PHYSIOLOGICAL AND ANATOMICAL RESPONSES OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) TO NICKLE NANOPARTICLES FOLIAR SPRAY

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ABSTRACT

Nickel is an essential nutrient for plant growth with low concentrations, its excessive amounts in soil above threshold values could be cause in toxicity. The main objectives of the present research were to determine the effects of nickel nanoparticles foliar spray with 20, 40 and 70 nm diameter on the physiological characters and anatomical aspects of *Phaseolus vulgaris* L. plants. Lowest reduction significantly ($P < 0.01$) in root and shoot biomass was recorded due to in 70 nm; the measurements 0.08 and 0.05 g per plant and highest root: shoot; 0.65 as compared with control treatment. As well as the lowest conserved water content; 40% was observed in size 70 nm. While in size 40 nm Nickle nanoparticles increased chlorophyll a, b, total and carotenoids pigment contents. When the nickel nanoparticles size increased, the shoot and root tissue Ni concentrations also increased. However, the rate of Ni in root was greater than that observed in the shoot. While the Mn, Fe, Cu and Zn levels decreased due to applying nickel nanoparticles. The size of nanoparticles effects on the anatomical characteristics or structures such as stem, and leaf, also effects on the size of stomata.

Key word: Nickel nanoparticle, Relative water content, chlorophyll, glandular trichrome, Xylem, Fiber

الاستجابات الفسيولوجية والتشريحية للفاصوليا الشائعة (*Phaseolus vulgaris* L.) لرش الورقي بجزيئات النيكل النانوية.

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المستخلص

على الرغم من أن النيكل عنصر غذائي أساسي لنمو النبات بتراكيز قليلة، إلا أن كميته الزائدة في التربة التي تتجاوز القيم المحددة يمكن أن تؤدي إلى السمية. تتمثل الأهداف الرئيسية للبحث الحالي في تحديد تأثير رش جزيئات النيكل النانوية بقطر 20 و 40 و 70 نانومتر على الصفات الفسيولوجية والجوانب التشريحية لنبات *Phaseolus vulgaris* L. تم تسجيل أقل انخفاض معنوي ($P <0.01$) في الكتلة الحيوية للجذور والبراعم بسبب انه في 70 نانومتر، القياسات 0.08 و 0.05 غم لكل نبات وأعلى جذر: ساق. 0.65 مقارنة بمعاملة السيطرة. وكذلك أقل محتوى مائي محفوظ ؛ 40٪ لوحظ في حجم 70 نانومتر. بينما زادت في حجم 40 نانومتر من جسيمات النيكل النانوية محتوى الكلوروفيل أ ، ب ، الكلوروفيل الكلي والكاروتينات. مع زيادة حجم الجسيمات النانوية للنيكل ، زادت أيضًا تراكيز النيكل في أنسجة الجذع والنبتة. ومع ذلك ، فإن معدل نسبة النيكل في جذر كان أكبر مما كان في الاجزاء الخضرية او الساق .بينما انخفضت مستويات المنغنيز والحديد والنحاس والزنك بسبب معاملة النبات بجزيئات النيكل النانوية. يؤثر حجم الجسيمات النانوية على الخصائص أو الصفات التشريحية مثل الساق والأوراق ، كما يؤثر على حجم الثغور او الخلايا الحارسة.

الكلمات المفتاحية: جزيئات نيكل النانوية، محتوى الماء النسبي, الكلوروفيل, الشعيرات الغدية, الصفات التشريحية للورقة, الخشب, الالياف

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INTRODUCTION

Common bean, *Phaseolus vulgaris* L., Fabaceae, is a widespread an important seed legume. Its fresh pods or dry seeds are rich of protein, dietary fiber, starch and minerals such as potassium, thiamine, vitamin B6 and folic acid (27). The demand in increasing crop productivity depends at great extent on the type of supplemental fertilizer to the essential growth nutrients of plants (2). Environmental factors that constrain bean production in most areas included nitrogen deficiency, soil acidity and drought (5). Many farmers overcome the problem by using different mineral or organic fertilizers (3, 31). The use of chemical or organic fertilizer, cause several issues; massive amount application results in soil and ground water pollution, insufficiency of micronutrients, and soil degeneration, eventually leading to reduce product quality **(**13**).** The crucial role of the micronutrients related to its impact to prompt photosynthesis and directly cause a positive impact on the crop yield (9). The prevalent characterization of nanotechnology is manipulation of matter on molecular, supra-molecular and atomic scale. Most nanoparticles are made up of little hundred atoms with one dimension sized from 1 to 100 nanometers. The nanoparticles are being used to improve or replace today's treatments (1). Because of the nanotechnology is recently area and wide variety applications in all forms of industries, from textiles to medicine, biomedical and treatments that related products, and electronics, everybody is based on nanotechnology (4, 6, 12). Newly, the continuous advance of fertilization technology introduces the nano-fertilizers; with at least one dimension between 1-100 nm. They possess small size and large surface to volume ratio give them unique and remarkable characters to be used in wide application areas; including food and biomedical sciences, gene therapy, drug delivery, and cell targeting. As well as it has had remarkable effects on plant growth and development (29). Nickle is a component of urease enzyme and it regarded as one of the important micronutrients for plant growth. Despite the necessity of Ni, its present in excess amount is toxic (28). Beside their beneficial effects on plant growth, the inverse effects should be also debated. Plant growth and development, including the physiology of acquisition of vital resources (water, minerals and carbon), basic anatomy of vascular plants, and how they grow and reproduce. Overview of the processes associated with the uptake and transport of water and mineral nutrients in plantsand the responses of plants to external stimuli and adverse growth conditions. The plant anatomy remains highly relevant to systematics, paleobotany, and the relatively new science of developmental genetics, which interfaces disciplines and utilizes a combination of techniques to examine gene expression in growing tissues (26). This study was aimed to determine the effects of nickel nanoparticles on the physiological growth and anatomical characters of common bean (*Phaseolus vulgaris* L.) plant. As well as its toxicity to be studied while it has been applied as a foliar spray with different diameters; 20, 40 and 70 nm as a nanoparticle.

MATERIALS AND METHODS

Nanoparticle preparation: The nickel nanoparticles were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Average particle sizes of experimental nanoparticle were; 20, 40 and 70 nm with 99.5% purity. The shape of Ni nanoparticles was spherical. The nanoparticles were weighed and dispersed in distilled water at concentrations 30 ppm. The nanoparticles were dispersed easily without precipitation by ultra-sonicator and agglomerates were broken up with sufficient shaking (18).

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.

Plant Sampling

Sixty days after emergence, whole plants were sampled. The plants were at the phenological phase of full development. The fresh samples were used to quantify the physiological and

anatomical characters. While the dried samples used to determine the mineral content. Five repetitions per treatment were used for each variable analyzed.

Experiment design and data analyses

The Complete Randomized based to design (CRD) the study in the nature of three dimensions of Nickle nanoparticle: 20, 40 and **70** nm given as a **foliar** spray. Each with **five** replications. The mean values were compared using Duncan's multiple range test DMRT. IBM SPSS Statistics version 25 was used to analyze the data.

Experimental traits

Physiological parameters:

Biomass: The whole plant become uprooted through pouring water into the plant's pot; roots had been cautiously wiped clean with tap water and later washed with distilled water then separated into shoot and root. The shoot and root have been dried in an oven at (70ºC) for 48hrs, then dry weight had been measured and the ratio of shoot dry weight (g plant^{-1})/ root dry weight (g plant^{-1}) was calculated (23). Then Root: shoot dry weight ratio plant^{-1} was calculated according to the following formula:

$$\boldsymbol{Root{:}shoot\ ratio} = (\boldsymbol{root\ dry\ weight}) / (\boldsymbol{shoot\ dry\ weight})$$

Relative water content (RWC)

The aerial parts and roots, their fresh weight (FW), dry weight (DW) and turgid weight (TW) were determined to evaluate the relative water content of the plants. Immediately after harvesting, the plants were weighed to obtain fresh weight. The plants were soaked in distilled water in the dark for 24h at 4°C to measure turgid weight. Then, they left at room temperature for 48 hours until completely dried and weighed to obtain dry weight (30).

$$\boldsymbol{RWC}\ (\%) = (\boldsymbol{Fresh\ weight} - \boldsymbol{dry\ weight}) (\boldsymbol{turgid\ weight} - \boldsymbol{dry\ weight}) \times \mathbf{100}$$

Photosynthetic pigment

From the aerial parts 300 mg were weighed and added to 25 ml of 80 % acetone. The solution was centrifuged at 4800 rpm for 20 minutes. The absorbance of supernatant was measured to examine the contents of chlorophyll a, b and carotenoids. The absorbance of the supernatant was read at 663, 645, and 470 nm (17). The following formula was used to calculate the pigment concentrations:

Chlorophyll a (µg/ml) = 12.25 × A663 – 2.79× A645

Chlorophyll b (µg/ml) = 21.5 × A645 – 5.1 × A663

Carotinoids (µg/ml) = (1000 ×A470 – 1.82 ×Chlorophyll a – 85.02 × Chlorophyll b)/198

Shoots and root nutrient concentrations:

Center analyzed by using an Energy-Dispersive x-Ray Fluorescenea analyzer CIT-300 SMP in research central laboratory of Agricultural Engineeering Science College-Salahaddin University- Erbil.

Anatomical characters

Prepare the sections (Paraffin method)

The fresh samples collected and kept in FAA. Dehydrated by a series concentration of alcohol then the samples were cleared by xylene (3-4 hrs.). After that infiltrated within xylene and paraffin for 30 min then transferred to pure paraffin wax and left overnight at 60^{O}C. Respectively embedded in paraffin wax and sections were prepared with the thickness of 8 µm by rotary microtome. Then the sections were stained with safranin and light green. Finally, the sections were mounted by DPX (21).

RESULTS AND DISCUSSION

Physiological parameters

Biomass: Nickel was found to be quite toxic to the growth of bean plant. The results summarized in Figure 1 show that nickel nanoparticles; 20, 40 and 70 nm decreased the root and shoot biomass of common bean plants significantly ($P < 0.01$) as compared to control treatment. The sharpest decrease was observed when 70nm Ni nanoparticles used at 30 ppm; 0.08 and 0.05 g per plant for shoot and root dry weights. While highest root: shoot; 0.65 due do the great loss of the shoot biomass. Jiang et al. (14) studied the toxicity of AgNO3 and silver nanoparticles on *Spiradelha polyrhiza*. As well as Miri et al. (18) revealed that as the concentration of nickel nanoparticles increases, the root and shoot dry weights decreased in *Coriandrum sativum* L. Reductions in shoot growth associated with Ni toxicity is generally thought to be twofold: (1) direct Ni toxicity, and (2) a Ni-induced deficiency of Fe or some other essential element (19). Nickel-induced Fe deficiencies have been reported by numerous authors, with younger leaves of plants suffering Ni toxicity showing an interveinal chlorosis (16, 22). In addition, the observed reductions in root mass possibly the result of a decrease in the translocation of carbohydrates to the roots as suggested by Baccouch et al. (7). The toxicity of nickel nanoparticles has been observed by other researchers; Gong et al. (11) have determined the toxicity of NiO nanoparticles at concentrations 10-

50 mg/L on *Chelorella vulgaris*. They suggested that the phytotoxicity of nanoparticle belongs to aggregation mechanism of the nanoparticles, which caused reduction in water uptake (10).

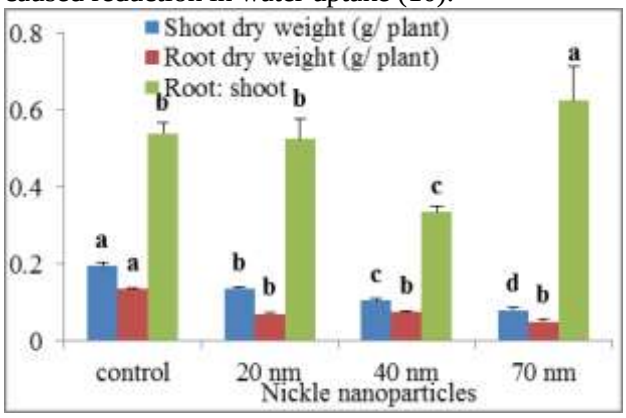

Figure 1. Effect of Nickle nanoparticles foliar spray on shoot and root dry weight (g plant $^{-1}$) and root: shoot ratio in *Phasoelus vulgaris* L. plant

Relative water content (RWC)

Relative water content (RWC) in plant cells regarded as good index to demonstrate the amount of water conserved in plant. Figure 2 shows a decline in the RWC of the common bean plants due to spraying with Ni nanoparticles. The lowest conserved water content; 40% was observed due to 70 nm Ni nanoparticle foliar spray. Many authors reported that Ni induced the decline in plant transpiration and water content. Therefore, higher the measured amount, the greater the ability of the treatment to preserve water and tolerate the Nickle nanoparticles toxicity (20). Miri et al. (18) as well as observed the decline of relative water content of *Coriandrum sativum* L. plants with increasing the of concentration nickel nanoparticles.

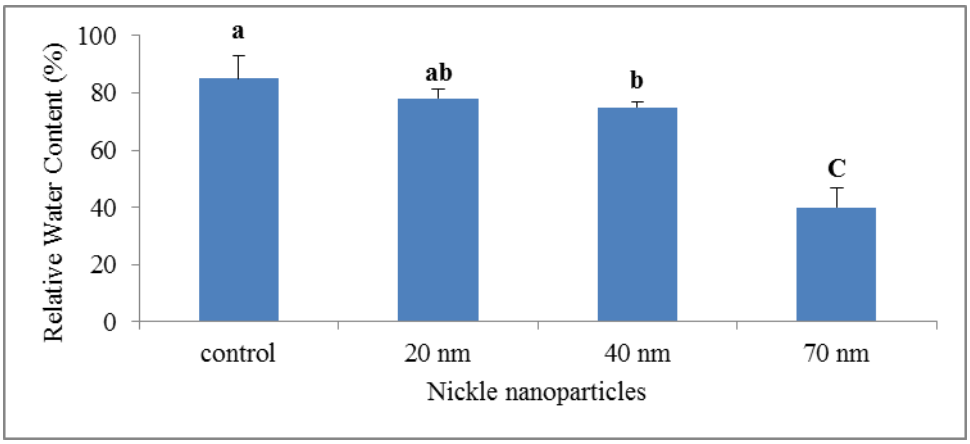

Figure 2. Effect of Nickle nanoparticles foliar spray on Relative Water Content (RWC %) in *P. vulgaris* plant

Photosynthetic pigment

Table (1) shows the effects of nickel nanoparticles on photosynthetic pigment contents, including chlorophyll a, b, total and carotenoids. Nickel nanoparticle with 40 nm diameter increases the photosynthetic pigment contents. The level of carotenoids was almost the same increased to spraying with 40nm Ni nanoparticles as compared to control plants. However, it decreased at 20 and 70 nm. This trend has also been observed by other research groups (15, 18). There are several proposed mechanisms suggested that different nanoparticles cause damages in the photosynthetic process. Thus, plants stimulate the translation of responsible genes for production of more pigments to make a balance and cellular homeostasis. Type of nanoparticle, plant species, size and shape of nanoparticles are other factors influencing this phenomenon.

Table1. Effect of Nickle nanoparticles foliar spray on photosynthetic pigment content (mg g^{-1} fresh weight) in *P. vulgaris* leaves

Nickle nanoparticles (nm)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Control	1.83 ± .04 a	0.53 ± 0.033 a	2.35 ± 0.067 a	0.67 ± 0.075 a
20 nm	1.44 ± .04 b	0.43 ± 0.008 b	1.86 ± 0.040 b	0.41 ± .003 ab
40 nm	2.55 ± 0.12 c	0.78 ± 0.010 c	3.33 ± 0.106 c	0.56 ± 0.008 b
70 nm	0.87 ± .05 d	0.25 ± .0080 d	1.12 ± 0.048 d	0.23 ± 0.007 c

Shoots and root nutrient concentrations

As the nickel nanoparticles diameter increased, the shoot and root tissue Ni concentrations also increased (Table 2). However, the rate of increases in root Ni was greater than that observed in the shoot Ni. The rate of; Mn, Fe, Ni, Cu and Zn was decreased due to applying nickel nanoparticles. One of the probable mechanisms for decreasing the uptake of macro- and micronutrients relies on the competition for the common binding sites due to the comparable ionic radii of Ni2+ and other cations (8). The decline in nutrient uptake may also result from the Ni-induced metabolic disorders that affect the structure and enzyme activities of cell membranes58. Thus, Ni2+ affected the sterol and phospholipid composition of the plasma membrane in *Oryza sativa* shoots, with concomitant changes in the ATPase activity59. Apparently, these changes affected the membrane permeability and in this way changed the ion balance in the cytoplasm. The effects of Ni on nutrient uptake depend in many aspects on Ni concentration in the environment (25).

Table 2. Effect of Nickle nanoparticles foliar spray on shoots and root nutrient concentrations in *P. vulgaris* plants

Plant parts	Nickle nanoparticles (nm)	Nutrient concentrations				
		Mn (ppm)	Fe (%)	Ni (ppm)	Cu (ppm)	Zn (ppm)
Root	Control	159.90	2.76	250.62	143.77	230.98
	20 nm	0.00	0.64	2070.10	95.39	181.94
	40 nm	0.00	0.38	1336.75	119.40	170.35
	70 nm	0.00	0.14	2070.10	95.40	181.94
Shoot	Control	137.00	1.47	129.49	152.25	157.82
	20 nm	0.00	0.73	219.48	80.53	126.69
	40 nm	0.00	1.03	404.77	88.90	145.52
	70 nm	0.00	1.09	1004.67	50.90	114.23

Anatomical characters

This research revearls that the nickle nanoparticle effect on anatomical characters such as stem, leaf, petiol, and number of ordinary epidermal cells with stomata (Table 3, 4, 5, 6 and 7). The transfervers section that the taken from stem after 45 dayes with 70nm Nickle nanoparticles, shown presence the starch seeds. The small sized stomata were observed in 40 and 70nm after 15 days, while after one month observed in 70nm as compare with control (Figure 3). The plants cant tolerate the 70nm after 2 months and died. The nanoparticles effect on plants takes place in physiological, morphological, and genotoxic changes. It is important to know the role of certain nanoparticle, for the effective use of nanotechnology in agriculture (24). Nanoparticles overlay a heterogeneous range of materials, but only a little of them are extensively used and now the environment is at risk when exposed to these materials. Metal and metal oxide nanoparticles such as titanium dioxide, silver, zinc oxide, cerium dioxide, copper, copper oxide, aluminum, nickel, and iron are most commonly used in industries and thus are mostly studied to observe the impacts on different plants. Several of non-metal nanoparticles as single-walled carbon nanotubes and fullerene have been well studied to reveal their Nano-toxicity mechanisms (24). Recently, converged on the potential toxicological effects of metal nanomaterials on the animals, humans and the environment through the exposure of them (18).

Table 3. Effect of Nickle nanoparticles foliar spray on leaf anatomy of *P. vulgaris* plants

Anatomical leaf parts	Nickle nanoparticles (nm)	After 15 days	After 30 days	After 45 days	After 60 days
Lamina cuticle	Control	1.99 ± 0.12 a	2.36 ± 0.28 a	2.35 ± 0.24 a	2.35 ± 0.19 a
	20 nm	1.57 ± 0.22ab	2.16 ± 0.22 a	1.39 ± 0.31 b	1.80 ± 0.11 b
	40 nm	1.47 ± 0.13 b	2.27 ± 0.13 a	1.83 ± 0.01 ab	1.53 ± 0.08 b
	70 nm	1.35 ± 0.08 b	1.17 ± 0.04 b	1.87 ± 0.17 ab	0.00 ± 0.00 c
Upper epidermis	Control	7.40 ± 0.62 ab	5.85 ± 0.30 b	5.94 ± 0.68 b	4.54 ± 0.32 b
	20 nm	8.33 ± 0.68 a	6.09 ± 0.28 ab	5.19 ± 0.69 b	5.89 ± 0.39 a
	40 nm	7.90 ± 0.86 ab	7.14 ± 0.06 a	7.91 ± 0.46 a	5.71 ± 0.10 a
	70 nm	5.81 ± 0.05 b	6.74 ± 0.46 ab	9.13 ± 0.51 a	0.00 ± 0.00 c
Lower epidermis	Control	4.54 ± 0.32 b	5.47 ± 0.38 a	2.91 ± 0.18 b	3.94 ± 0.67 a
	20 nm	5.88 ± 0.39 a	4.30 ± 0.51 ab	3.40 ± 0.11 b	4.07 ± 0.11 a
	40 nm	5.71 ± 0.10 a	4.94 ± 0.71 ab	6.00 ± 0.17 a	4.74 ± 0.06 a
	70 nm	0.00 ± 0.00 c	3.59 ± 0.29 b	5.43 ± 0.32 a	0.00 ± 0.00 b
Vascular bundles	Control	5.28 ± 0.28 c	6.28 ± 0.40 b	6.55 ± 0.23 c	11.01 ± 0.25 a
	20 nm	10.58 ± 0.15 a	11.88 ± 1.46 a	7.47 ± 0.21 bc	6.47 ± 0.56 b
	40 nm	7.55 ± 1.71 b	14.26 ± 0.46 a	9.32 ± 0.32 ab	9.901 ± 0.78 a
	70 nm	7.02 ± 1.70 b	8.83 ± 0.12 b	8.43 ± 0.45 a	0.00 ± 0.00 c
Glandular trichomes	Control	18.09 ± 0.97 a	13.37 ± 1.26 a	6.09 ± 0.36 b	10.16 ± 0.44 b
	20 nm	12.98 ± 2.56 b	9.87 ± 1.71 ab	6.82 ± 0.04 ab	6.94 ± 0.44 c
	40 nm	9.40 ± 0.85 b	11.61 ± 1.51 ab	8.99 ± 0.40 ab	12.35 ± 0.30 a
	70 nm	8.98 ± 0.35 b	8.61 ± 0.08 b	10.22 ± 2.10 a	0.00 ± 0.00 d
Non-glandular trichomes	Control	17.33 ± 1.58 b	51.77 ± 15.13 a	12.66 ± 0.61 b	11.41 ± 0.35 bc
	20 nm	26.76 ± 0.72 ab	19.99 ± 6.79 b	34.59 ± 3.38 a	29.58 ± 0.37 a
	40 nm	31.66 ± 2.62 a	13.70 ± 0.26 b	31.70 ± 1.49 a	23.67 ± 7.96 ab
	70 nm	26.51 ± 5.80 ab	15.34 ± 0.74 b	28.66 ± 3.71 a	0.00 ± 0.00 c
Mesophyll layer	Control	26.15 ± 2.00 b	45.80 ± 2.17 ab	39.07 ± 0.68 a	47.12 ± 1.98 b
	20 nm	40.36 ± 1.40 a	44.37 ± 3.98 ab	33.49 ± 0.16 d	20.63 ± 0.18 c
	40 nm	41.55 ± 3.06 a	48.93 ± 0.71 a	54.91 ± 1.69 a	51.38 ± 1.86 a
	70 nm	36.15 ± 0.90 a	39.52 ± 0.03 b	44.95 ± 2.00 b	0.00 ± 0.00 d

Table 4. Effect of Nickle nanoparticles foliar spray on stem anatomy of *P. vulgaris* plants

Anatomical leaf parts	Nickle nanoparticles (nm)	After 15 days	After 30 days	After 45 days	After 60 days
Epidermis	Control	2.86 ± 0.39 a	3.05 ± 0.45 a	3.38 ± 0.26 a	3.05 ± 0.16 a
	20 nm	2.82 ± 0.45 a	2.34 ± 0.27 a	2.14 ± 0.06 b	1.90 ± 0.05 b
	40 nm	2.82 ± 0.45 a	1.76 ± 0.16 a	1.72 ± 0.05 b	3.19 ± 0.08 a
	70 nm	2.80 ± 0.09 a	2.17 ± 0.62 a	2.12 ± 0.03 b	00.00 ± 00.00 d
Phloem	Control	15.30 ± 1.32 a	14.61 ± 2.61 a	9.11 ± 0.45	10.72 ± 1.45 a
	20 nm	15.30 ± 1.32 a	17.05 ± 0.92 a	13.47 ± 2.88 a	11.20 ± 0.67 a
	40 nm	9.95 ± 0.25 b	13.07 ± 0.46 a	9.47 ± 1.62 a	10.04 ± 0.43 a
	70 nm	15.60 ± 1.11 a	8.68 ± 0.42 b	10.22 ± 1.07 a	00.00 ± 00.00 d
Xylem	Control	18.40 ± 5.97 b	32.37 ± 4.78 a	18.64 ± 0.26 b	21.44 ± 2.44 a
	20 nm	26.67 ± 0.91 ab	31.00 ± 0.74 a	28.13 ± 1.88 a	10.04 ± 0.28 b
	40 nm	24.98 ± 1.28 ab	20.98 ± 0.93 b	31.40 ± 3.10 a	10.82 ± 0.67 b
	70 nm	35.56 ± 1.12 a	28.25 ± 2.27 ab	29.97 ± 1.42 a	0.00 ± 0.00 c
Fiber	Control	8.60 ± 1.71 a	7.69 ± 0.15 a	4.90 ± 0.56 ab	4.26 ± 0.43 a
	20 nm	5.24 ± 0.04 b	4.30 ± 0.11 b	4.31 ± 0.12 b	3.73 ± 0.37 a
	40 nm	4.15 ± 0.31 b	4.50 ± 0.64 b	5.51 ± 0.40 ab	4.05 ± 0.56 a
	70 nm	3.56 ± 0.44 b	3.89 ± 0.62 b	5.98 ± 0.24 a	00.00 ± 00.00 b
Trichomes	Control	15.36 ± 0.48 a	17.34 ± 2.55 a	15.82 ± 0.61 a	11.78 ± 2.36 a
	20 nm	4.34 ± 0.17 b	6.46 ± 0.23 b	4.43 ± 0.34 b	12.89 ± 0.75 a
	40 nm	3.75 ± 0.72 b	7.02 ± 0.60 b	7.85 ± 1.50 b	00.00 ± 00.00 b
	70 nm	00.00 ± 00.00 c	4.73 ± 0.10 b	13.07 ± 2.58 a	00.00 ± 00.00 b
Cortex	Control	63.80 ± 5.99 a	39.44 ± 0.58 b	18.10 ± 0.85 a	22.08 ± 0.24 b
	20 nm	37.00 ± 15.83 ab	21.63 ± 0.45 c	20.35 ± 0.64 a	61.35 ± 1.59 a
	40 nm	28.47 ± 1.16 b	24.85 ± 1.36 c	24.18 ± 0.78 a	19.31 ± 0.56 b
	70 nm	35.74 ± 0.96 ab	65.55 ± 4.92 a	25.46 ± 0.84 a	00.00 ± 00.00 c

Table 5. Effect of Nickle nanoparticles foliar spray on petiole anatomy of *P. vulgaris* plants

Anatomical leaf parts	Nickle nanoparticles (nm)	After 15 days	After 30 days	After 45 days	After 60 days
Non-glandular trichomes	Control	12.39 ± 0.33 a	19.24 ± 0.27 a	3.09 ± 0.04 b	12.88 ± 0.20 a
	20 nm	8.21 ± 0.48 b	9.96 ± 0.51 c	9.51 ± 1.46 a	12.25 ± 0.37 a
	40 nm	12.39 ± 0.33 a	19.24 ± 0.27 a	3.09 ± 0.04 b	12.88 ± 0.20 a
	70 nm	8.21 ± 0.48 b	9.96 ± 0.51 c	9.51 ± 1.46 a	12.25 ± 0.37 a
Glandular-trichomes	Control	2.61 ± 0.36 b	3.25 ± 0.13 b	2.89 ± 0.19 b	2.67 ± 0.40 b
	20 nm	3.94 ± 0.53 b	7.83 ± 1.34 a	00.00 ± 00.00 c	4.05 ± 0.36 a
	40 nm	27.47 ± 1.27 a	00.00 ± 00.00 c	4.91 ± 0.62 a	00.00 ± 00.00 c
	70 nm	00.00 ± 00.00 c	3.44 ± 0.21 b	00.00 ± 00.00 c	00.00 ± 00.00 c
Xylem	Control	22.33 ± 0.37 a	17.07 ± 2.82 ab	12.80 ± 0.08 d	17.56 ± 0.24 a
	20 nm	22.10 ± 1.76 a	14.46 ± 0.33 b	16.75 ± 0.66 c	14.73 ± 0.72 b
	40 nm	23.20 ± 0.32 a	21.35 ± 0.89 a	20.98 ± 0.58 a	17.30 ± 0.53 a
	70 nm	22.28 ± 0.40 a	16.45 ± 0.83 ab	18.87 ± 0.60 b	00.00 ± 00.00 c
Fiber	Control	2.67 ± 0.10 b	2.64 ± 0.32 a	3.30 ± 0.06 a	4.27 ± 0.05 a
	20 nm	3.49 ± 0.13 a	3.11 ± 0.47 a	3.56 ± 0.18 a	1.74 ± 0.12 c
	40 nm	2.65 ± 0.32 b	3.24 ± 0.06 a	2.43 ± 0.24 b	2.60 ± 0.24 b
	70 nm	2.95 ± 0.14 ab	3.34 ± 0.10 a	2.48 ± 0.21 b	00.00 ± 00.00 d
Accessory vascular bundles	Control	19.67 ± 0.52 b	20.91 ± 0.47 b	18.50 ± 0.90 b	21.68 ± 0.10 a
	20 nm	28.3 ± 0.90 a	19.00 ± 0.49 b	21.98 ± 0.25 a	23.02 ± 4.20 a
	40 nm	14.85 ± 0.90 c	24.85 ± 1.60 a	21.41 ± 0.62 a	19.58 ± 0.41 a
	70 nm	20.45 ± 1.10 b	14.17 ± 0.32 c	18.29 ± 0.54 b	00.00 ± 00.00 b
Phloem	Control	5.59 ± 0.29 b	5.81 ± 0.53 c	5.13 ± 0.31 b	6.71 ± 0.08 b
	20 nm	6.98 ± 0.62 b	4.21 ± 0.05 b	6.39 ± 0.22 a	5.00 ± 0.55 b
	40 nm	5.56 ± 0.14 b	7.78 ± 0.17 a	7.06 ± 0.59 a	8.42 ± 0.62 a
	70 nm	9.77 ± 0.91 b	4.52 ± 0.13 c	6.69 ± 0.28 a	00.00 ± 00.00 c
Diameter	Control	183.30 ± 2.65 c	212.45 ± 3.90 a	148.16 ± 0.87 c	164.49 ± 1.22 a
	20 nm	210.04 ± 3.91 ab	165.27 ± 0.53 b	190.93 ± 2.49 a	137.81 ± 0.71 c
	40 nm	228.34 ± 9.90 a	186.88 ± 2.71 c	163.27 ± 3.69 b	152.58 ± 0.69 b
	70 nm	205.85 ± 2.66 b	148.28 ± 1.09 d	146.95 ± 2.26 c	00.00 ± 00.00 d

Table 6. Effect of Nickle nanoparticles foliar spray on midrib anatomy of *Phasoelus vulgaris* L. plants

Anatomical leaf parts	Nickle nanoparticles (nm)	After 15 days	After 30 days	After 45 days	After 60 days
Trichomes	Control	23.17 ± 10.44 ab	23.58 ± 0.53 a	26.69 ± 4.44 a	9.79 ± 0.17 c
	20 nm	38.52 ± 2.98 a	14.83 ± 0.74 b	8.39 ± 0.71 b	22.74 ± 1.01 b
	40 nm	30.50 ± 7.22 ab	00.00 ± 00.00 d	31.87 ± 0.79 a	72.54 ± 1.17 a
	70 nm	11.58 ± 5.80 b	6.07 ± 0.35 c	19.97 ± 5.69 ab	00.00 ± 00.00 d
Vascular bundles	Control	41.03 ± 0.39 b	31.39 ± 1.55 a	33.14 ± 0.84 c	56.22 ± 3.08 a
	20 nm	49.62 ± 1.88 a	31.55 ± 1.65 a	41.58 ± 2.87 b	16.24 ± 0.64 c
	40 nm	35.51 ± 0.54 c	24.20 ± 0.61 b	53.14 ± 3.26 a	37.782 ± 0.53 b
	70 nm	37.20 ± 0.40 c	17.07 ±0.41 c	44.69 ± 0.89 b	00.00 ± 00.00 d
Diameter	Control	167.92 ± 1.16 b	151.02 ± 1.55 ab	129.83 ± 15.91 a	167.95 ± 0.66 b
	20 nm	211.18 ± 0.34 a	144.51 ± 4.68 b	175.94 ± 0.85 a	115.20 ± 0.40 c
	40 nm	163.42 ± 1.60 b	153.16 ± 1.39 ab	148.34 ± 33.99 a	175.71 ± 1.32 a
	70 nm	167.36 ± 3.36 b	157.04 ± 0.62 a	179.02 ± 0.83 a	00.00 ± 00.00 d

Table 7. Effect of Nickle nanoparticles foliar spray on ordinary epidermal cells and stomata of leaf of *Phasoelus vulgaris* L. plants

Anatomical leaf parts	Nickle nanoparticles (nm)	After 15 days	After 30 days	After 45 days	After 60 days
Adaxial surface	Control	590.33 ± 5.04 c	572.33 ± 6.77 d	2184.33 ± 632.83 a	587.00 ± 7.50 c
	20 nm	910.67 ± 2.33 b	919.00 ± 2.08	617.67 ± 1.45 a	883.00 ± 1.15 a
	40 nm	92.67 ± 1.45 d	1017.00 ± 2.89 b	1278.33 ± 0.88 a	767.67 ± 11.20 b
	70 nm	1097.00 ± 5.29 a	1538.67 ± 0.88 a	1227.00 ± 2.87 a	00.00 ± 00.00 d
Adaxial surface stomata	Control	74.33 ± 1.45 c	78.00 ± 1.53 c	75.33 ± 3.83 c	75.67 ± 1.76 c
	20 nm	62.33 ± 0.88 d	452.67 ± 1.76 a	193.33 ± 1.76 a	143.67 ± 2.03 a
	40 nm	170.67 ± 0.88 a	53.00 ± 1.15 d	89.33 ± 1.20 b	92.67 ± 1.45 b
	70 nm	103.00 ± 1.15 b	95.33 ± 2.03 b	33.67 ± 1.76 d	00.00 ± 00.00 d
Abaxial surface	Control	384.00 ± 2.31 d	388.33 ± 1.20 d	388.33 ± 3.18 d	383.00 ± 1.73 b
	20 nm	642.67 ± 1.77 b	750.67 ± 1.45 a	690.00 ± 1.15 b	569.00 ± 3.06 a
	40 nm	1028.33 ± 1.77 a	581.33 ± 0.88 c	712.67 ± 1.45 a	359.00 ± 3.61 c
	70 nm	594.33 ± 2.60 c	717.67 ± 2.03 b	468.67 ± 1.86 d	00.00 ± 00.00 d
Abaxial surface stomata	Control	After 15 days	After 30 days	After 45 days	After 60 days
	20 nm	191.67 ± 0.88 b	194.33 ± 2.03 d	196.00 ± 0.58 c	194.00 ± 2.08 a
	40 nm	203.33 ± 1.76 a	510.00 ± 1.15 a	284.67 ± 2.60 a	144.67 ± 2.03 c
	70 nm	95.00 ± 2.08 d	303.33 ± 2.03 b	204.33 ± 1.76 b	155.33 ± 2.03 b

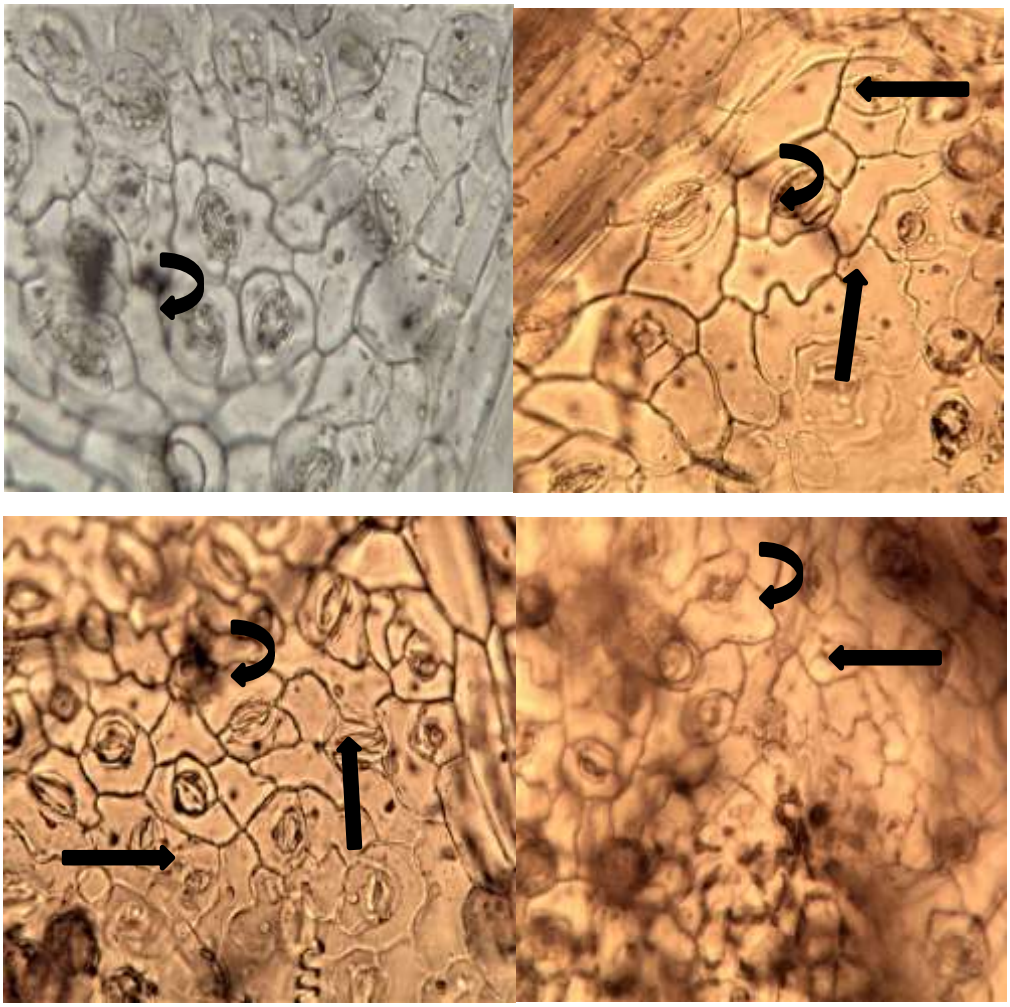

Figure 3. Epidemis of *phaseolus vulgaris* 3. *L.*: A. control, B. treatment with 40nm after 15 days, C. treatment with 70nm after 15 days, D. treatment with 70nm after one month. Small size stomata (large black arrow), stomata (curved black arrow). A,B,C,D

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