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Abstract

"Background and objectives: Zinc oxide (ZnO) is one of the best applied nanoscale technologies in agriculture. It showed both positive and negative effects on plant growth. The current study aimed to evaluate the effect of ZnO NPs positive or negative effect as a foliar fertilizer on the growth and development of Brophyllum pinnatum seedlings and which concentration play a crucial role in its positivity Methods: The study was conducted as completely randomized design to detect the effect of three levels of green ZnO NPs foliar spray; 0.20, 0.40 and 0.60 ppm as compared to control on some physiological anatomical characters of Brophyllum pinnatum seedlings. Results: The results had obtained showed that maximum stem height 32.33 and 44.00 cm due to 0.60 ppm after first and second sprays over control. The same level led to a significant increase in leaf area, shoot dry weight (SDW), root dry weight (RDW), total seedling dry weight (TDW) and root: shoot (R:S) ratio; 58.67 cm2, 1.60 g, 0.44 g and 2.09 g and 3.79 respectively. The highest chlorophyll a, b and total recorded duo to 0.40 ppm; 0.72, 0.04 and 0.76 mg g-1 of fresh leaves respectively. The transverse sections prepared by paraffin methods shows the anatomy of plant parts such as stem, petiole and leaf effect with treated by ZnO NPs; through two Periods (30 days, and 60 days). 0.40 and 0.60 ppm represent the best promoter of the physiological behavior and anatomical characters; midrib, petiole, lamina and stem anatomy. Conclusions: It could be concluded that foliar application with green ZnO NPs behaved as a growth promoter for Brophyllum pinnatum. 0.40 and 0.60 ppm represent the best concentration to enhance best performance in the plant body that affect the physiological behavior and anatomical characters "

Keywords

ZnO NPs, leaf chlorophyll, leaf area, lamina, stem anatomy

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RESEARCH ARTICLE

Influence of Zinc Oxide Nano Spray on the Growth and Development of *Bryophyllum pinnatum*

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ABSTRACT

Background and objectives: Zinc oxide (ZnO) is one of the best applied nanoscale technologies in agriculture. It showed both positive and negative effects on plant growth.
The current study aimed to evaluate the effect of ZnO NPs positive or negative effect as a foliar fertilizer on the growth and development of *Brophyllum pinnatum* seedlings and which concentration play a crucial role in its positivity
Methods: The study was conducted as completely randomized design to detect the effect of three levels of green ZnO NPs foliar spray; 0.20, 0.40 and 0.60 ppm as compared to control on some physiological anatomical characters of *Brophyllum pinnatum* seedlings.
Results: The results had obtained showed that maximum stem height 32.33 and 44.00 cm due to 0.60 ppm after first and second sprays over control. The same level led to a significant increase in leaf area, shoot dry weight (SDW), root dry weight (RDW), total seedling dry weight (TDW) and root: shoot (R:S) ratio; 58.67 cm2, 1.60 g, 0.44 g and 2.09 g and 3.79 respectively. The highest chlorophyll a, b and total recorded duo to 0.40 ppm; 0.72, 0.04 and 0.76 mg g-1 of fresh leaves respectively. The transverse sections prepared by paraffin methods shows the anatomy of plant parts such as stem, petiole and leaf effect with treated by ZnO NPs; through two Periods (30 days, and 60 days). 0.40 and 0.60 ppm represent the best promoter of the physiological behavior and anatomical characters; midrib, petiole, lamina and stem anatomy.
Conclusions: *It could be concluded that foliar application with green ZnO NPs behaved as a growth promoter for Brophyllum pinnatum. 0.40 and 0.60 ppm represent the best concentration to enhance best performance in the plant body that affect the physiological behavior and anatomical characters*
Keywords: ZnO NPs, leaf chlorophyll, leaf area, lamina, stem anatomy

INTRODUCTION

Bryophyllum pinnatum (Lam) Kurz form Crassulaceae family is a perennial herb widely cultivated throughout the hot and humid climate. It is native to Madagascar, which is a popular houseplant and has become naturalized in tropical and subtropical areas (Lans, 2006). Bryophyllum species have a unique mode of vegetative reproduction whereby young plantlets develop on the edges of leaves before being shed for propagation (Fürer et al., 2016). The demand in increasing plant productivity depends at great extent on the type of supplemental fertilizer to the essential growth nutrients of plants. By adding inorganic fertilizers, organically by manures, and other vegetative materials, plant growth would cease (Fathulla et al., 2023). Nanotechnology has a great role in science and technology development nowadays. The nanostructures produced from metal transition such as metal and metal oxide have acquired admirable interest to be next generation technologies. Zinc oxide (ZnO) is one of the best metal oxides widely applied in nanoscale technologies as due to its unique physicochemical properties (Mohammadi et al., 2017). Widespread use of ZnO NP in agriculture shows both positive and negative effects on plant growth (Zhang et al., 2015, Javed et al., 2017, Faizan et al., 2021 and Rajput et al., 2021). The foliar spraying 10 mg L-1 ZnO NPs resulted a positive influence in Cyamopsis tetragonoloba of chlorophyll, phosphorus, and protein contents (Raliya and Tarafdar, 2013). Yusefi-Tanha et al. (2020) considered that 160 mg kg-1 of ZnO NPs increased the seed yield of Glycine max as well as using 8 mg L-1 enhanced the the enzymatic activity of Lycopersicon esculentum (Faizan et al., 2018). However, treatment of Arabidopsis thaliana seeds with 300 mg L-1 ZnO NPs reduced the chlorophyll content by more than 50 % and growth by 80 % (Wang et al., 2016). The toxic effect on the A. thaliana plants growth and development was also revealed when ZnO NPs applied at lower concentrations (Wan et al., 2019). Javed et al. (2017) concluded both positive and negative effects depending on the ZnO NPs concentration applied in Stevia rebaudiana. According to the previous studies, the Zn-based NPs behaves as promoter or inhibitor that related to the species, size and type of NPs, ultimately the used concentration. Once NPs enter the plant cells through vascular bundle (xylem) and stele, they reach the aerial parts via apoplast or symplast pathways and interact with cellular and sub-cellular organelles. After the penetration and translocation process, it could damage the sub-cellular organization (Rajput et al., 2019) and might influence the photosynthesis (Giraldo et al., 2014). So, it's believed that Zn may be accumulated in plant tissues, cellular and sub-cellular organelles; chloroplasts, cell membranes, vacuoles and nuclei (Bradfield., 2017), and modulate cellular organizations (Radi et al., 2018). Ghosh et al. (2016), Ali et al. (2018), Peng et al. (2020) and Zoufan et al. (2020) results showed the high accumulation of Zn in plant tissues performed with Zn-based NPs in pots and hydroponic conditions.

Physiology and anatomy are tightly correlated, as cell and tissue structure has changed with respect to the evolution of novel functional mechanisms (Simpson, 2019). Zinc is necessary and the least available micronutrient for plants. Abiotic factors effect on the plant structure have been the object of some experimental studies. The current study aimed to evaluate the effect of ZnO NPs positive or negative effect as a foliar fertilizer on the growth and development of Brophyllum pinnatum seedlings and which concentration play a crucial role in its positivity.

SUBJECTS AND METHODS

Green synthesis of zinc oxide NPs:

ZnO NPs have been previously synthesized by (Mohammadi et al., 2017) using Euphorbia petiolate. Its efficient antibacterial ability tested against Escherichia coli. "50 g of dried powder leaf of the plant was boiled in 500 mL double distilled water for 30 minutes at 80 °C. The aqueous extract was filtered and kept freezing for further use. 30 mL of plant extract was added to 50 mL zinc nitrate (1 M) dropwise under reflux condition at 80 °C for 2 hours until changing the color and formation of some white precipitation. By centrifugation at 7000 rpm the precipitant was completely separated. Obtained powder precipitant was washed with methanol and distilled water to remove possible contaminations. Finally annealing was carried out in a muffle furnace at 400 °C for 2 hours. The obtained nanoparticles were identified using FT-IR, Uv-Vis, XRD, SEM and EDS techniques".

Treatment and experimental design:

The study was conducted to determine the effect of different concentrations of green ZnO NPs foliar spray; 0.00, 0.20, 0.40 and 0.60 ppm on the growth and development of Brophyllum pinnatum seedlings. The experiment was done in the glass houses of Biology department of the College of Science/ University of Salahaddin- Erbil. Pots containing 4 kg of sieved loamy soil and 100 g of peat moss were prepared. Two seedlings of about two months' age were transplanted per pot. The first foliar spray done after 30 days from seedling transplanting. And the second spray after 60 days transplanting. The experiment was laid out in a completely randomized design (CRD) with four replications. Duncan's multiple range test (DMRT) applied to compare among the mean of studied measurements. Analysis of variance (ANOVA) of the data and mean comparison computed using the Statistical package for the Social Sciences (SPSS) model 26 (Statistics, 2013).

Studied characters:

Physiological characters: Plant height, Leaf area and biomass characters: The whole plant become uprooted through pouring water into the plant's pot; roots were 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 48 hours, then shoot and root dry weights (g) had been measured (BAĞCI et al., 2003 and Qadir et al., 2019).

Then Root: shoot dry weight ratio plant-1 was calculated according to the following formula:

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

Chlorophyll content: The amount of chlorophyll (µg ml-1 solution) a, b and carotenoids can be estimated as mentioned in (Wintermans and Demots, 1965); 0.3 g of fresh leaves left in 10 ml of absolute ethanol for 24 hours, in dark condition. Take the extract and put it in a flask. And add another 10ml of ethanol also for 24 hrs. This process was repeated three times for the complete extraction of the chlorophyll, the end volume reached 30ml. Following the extraction of chlorophyll from the plant leaves, chlorophyll a and b were spectrophotometric ally estimated 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

Anatomical characters: Section preparation (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 (Fathulla et al., 2023).

RESULTS AND DISCUSSION

Zinc is necessary and the least available micronutrient for plants. Water repellence from leaf surface acts as one of the limiting factors limit the Zn uptake through foliar spray (Holder, 2007). ZnO NPs with less hydrophilicity and more dispersible capacity to penetrate the leaf surface and release ions across the cuticle which increase the solubility of the zinc ions in water through the cuticle lipophilic nature (García-López et al., 2019). Results pertaining to plant height (figure 1) showed that ZnO NPs significantly increased the trend after the first and second spray. The maximum height was 32.33 and 44.00 cm after both sprays with 0.60 ppm over control. same positive response of ZnO-NPs on plant height and the diameter of the stem and root found by Pérez Velasco et al. (2020) in Solanum lycopersicum. As well as same positive response recorded by García-López et al. (2019) at a concentration of 1000 mg L−1 that positively affected plant height, stem diameter, and chlorophyll content, and biomass accumulation compared to control in *Habanero* peppers.

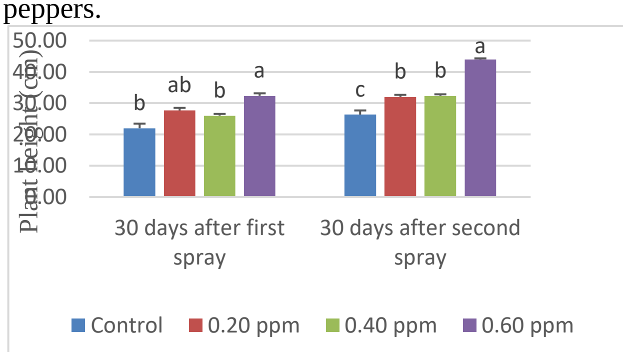


Figure 1: effect of ZnO NP- levels (ppm) on Bryophyllum pinnatum seedling height (cm)

Data on leaf area indicate that the area of a leaf was significantly more in all the treatments and maximum area i.e., 58.67 cm^2 was exhibited in seedlings treated with 0.6 ppm ZnO NPs as against 28.35 cm^2 in control plants (figure 2). ZnO NPs was found to had positive influence. All levels; 0.20, 0.40 and 0.60 ppm significantly increased the SDW, RDW, TDW and shoot: root ratio (table 1). The highest increase was observed when 0.60 ppm; 1.66 g, 0.44 g, 2.09 g and 3.79 respectively as compared to control treatment. As a result of the foliar spray and the presence of adequate water in the soil that the plant not exposed to water tension in the soil, the root grew in a constant level and the biomass increased as well as the shoot: root ratio increased significantly as the ZnO NPs foliar sprayed. Because of higher bioavailability of the ZnO NPs due to their Nano size and lower water solubility (Prasad *et al.*, 2012). Zinc is an essential micronutrient that enhances enzyme reactions, and it can improve the effectiveness of photosynthesis and enhance the antioxidant system (Hassan *et al.*, 2022). The results in parallel with Tirani *et al.* (2109) and García-López *et al.* (2019); nano-ZnO at most of the levels and 1 µM bulk-ZnO positively affected growth (root and shoot length and dry weight), leaf surface area.

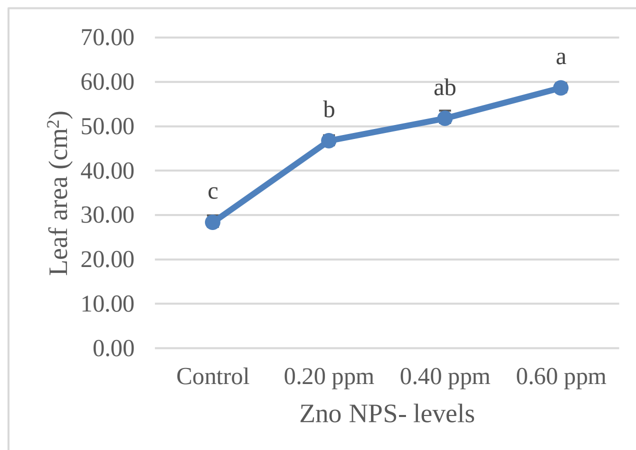


Figure 2: effect of ZnO NP- levels (ppm) on *Bryophyllum pinnatum* leaf area (cm^2)

Table 1: effect of ZnO NP- levels (ppm) on Bryophyllum pinnatum biomass characters

ZnO NPs-levels	SDW (g)	RDW (g)	TDW (g)	Root: shoot
Control	0.61 ± 0.03 b	0.34 ± 0.003 c	0.95 ± 0.03 b	1.77 ± 0.04 b
0.20 ppm	1.17 ± 0.13 a	0.38 ± 0.01 b	1.56 ± 0.13 a	3.06 ± 0.14 a
0.40 ppm	1.34 ± 0.04 a	0.42 ± 0.003 a	1.76 ± 0.04 a	3.19 ± 0.06 a
0.60 ppm	1.66 ± 0.12 a	0.44 ± 0.010 a	2.09 ± 0.13 a	3.79 ± 0.05 a

A positive influence of the foliar Nano spray of all ZnO concentrations; 0.20, 0.40 and 0.60 ppm on chlorophyll content was shown in figure (3). The highest chlorophyll a, b and total were; 0.72, 0.04 and 0.76 mg g^{-1} fresh weight were recorded duo to 0.40 ppm ZnO NP spray at the end of the experiment. Zn as this metal integrates the structure of Rubisco for carbon fixation in the light reaction in the photosynthesis process. This nutrient also modulates the conversion of carbon dioxide to reactive bicarbonate species required for the fixation to carbohydrates. Zn has an important role to shift the equilibrium in favor of $HCO3^-$, the substrate for PEP carboxylase (Cousins *et al.*, 2007). The foliar application of 10 mg L^{-1} ZnO NPs expressed positive impacts on chlorophyll, phosphorus, and protein contents in *Cyamopsis tetragonoloba* (Elmer & White, 2016). As well as Pullagurala *et al.* (2018) found that ZnO NPs increased the chlorophyll content by at least 50%, compared to control in *Coriandrum sativum*.

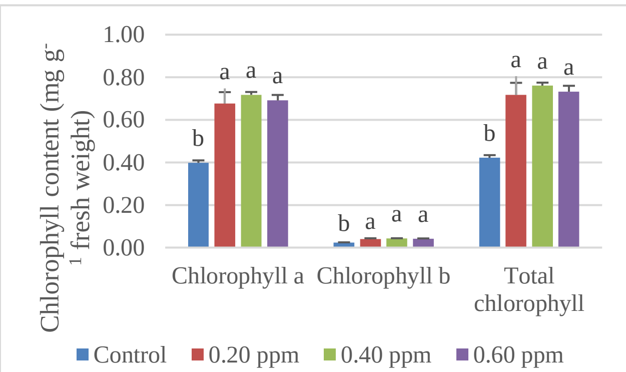


Figure 3: effect of ZnO NP- levels (ppm) on *Bryophyllum pinnayum* chlorophyll content (mg g^{-1} fresh weight)

The critical investigation revearls that green ZnO nanoparticle affected anatomical characters such as midrib, petiol, lamina, and stem (table 2, 3, 4 and 5) and (figure 5). The transfervers section that the taken from the midrib after 30 dayes spraying with 0.20 ppm and 60 dayes with 0.20 ppm and 0.60 ppm accessory vascular bundles was observed, meanwhile its absent in others (figure 4). 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 (Rastogi, *et. al.*, 2017). Nanoparticles interact with plant tissues by various paths of implementation in agriculture, accidental discharges, water curing, irrigation water, and aerial deposition or presence in the soil. The Plants need few amounts of trace elements; therefore, the increase concentration in soil may affect plant performance. The structure and ultrastructure inconstancy by nanoparticles is a very difficult mission, and even more difficult to find the accumulation of nanoparticles in plant tissues. However, there are sundry researches indicate the lucid havoc to plant cell organelles, and most common changes were shown in cell organelles such as plastids, thylakoids, mitochondria, peroxisomes, plastoglobules, starch granules, protoplasm, vascular bundles, plasma membrane, cell wall, root morphology, epidermis, endodermis, and central cylinder. However, Chloroplasts are the most vulnerable organelles in plant, the anatomical modifications in chloroplast would affect the light way and consequently affect the photosynthesis (Rajput *et al.*, 2019). The changes in root histology were clearly visible after treatment of neutral gold nanoparticles (AuNPs) (Milewska-Hendel *et al.*, 2019). Also, Mohajjel Shoja *et al.*, (2021) reported the titanium dioxide nanoparticles significantly increased the vascular bundle diameter of the root while the vascular bundle diameter decreased in the stem of some treatments.

Table 2: Effect ZnO NP- levels (ppm) on midrib anatomy of *Bryophyllum pinnayum*

Anatomical parts	ZnO NP levels (ppm)	30 days after 1st spray	30 days after 2nd spray
Diameter (Mµ)	Control	311.49 ± 1.24 a	329.45 ± 2.07 a
	0.2	279.84 ± 4.92 a	339.62 ± 0.40 a
	0.4	216.86 ± 2.39 b	317.78 ± 1.36 b
	0.6	200.70 ± 5.29 b	317.29 ± 2.84 b
Vascular bundle (Mµ)	Control	42.06 ± 0.83 a	57.33 ± 0.61 a
	0.2	23.99 ± 1.67 c	67.42 ± 2.71 a
	0.4	30.54 ± 0.98 b	55.12 ± 4.21 ab
	0.6	32.22 ± 0.67 b	41.85 ± 0.18 b
Accessory vascular bundle	Control	0.00 ± 0.00 b	0.00 ± 0.00 b
	0.2	13.31 ± 0.16 a	18.33 ± 0.56 a
	0.4	0.00 ± 0.00 b	0.00 ± 0.00 b
	0.6	0.00 ± 0.00 b	12.88 ± 0.30 a

Table 3: Effect ZnO NP- levels (ppm) on petiole anatomy of *Bryophyllum pinnayum*

Anatomical parts	ZnO NP levels (ppm)	30 days after 1st spray	30 days after 2nd spray
Diameter	Control	393.52 ± 3.04 a	421.79 ± 5.19 a
	0.20	338.26 ± 1.18 b	381.38 ± 1.94 b
	0.40	390.67 ± 4.96 a	382.29 ± 3.26 b
	0.60	345.20 ± 0.62 a	335.29 ± 7.58 a
Vascular bundle	Control	80.16 ± 1.99 a	79.24 ± 1.10 a
	0.20	45.00 ± 0.73 c	63.90 ± 0.23 b
	0.40	57.09 ± 2.59 b	65.34 ± 1.92 b
	0.60	74.19 ± 0.63 a	59.26 ± 0.72 b
Cortex	Control	172.31 ± 6.96 a	151.94 ± 1.10 ab
	0.20	139.15 ± 1.43 b	148.88 ± 5.54 ab
	0.40	156.92 ± 3.47 ab	155.24 ± 1.25 a
	0.60	140.09 ± 4.68 b	133.51 ± 2.57 b
Epidermis	Control	6.59 ± 0.17 a	5.49 ± 0.03 c
	0.20	6.48 ± 0.14 a	7.25 ± 0.27 a
	0.40	7.14 ± 0.59 a	5.85 ± 0.34 bc
	0.60	6.08 ± 0.31 a	6.46 ± 0.03 ab

Table 4: Effect ZnO NP- levels (ppm) on lamina anatomy of *Bryophyllum pinnayum*

Anatomical parts	ZnO NP levels (ppm)	30 days after 1st spray	30 days after 2nd spray
Cuticle	Control	3.20 ± 0.28 a	4.71 ± 0.22 a
	0.20	2.47 ± 0.08 a	2.63 ± 0.05 b
	0.40	2.56 ± 0.05 a	2.34 ± 0.12 b
	0.60	2.79 ± 0.25 a	4.77 ± 0.22 a
Upper epidermis	Control	20.49 ± 0.58 a	9.06 ± 0.28 a
	0.20	12.71 ± 1.01 bc	10.57 ± 0.32 ab
	0.40	15.57 ± 1.62 ab	11.47 ± 0.54 ab
	0.60	7.78 ± 1.06 c	12.18 ± 0.67 a

Lower epidermis	Control	11.15 ± 0.32 ab	8.21 ± 0.79 b
	0.20	13.79 ± 0.23 a	13.22 ± 0.37 a
	0.40	8.93 ± 1.08 b	11.34 ± 0.32 a
	0.60	9.04 ± 0.86 b	8.45 ± 0.37 b
Vascular bundles	Control	14.53 ± 0.02 b	26.91 ± 0.82 a
	0.20	16.45 ± 0.94 ab	30.12 ± 3.01 a
	0.40	19.36 ± 0.61 a	14.71 ± 0.69 b
	0.60	13.20 ± 0.87 b	12.67 ± 0.45 b
Mesophyll layer	Control	227.68 ± 20.58 a	163.79 ± 11.43 ab
	0.20	165.94 ± 4.95 b	201.46 ± 7.78 a
	0.40	160.92 ± 2.19 b	137.47 ± 3.70 b
	0.60	168.50 ± 11.78 b	168.79 ± 6.24 ab

Table 5: Effect ZnO NP- levels (ppm) on stem anatomy of *Bryophyllum pinnayum*

Anatomical parts	ZnO NP levels (ppm)	30 days after 1st spray	30 days after 2nd spray
Periderm	Control	12.99 ± 0.48 a	11.83 ± 0.43 a
	0.20	10.15 ± 0.75 ab	11.35 ± 0.27 a
	0.40	11.23 ± 0.06 ab	14.22 ± 0.90 a
	0.60	9.27 ± 0.58 b	10.81 ± 0.50 a
Phloem	Control	5.04 ± 0.17 a	7.09 ± 0.42 a
	0.20	5.22 ± 0.05 a	7.32 ± 0.19 a
	0.40	5.89 ± 0.19 a	8.71 ± 0.75 a
	0.60	6.76 ± 0.96 a	8.28 ± 0.23 a
Xylem	Control	11.72 ± 0.46 a	26.04 ± 1.12 a
	0.20	9.00 ± 0.40 a	16.96 ± 0.89 b
	0.40	11.33 ± 0.52 a	23.64 ± 0.81 ab
	0.60	10.87 ± 1.10 a	18.96 ± 2.26 ab
Fiber	Control	10.17 ± 0.11 b	14.37 ± 0.43 a
	0.20	7.79 ± 0.39 c	15.63 ± 0.29 a
	0.40	13.96 ± 0.27 a	15.30 ± 0.33 a
	0.60	8.10 ± 0.13 c	20.64 ± 2.04 b
Cortex	Control	110.36 ± 1.20 bc	147.69 ± 2.67 ab
	0.20	106.90 ± 0.72 c	136.89 ± 0.64 ab
	0.40	128.04 ± 2.15 ab	132.16 ± 3.37b
	0.60	136.28 ± 6.28 a	150.85 ± 3.57 a

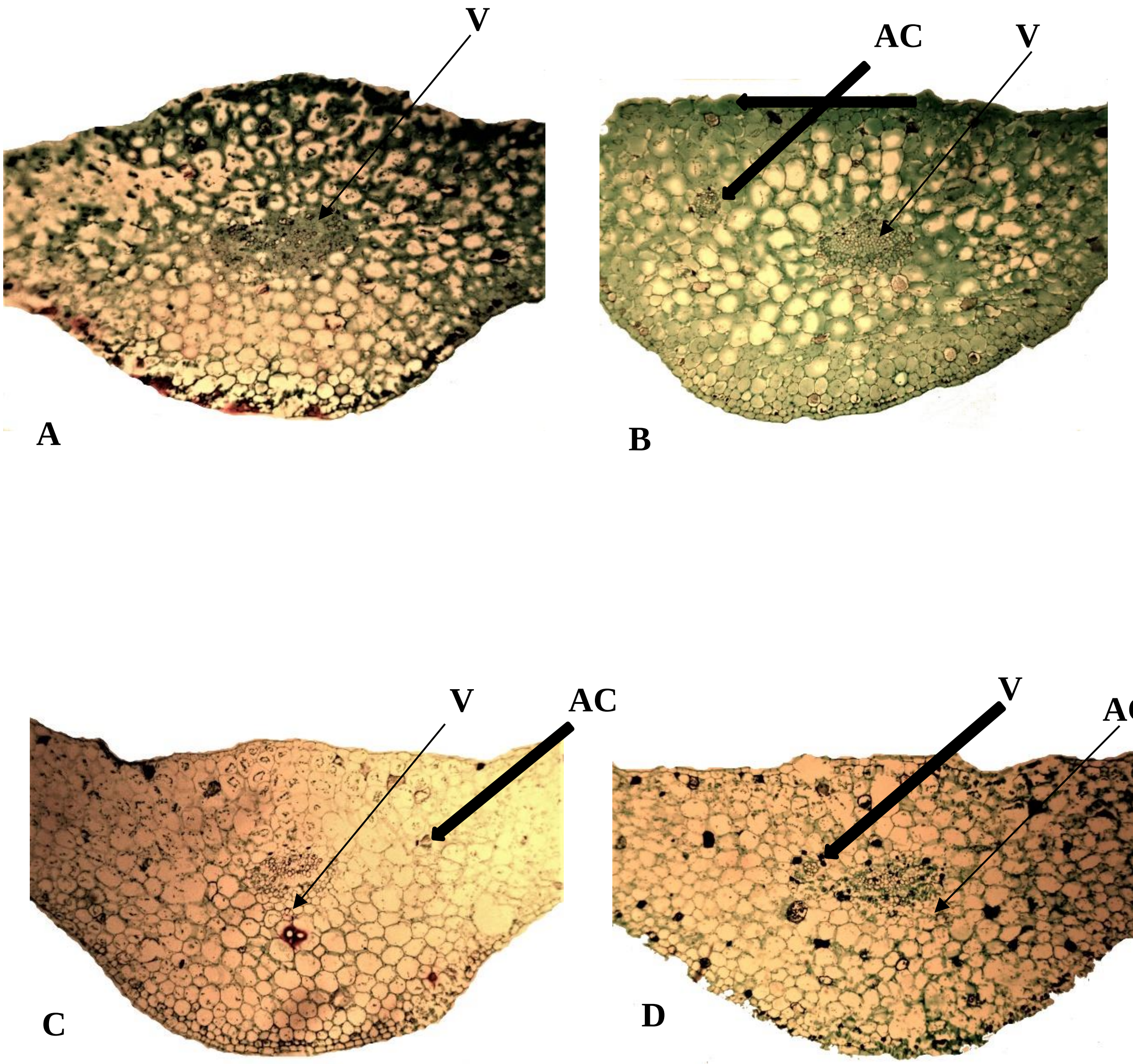
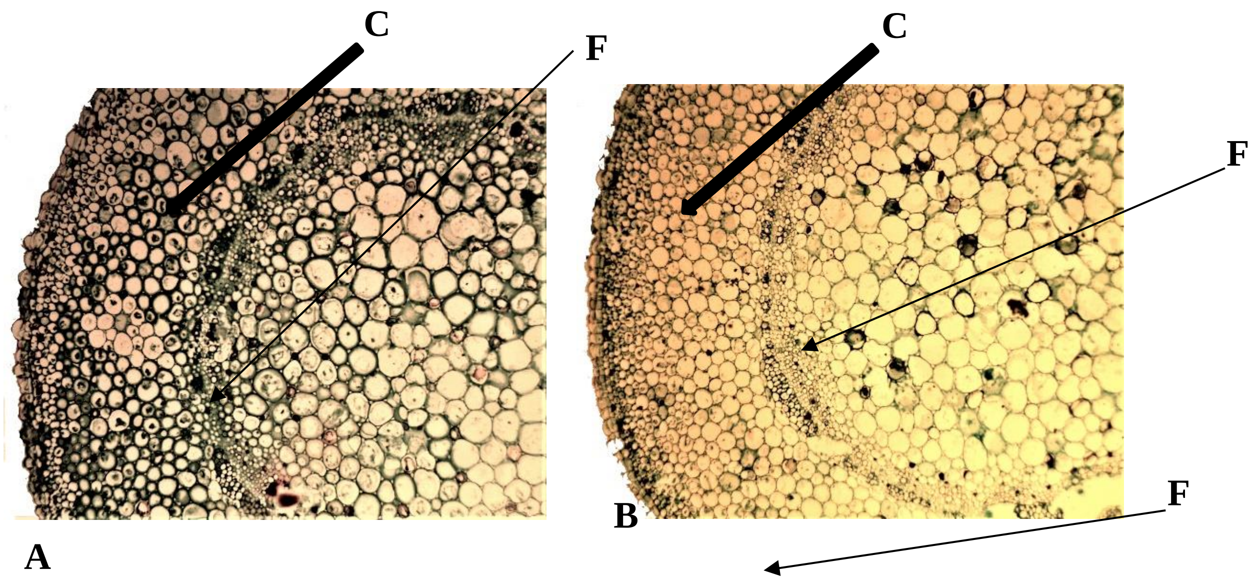


Figure 4: TS midrib of *Bryophyllum pinnatum*. A. control, B. treated with ZnO NPs (0.20 ppm; after 30 days), C. treated with ZnO NPs (0.20 ppm; after 60 days), D. treated with ZnO NPs (0.60 ppm; after 60 days). V: vascular bundle, AC: accessory vascular bundle. A, B, C, D=4X.



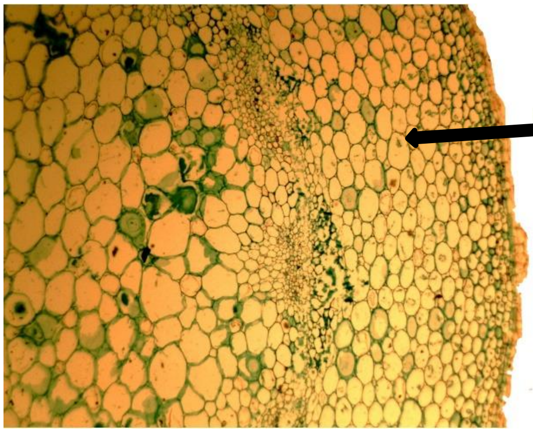


Figure 5. TS stem of *Bryophyllum pinnatum*. A. control, B. treated with ZnO NPs (0.60 ppm; after 30 days), C. treated with ZnO NPs (0.60 ppm; after 60 days), C: cortex, F: fiber. A, B, C=4X.

CONCLUSION

It could be concluded that foliar application with green ZnO NPs behaved as a growth promoter for Brophyllum pinnatum. 0.40 and 0.60 ppm represent the best concentration to enhance best performance in the plant body that affect the physiological behavior and anatomical characters; midrib, petiole, lamina and stem anatomy. As well as formation of accessory vascular bundles in the midrib due to 0.20 and 0.60 ppm.

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