways; amino acids, polyamines, and different nitrogen compounds [5]. Small quantities of Ni (0.01–5 lg/g dry weight) essential for some plant species as it is a component of the active site of urease. However, its High

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levels of Ni inhibit root apical meristem cells division and decrease plant growth in sensitive plants excess levels are phytotoxic for plants cause; chlorosis, necrosis and limit the Fe uptake and metabolism [6]. Cause alteration in chloroplast followed by a decrease in chlorophyll content and ultimately retard greatly the photosynthesis process that reduce yield and its quality [7]. Nanotechnology invention enhances the use of foliar spray of nanoparticles (NPs) on a wide range in agriculture. But high doses of NPs stressed the plant. Damage the membranous system in the cell and lead to electrolyte leakage. Reduce the chlorophyll and carotenoids contents. Induce oxidative stress and free radicle formation which influence the gene expression [8]. Qadir and Fathulla [9], showed that 30 ppm of sprayed nickel NPs (40 nm) caused reduction in shoot and root dry weight (g plant $^{-1}$) and root: shoot ratio of Phaseolus vulgaris L., while 70 nm at same concentration caused a significant reduction in relative water, chlorophyll and carotinoids content of Phasoelus vulgaris L. [10], concluded that 20 nm nickel nanoparticles with 20, 40 and 80 ppm had a phytotoxic effect on Coriandrm sativum leaves. [11] found that 1 mM NiSO4 solution decreased the mesophyll thickness, the size of vascular bundles and the vessel diameter in the main and lateral vascular bundles of Triticum aestivum L. [12] concluded that Ni at high concentrations had a detrimental effect on the growth of *Calendula officinalis*. Upon view of the cited evidence; Ni at low concentrations necessary for plant growth. It becomes phytotoxic at high doses, because it alters the metabolic and physiologic processes according to plant species, dosage and particle size of nano spray. The aim of the study to investigate the effect of different size and concentration of nickel nanoparticle foliar spray on the growth, physiological and anatomical behavior of *Brohyllum pinnatum* L. seedlings.

2. Materials and Methods

2.1. Preparation of Ni NPs

The Ni NPs were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The NPs size used in the experiment were; 20 and 40 nm of 99.5 % purity degree with a spherical shape. The applied concentrations; 0.20 and 0.40 ppm were prepared. Each of 2 and 4 mg Ni NPs were dispersed easily in distilled water. Their precipitation diminished by using ultra-sonicator and agglomerates that broke the precipitates with sufficient shaking at low teperature [10].

2.2. Planting and Harvesting

The seeds of common bean were planted in 20 pots of 6 kg soil previously sieved and prepared at glass house of college of Science- Salahaddin University- Erbil. In each pot 3 seeds were sowed. They were irrigated internally to its soil holding capacity for 4 months. After one month from seed sowing the seedlings of nearly 15 cm height were started to be foliar sprayed with 30 ppm of; 20, 40 and 70 nm Nickle nanoparticles. The plants were sprayed with two spraying through the entire life. Each spray applied after 30 days.

2.3. Experiment Design and Treatment Applied

The study was conducted to determine the effect of two size particles of Ni NPs; 20 and 40 nm each with two concentrations; 0.20 and 0.40 ppm on the growth and development of Brophyllum pinnatum seedlings. The experiment laid out in as pot experiment in the glass house of the biology department-College of Science at Salahaddin University- Erbil. Each pot filled with 4 kg of previously sieved loamy soil. The soil of each pot mixed with 100 g of peat moss. The seedlings aged about two months; two seedlings were transplanted to each pot. The seedlings were sprayed two times and the data obtained after 30 days of each spray. The experiment designed as a completely randomization (CR) with four replications. Duncan's multiple range test (DMRT) used to find pout the pair wise comparison among the average of the studied characters. The analysis of variance (ANOVA) and mean comparison computed by Statistical package for the Social Sciences (SPSS) model 26.

2.4. Studied Parameters

Physiological parameters: the physiological parameters obtained includes the visual plant growth characters; seedling height, leaf area and dry matter content (biomass): The soot system carefully

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washed with tap water and later washed with distilled water and then wiped. The shoots were dried by keeping them in an oven for 48 hours at 70ºC. Then the dry weight obtained by weighting them [9].
Chlorophyll content: chlorophyll a, b and carotenoids estimated as mentioned by [13]. About 0.3 g fresh leaves kept in 10 ml of absolute ethanol in dark bottles for 24 hours. The first 10 ml extracted separated. And another 10ml of ethanol added for 24 hours. The second extracted 10 ml taken after 24 hours too. And the third extraction process done for complete extraction (30 ml). The amount of chlorophyll a and b were measured using spectrophotometer by taking the absorbance of extracted samples on two wave lengths 649 nm and 665 nm as follows:

$$Chlorophyll\ a = (13.7)(A663\ nm) - (5.76)(A645\ nm)$$

$$Chlorophyll\ b = (25.8)(A645) - (7.60)(A663)$$

$$Total\ chlorophyll = chlorophyll\ a + chlorophyll\ b$$

2.5. Anatomical Characters
The sections prepared by Paraffin method: fresh samples of leaves and roots were kept in FAA. Dehydrated by a serial concentration of alcohol. They were cleared by keeping them in xylene for 3- 4 hours. Then infiltrated within xylene and paraffin for about 30 mints. After that transferred to pure paraffin wax and left overnight at 60 ^{O}C. The embedded samples were sectioned with the thickness of 8 µm by the rotary microtome. Lastly the sections were stained by using safranin and light green stains. Finally, the sections were mounted by DPX [14].

3. Results and Discussion

Physiological parameters: Ni is an important heavy metal for plant growth at lower concentrations, Ni nanoparticles can have positive effects on plant growth and development. As shown in figure (1), nickel nanoparticles affected significantly on shoot elongation. The highest mean value of the plant height was 29 cm after the first Ni spray 40 nm/ 0.4 ppm. While after 30 days from the second spray the highest height was 34 cm due to spraying 40 nm/ 0.2 ppm. Because of its large size penetrates in lower rate to the leaves. Which becomes a promoter to enhance the plant height. One possible explanation is that Ni NPs may have increased the activity of growth-promoting hormones such as auxins and gibberellin, which could have stimulated elongation of the stem and resulted in increased plant height [15]. The size of Ni nanoparticles can play a role in their phytotoxicity. It has been reported that smaller-sized nanoparticles can penetrate the plant tissues more easily than larger-sized ones, which may result in more severe toxic effects but [16]. The smaller sized Ni NPs (20 nm) mostly penetrates the plants and accumulates in high concentrations in the cells and becomes toxic. Thus, the lowest height; 19.83 cm recorded due to spraying with 20 nm/ 0.4 ppm after first spray. The same size 20 nm of Ni caused the significant decrease of the height (21.33 cm) but at 0.2 ppm after 30 days from second spray. In general, exposure to high concentrations due to their aggregation as a possible mechanism of their phytotoxicity and negative impact on plant growth and development, leading to stunted growth. Plant height is mainly determined by cell elongation, which involves processes such as cell wall expansion and turgor pressure. Ni NPs are known to induce oxidative stress, disrupt membrane integrity, and affect the uptake and transport of essential nutrients in plants, which can lead to inhibition of cell elongation and ultimately, stunted plant growth [17]. The results demonstrate the significant positive effect of the small sized Ni NPs (20 nm) but at low concentration act as growth promoter and increased the leaf area (45.33 cm^2) as shown in figure (2) but inhibitor for shoot length (figure 1) after thirty days from the second spray. As for why leaf area increased, it is possible that the Ni NPs had a greater effect on stem growth than on leaf growth. Alternatively, the plants may have compensated for the reduced stem growth by increasing leaf growth, which would maintain a constant leaf area despite the reduction in plant height [18]. It is important to note that the effects of Ni NPs on plant growth and development can be complex and depend on various factors. Further research is needed to understand the mechanisms underlying the observed changes in plant height and leaf area after the foliar spray of 20 nm Ni NPs. The application of 40 nm Ni NPs via foliar spray caused a significant increase in shoot dry weight (figure 3). The maximum shoot dry weight was 1.28 g due to spraying of 40 nm/ 0.2 ppm. Because Ni NPs can improve the absorption of nutrients, such as

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nitrogen, phosphorus, and potassium, from the soil and increase the efficiency of photosynthesis. This can lead to an increase in shoot biomass production [19]. Ni NPs can stimulate plant growth and development by acting as a bio stimulant. They can enhance the activity of enzymes involved in plant metabolism, leading to increased shoot biomass production. Vice versa the aggregation of small sized Ni NPs in plant cells due to their high penetration ability caused significant decrease in biomass production. That is why the lowest value of the shoot dry weight in the study recorded due to spraying 20 nm sized Ni NPs. Because they can generate reactive oxygen species (ROS) that can cause oxidative damage to plant cells. ROS can cause membrane damage, protein denaturation, and DNA fragmentation, which can lead to cell death and reduced plant growth [20]. Ni NPs may also interfere with the uptake and utilization of essential nutrients by the plants. This can lead to nutrient imbalances, which can affect plant growth and development. Spraying 40 nm/ 0.2 ppm Ni NPs caused a significant and highest increase in the chlorophyll a, b and total content of the leaves (table 1). By providing Ni NPs via foliar spray, the plants may have received a boost in their nickel uptake, which could have increased their overall nutrient availability and enhanced chlorophyll synthesis. It's possible that the presence of Ni NPs somehow reduces stress at low doses, which can in turn improve chlorophyll synthesis. Which may act as antioxidants to protect the plant from oxidative stress and other types of damage [15]. While 20 nm/ 0.4 ppm spray not harmed the leaf nor reduced Its chlorophyll content. That's why the chlorophyll content of the leaves not differed significantly with the control plant group when sprayed with 20 ppm. It is also possible that the plant's defense mechanisms were able to mitigate the effects of Ni NPs on chlorophyll content. Previous researches found the positive and negative effect of Ni on other plants; [9], showed its negative effect on Phaseolus vulgaris L. [10], found its phytotoxic effect on Coriandrm sativum. [11] its negative effect on Triticum aestivum L. Different plant species may respond differently to the presence of Ni NPs due to variations in their metabolic processes, growth patterns, and responses to environmental stressors. The duration of exposure is another factor that can influence the plant's response to Ni NPs. Longer exposure times may result in more significant changes in the plant's morphology, physiology, and anatomy. Additionally, some plants may be more tolerant of prolonged exposure to Ni NPs than others.

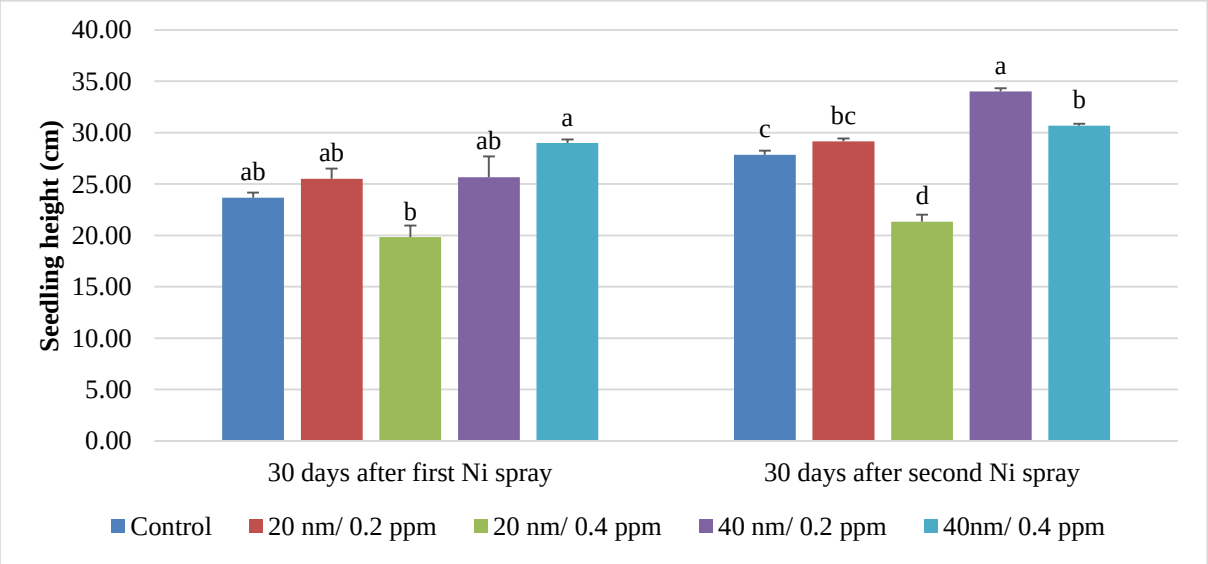

Figure 1. Effect of Ni NP treatments on *Bryophyllum pinnayum* height (cm) plants.

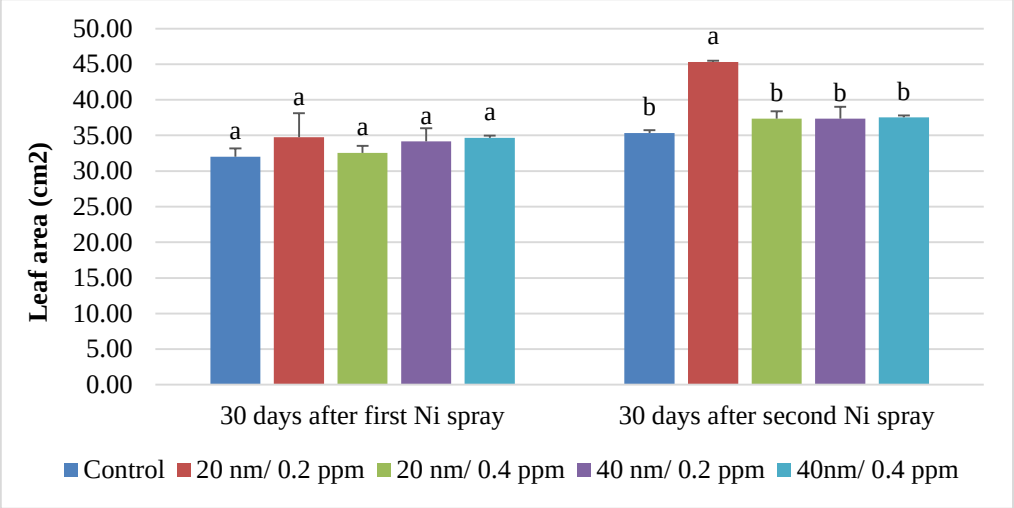

Figure 2. Effect of Ni NP treatments on leaf area (cm^2) of *Bryophyllum pinnayum.*

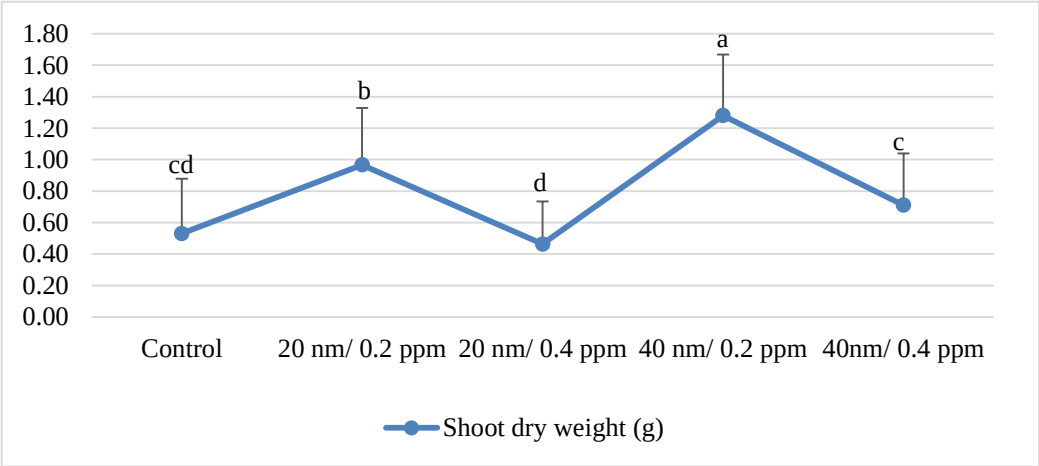

Figure 3. Effect of Ni NP treatments on shoot dry weight of (g) of *Bryophyllum pinnayum* plants.

Table 1. Effect of Ni NP treatments on chlorophyll content of *Bryophyllum pinnayum* leaves.

Ni NP treatments	Ch a (mg/ g F wt)	Ch b (mg/ g F wt)	Ch T (mg/ g F wt)
Control	0.39 ± 0.03 b	1.11 ± 0.12 b	1.50 ± 0.14 b
20 nm/ 0.2 ppm	0.50 ± 0.02 ab	2.03 ± 0.07 b	2.53 ± 0.09 b
20 nm/ 0.4 ppm	0.39 ± 0.08 b	1.41 ± 0.57 b	1.81 ± 0.65 b
40 nm/ 0.2 ppm	0.73 ± 0.05 a	3.92 ± 0.25 a	4.65 ± 0.28 a
40nm/ 0.4 ppm	0.28 ± 0.04 b	0.46 ± 0.04 b	0.75 ± 0.05 b

Table 2. Effect of Ni NP treatments on midrib anatomy of *Bryophyllum pinnayum.*

Anatomical parts	Ni NP treatments	30 days after 1st spray	30 days after 2nd spray
Diameter	Control	199.21 ± 7.33 b	202.98 ± 3.42 c
	20 nm/ 0.2 ppm	203.76 ± 3.23 b	352.21 ± 0.59 a
	20 nm/ 0.4 ppm	136.75 ± 20.74 c	349.70 ± 1.40 a
	40 nm/ 0.2 ppm	395.29 ± 40.44 a	337.75 ± 10.62 a
	40nm/ 0.4 ppm	164.03 ± 7.18 bc	314.67 ± 2.93 b
Vascular bundle	Control	33.37 ± 2.50 bc	36.92 ± 0.94 b
	20 nm/ 0.2 ppm	25.85 ± 1.90 c	56.54 ±2.97 a
	20 nm/ 0.4 ppm	37.24 ± 4.21 b	60.86 ± 2.58 a
	40 nm/ 0.2 ppm	60.23 ± 0.89 a	39.25 ± 2.78 b

Anatomical parts	Ni NP treatments	30 days after 1st spray	30 days after 2nd spray
Accessory vascular bundle	40nm/ 0.4 ppm	25.30 ± 1.93 c	39.25 ± 2.78 b
	Control	0.00 ± 0.00 c	0.00 ± 0.00 b
	20 nm/ 0.2 ppm	0.00 ± 0.00 c	0.00 ± 0.00 b
	20 nm/ 0.4 ppm	14.15 ± 0.33 b	0.00 ± 0.00 b
	40 nm/ 0.2 ppm	25.86 ± 0.88 a	13.19 ± 0.27 a
	40nm/ 0.4 ppm	0.00 ± 0.00 c	0.00 ± 0.00 b

Table 3. Effect of Ni NP treatments on lamina anatomy of *Bryophyllum pinnayum.*

Anatomical parts	Ni NP treatments	30 days after 1st spray	30 days after 2nd spray
Cuticle	Control	2.31 ± 0.26 a	3.40 ± 0.30 a
	20 nm/ 0.2 ppm	3.38 ± 0.68 a	3.55 ± 0.15 a
	20 nm/ 0.4 ppm	3.19 ± 0.84 a	3.15 ± 0.71 a
	40 nm/ 0.2 ppm	2.78 ± 0.33 a	2.40 ± 0.12 a
	40nm/ 0.4 ppm	3.19 ± 0.43 a	2.35 ± 0.14 a
Upper epidermis	Control	6.25 ± 0.57 c	6.61 ± 1.07 a
	20 nm/ 0.2 ppm	14.16 ± 2.09 a	10.52 ± 1.64 a
	20 nm/ 0.4 ppm	11.34 ± 1.60 ab	10.27 ± 2.13 a
	40 nm/ 0.2 ppm	8.96 ± 0.25 bc	10.86 ± 1.54 a
	40nm/ 0.4 ppm	7.97 ± 0.64 bc	9.73 ± 0.24 a
Lower epidermis	Control	10.08 ± 0.52 c	10.58 ± 1.02 a
	20 nm/ 0.2 ppm	14.49 ± 0.12 a	10.27 ± 0.31 a
	20 nm/ 0.4 ppm	11.32 ± 1.96 bc	7.31 ± 1.25 a
	40 nm/ 0.2 ppm	8.66 ± 0.12 c	7.37 ± 0.39 a
	40nm/ 0.4 ppm	14.00 ± 0.41 ab	10.66 ± 1.63 a
Vascular bundles	Control	15.57 ± 1.82 c	25.11 ± 3.16 a
	20 nm/ 0.2 ppm	13.06 ± 1.31 c	15.60 ± 0.20 bc
	20 nm/ 0.4 ppm	29.25 ± 4.95 b	22.41 ± 2.91 ab
	40 nm/ 0.2 ppm	40.92 ± 0.71 a	20.44 ± 1.53 bc
	40nm/ 0.4 ppm	14.57 ± 1.43 c	14.19 ± 1.30 c
Mesophyll layer	Control	172.09 ± 13.63 b	160.20 ± 12.51 b
	20 nm/ 0.2 ppm	168.82 ± 15.16 b	159.38 ± 17.30 b
	20 nm/ 0.4 ppm	140.12 ± 11.98 b	156.88 ± 3.12 b
	40 nm/ 0.2 ppm	322.07 ± 16.25 a	221.52 ± 12.94 a
	40nm/ 0.4 ppm	143.56 ± 4.51 b	173.17 ± 12.94 b

Table 4. Effect of Ni NP treatments on stem anatomy of *Bryophyllum pinnayum.*

Anatomical parts	Ni NP treatments	30 days after 1st spray	30 days after 2nd spray
Epidermis	Control	11.01 ± 1.07 a	9.71 ± 0.65 b
	20 nm/ 0.2 ppm	10.69 ± 1.21 a	10.99 ± 1.50 b
	20 nm/ 0.4 ppm	11.52 ± 0.09 a	11.66 ± 0.87 b
	40 nm/ 0.2 ppm	8.91 ± 0.12 a	15.20 ± 0.41 a
	40nm/ 0.4 ppm	9.62 ± 1.06 a	11.16 ± 0.31 b
Phloem	Control	4.79 ± 0.13 d	7.76 ± 0.94 a
	20 nm/ 0.2 ppm	7.32 ± 0.49 bc	7.48 ± 0.82 a
	20 nm/ 0.4 ppm	9.56 ± 0.21 a	7.84 ± 0.61 a
	40 nm/ 0.2 ppm	7.94 ± 1.2 ab	7.47 ± 0.42 a
	40nm/ 0.4 ppm	5.80 ± 0.08 cd	7.70 ± 0.58 a
Xylem	Control	10.32 ± 1.22 c	16.34 ± 2.64 b
	20 nm/ 0.2 ppm	19.45 ± 0.36 a	19.49 ± 0.63 b
	20 nm/ 0.4 ppm	15.34 ± 0.50 b	17.166 ± 0.29 b
	40 nm/ 0.2 ppm	12.93 ±0.59 b	30.49 ± 3.62 a
	40nm/ 0.4 ppm	15.38 ± 1.06 b	18.92 ± 0.26 b
Fiber	Control	8.46 ± 0.59 c	12.61 ± 1.07 bc
	20 nm/ 0.2 ppm	16.71 ± 1.32 a	16.18 ± 1.68 ab
	20 nm/ 0.4 ppm	13.18 ± 0.79 ab	17.06 ± 0.65 a

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Anatomical parts	Ni NP treatments	30 days after 1st spray	30 days after 2nd spray
Cortex	40 nm/ 0.2 ppm	11.33 ± 1,97 bc	13.68 ± 1.29 abc
	40nm/ 0.4 ppm	14.68 ± 1.27 ab	11.37 ± 0.94 c
	Control	109.11 ± 1.10 b	125.85 ± 0.63 b
	20 nm/ 0.2 ppm	122.28 ± 3.27 ab	121.53 ± 3.91 b
	20 nm/ 0.4 ppm	116.77 ± 1.12 ab	111.28 ± 1.72 c
	40 nm/ 0.2 ppm	127.53 ± 3.27 a	145.89 ± 2.60 a
	40nm/ 0.4 ppm	109.21 ± 8.81 b	123.40 ± 3.00 b

Conclusion

The nickel nanoparticles with small diameter (20) nm showed a phytotoxic effect. While the large dimension of the Ni NPs (40 nm) act as an essential promoter element for studied physiological and anatomical properties of *Bryophyllum pinnatum*. However, underlying precise mechanisms should be investigated in future studies.

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