

EFFECT OF ZINC OXIDE NANOPARTICLES ON THE MORPHOLOGICAL TRAITS AND NUTRITIONAL QUALITY OF ASPARAGUS (*Asparagus officinalis* L.) IN *in vitro* CULTURE

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ABSTRACT

Zinc oxide nanoparticles (ZnONPs) are used to stimulate the growth and development of various plant species, including those grown in tissue cultures. However, there is still a lack of data regarding their effect on the micropropagation of asparagus (*Asparagus officinalis* L.). Asparagus is a valuable vegetable crop due to its taste, easy digestibility, low caloric value, and significant health-promoting properties. In the present study, the effects of different concentrations of ZnONPs on morphological traits and on the content of metals, total phenolic content (TPC), total antioxidant capacity (TAC), and photosynthetic pigments in plantlets of *A. officinalis* derived from tissue cultures were evaluated. Shoot fragments obtained from seedlings grown in *in vitro* culture were used for micropropagation. Explants measuring approximately 0.5 cm were placed on MS medium supplemented with 0.20 mg dm⁻³ BAP, 0.25 mg dm⁻³ Kin, 0.20 mg dm⁻³ NAA, and ZnONPs at concentrations of 0 (control), 10, 20, 30, and 40 mg dm⁻³. The *in vitro* culture was conducted over two subcultures lasting a total of four months. After four months of *in vitro* culture, the developed plantlets were removed from the culture vessels, and their morphological parameters were assessed. A portion of the plantlets was used for further analyses, including the determination of their metal content, photosynthetic pigments, TPC, and TAC using the DPPH assay. ZnONPs at concentrations of 20–40 mg dm⁻³ negatively affected shoot number, root length, and fresh weight of plantlets. At concentrations of 10–20 mg dm⁻³, ZnONPs did not affect shoot length or root number, whereas the 40 mg dm⁻³ treatment negatively affected these parameters. In samples of asparagus plantlets grown under treatment conditions involving 10–40 mg dm⁻³ of ZnONPs, higher levels of K, Zn, and Cu and lower levels of Ca and Mn were found compared with the control group. Samples grown at the 10–20 mg dm⁻³ treatments contained more Mg and Fe than in the control. Strong positive correlations were found between the ZnONPs treatments and the concentrations of Zn and K, and strong negative correlations were found between these treatments and the concentrations of Ca, Mn, and Mg. The total content of phenols and antioxidants measured by the DPPH method, as well as photosynthetic pigments, in plantlets grown at the 10–20 mg dm⁻³ ZnONPs treatments was higher than in the control.

Keywords: *Asparagus officinalis* L., tissue culture, ZnONPs, antioxidants, minerals, photosynthetic pigments, polyphenols

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INTRODUCTION

There are approximately 200 plant species known to belong to the genus *Asparagus* (family *Asparagaceae*). These include edible species such as *A. albus*, *A. acutifolius*, and *A. officinalis*; medicinal species such as *A. adscendens* and *A. racemosus*; and ornamental species such as *A. asparagoides* and *A. densiflorus* [Encina and Regalado 2022]. *Asparagus officinalis* L. grows naturally in the Mediterranean region, adjacent areas, and Central Asia. It is the only edible perennial species of this family cultivated worldwide, particularly in China. Its popularity stems from its ability to provide an early spring supply of fresh, high-quality produce. Unlike many other crops, its underground organs persist after the growing season and overwinter in the soil, enabling the development of new aerial shoots in the following season, which subsequently flower and set fruit [Fuentes-Alventosa 2009, Kobus-Cisowska 2019]. *Asparagus officinalis* L. is valued for its high nutritional quality, low caloric content, delicate taste and health-promoting properties. Young shoots provide approximately 20 kcal per 100 g of fresh weight, and consist mainly of water (approximately 93%), with carbohydrates (2.04%), proteins (1.91%), dietary fiber (1.31%), nitrogen (0.31%), and a low fat content (0.16%) [Olas 2024]. The presence of dietary fiber and inulin contributes to proper digestive function and supports the growth of beneficial intestinal microbiota [Mudannayake et al. 2025]. Asparagus is also a source of minerals, including potassium, phosphorus, magnesium, calcium, iron, zinc, and manganese [Kohli et al. 2023], as well as vitamins A, C, B1, B2, E, and folic acid. Its health-promoting properties are associated with numerous biologically active compounds, including arginine, tyrosine, asparagine, flavonoids (kaempferol, quercetin, and rutin), essential oils, steroidal saponins, and carotenoids [Hamdi et al. 2018, Zhang et al. 2019]. These compounds contribute to antioxidant activity, while chemical analyses have also revealed the presence of organic acids, amino acids, and peptides [Guo et al. 2020, Rolbiecki et al. 2024, Zhang et al. 2024].

Asparagine is a characteristic component of asparagus and is associated with its diuretic properties and high content of non-protein nitrogen [Gupta and Chauhan 2025]. Regular consumption of asparagus may support digestive, cardiovascular, and metabolic health, while its low glycemic index makes it suitable for individuals with carbohydrate metabolism disorders [Hamdi et al. 2018, Guo et al. 2020, Zhang et al. 2024]. Asparagus also contains folic acid, vitamins C and E, carotenoids, potassium, dietary fiber, and glutathione, while its young shoots and roots exhibit diuretic and mild laxative properties [Kulczyński et al. 2016, Javaid et al. 2022]. Furthermore, compounds such as protodioscin, rutin, and quercetin may contribute to the potential cancer-preventive properties of green asparagus [Wang 2022]. The nutritional value and abundance of bioactive compounds, together with the documented health-promoting properties of asparagus, make this vegetable a valuable source of nutrients and biologically active substances [Olas 2024].

For commercial purposes, asparagus is propagated by seeds or vegetatively through division. These traditional propagation methods are time-consuming and may increase the risk of disease transmission. The genetic diversity of cultivated asparagus (*A. officinalis* L.) is relatively limited, which may constrain breeding progress. Therefore, the identification and utilization of diverse genetic resources are important for broadening the genetic base and developing new cultivars with improved agronomic, nutritional, and quality traits [Knaflewski and Kałużewicz 1998].

The cultivation of asparagus in *in vitro* culture is a widely used method for propagation and obtaining plant material of high phytosanitary quality and genetic uniformity [Sharma et al. 2025]. This technique is applied in horticultural practice, particularly for producing pathogen-free plants and rapidly multiplying valuable genotypes. Asparagus is a perennial species with a relatively long production cycle; therefore, micropropagation significantly shortens the time required to obtain planting material [Esmaeili et al. 2016].

Plant growth regulators are widely used in the micropropagation of *Asparagus* species to promote shoot multiplication, rooting, and plant regeneration. In *A. officinalis*, 6-benzylaminopurine (BAP) has been applied at concentrations ranging from 0.1 to 2.0 mg dm⁻³, usually in combination with 1-naphthaleneacetic acid (NAA) at 0.015–0.5 mg dm⁻³ [Štajner et al. 2013, Esmaeili et al. 2016, Minh et al. 2022, Sarabi and Almasi 2010]. Other studies have used kinetin (Kin) in combination with NAA or indole-3-butyric acid (IBA), whereas IBA has been mainly applied for rooting [Pontaroli and Camadro 2005, Esmaeili et al. 2016,

Sarabi and Almasi 2010]. In *A. racemosus*, direct organogenesis has been achieved using BAP in combination with indole-3-acetic acid (IAA) [Singh et al. 2023].

In plant cultures conducted under *in vitro* conditions, nanotechnology is playing an increasingly important role as a tool supporting both propagation and modification of plant material. Nanoparticles are primarily used to reduce microbial contamination and serve as an alternative or complement to traditional disinfectants. They also influence plant morphogenesis, as they have been shown to stimulate somatic embryogenesis, organogenesis, and accelerate tissue growth and differentiation. In many cases, their application leads to increased plant regeneration efficiency and improved quality of the obtained material [Singh et al. 2023]. Additionally, beneficial effects on the biosynthesis of secondary metabolites, such as phenolic compounds, flavonoids, and alkaloids, have been reported, which may enhance the production of plant-derived bioactive substances [Alghamdi et al. 2022, Alharby et al. 2016, Devasia et al. 2020]. In *in vitro* cultures, metal nanoparticles and their oxides are most commonly used, including silver, copper, titanium, zinc, cobalt, nickel, and iron. Carbon-based nanomaterials, such as graphene and carbon nanotubes, as well as nanosilica, are also employed. The diverse physicochemical properties of these materials are contributing to their increasing use in plant biotechnology.

Zinc oxide nanoparticles may stimulate cell division and proliferation, accelerate plant tissue regeneration [Alghamdi et al. 2022, Geremew et al. 2023], and support callus formation, rooting, and somatic embryogenesis [Shafique et al. 2020, Al-Mayahi 2021, Geremew et al. 2023]. Appropriately selected concentrations of zinc oxide nanoparticles (ZnONPs) can have a positive effect on the micropropagation, growth, and *in vitro* development of olive tree explants (*Olea europaea* L. cv. Moraiolo) [Regni et al. 2022]. Beneficial effects of ZnONPs have also been reported for shoot regeneration in blackberry (*Rubus fruticosus* L.) cv. Nawaho [Krzepiłko et al. 2024a], stevia (*Stevia rebaudiana* Bertoni) [Javed et al. 2018, Krzepiłko et al. 2024b], mustard (*Brassica nigra*) [Zafar et al. 2016], *Panicum virgatum* [Nair et al. 2010], *Thymus kotschyianus* and *Zataria multiflora* [Mosavat et al. 2019], and potato (*Solanum tuberosum*) [Alghamdi et al. 2022].

In *in vitro* culture, zinc oxide nanoparticles serve as a source of zinc for plants. This mineral element is essential for chlorophyll synthesis and the production of plant hormones – especially auxins and gibberellins; it also enhances photosynthetic efficiency, and influences nutrient uptake [Hayat et al. 2023]. Zinc also affects cell proliferation and key metabolic processes such as protein biosynthesis, DNA replication, and transcription, and is a component of prosthetic groups of many enzymes [Cabot et al. 2019, Hayat et al. 2023, Shao et al. 2023]. Zinc oxide nanoparticles mitigate stress caused by heavy metals, drought, and salinity [Sun et al. 2021, Jaithon et al. 2022, Liu et al. 2022]. They also exhibit antifungal and antibacterial activity, thereby reducing the incidence of plant diseases [Pena et al. 2022, Zou et al. 2022, Subba et al. 2024].

The aim of this study was to investigate the effects of zinc oxide nanoparticles (ZnONPs), applied at four concentrations (10, 20, 30, and 40 mg dm⁻³), on morphological traits, growth, and the contents of selected metals (potassium, calcium, magnesium, iron, zinc, manganese, and copper), total phenolic content (TPC), total antioxidant capacity (TAC), and photosynthetic pigments (chlorophyll a and b, total chlorophyll: a + b, and carotenoids) in asparagus plants cultured *in vitro*.

MATERIAL AND METHODS

Zinc oxide nanoparticles (ZnONPs) were obtained from Sigma-Aldrich (catalog no. 721077). Particle size was measured using an Aerodynamic Particle Sizer (APS). The average size of individual particles was <35 nm [Wang et al. 2013].

Plant material. The experimental material consisted of garden asparagus (*Asparagus officinalis* L.) cv. Argenteuil (PL-14/32/17672), an early cultivar, characterized by thick, white (or green) shoots with rounded tips and short, pinkish scales. Seeds were obtained from the Polish Breeding and Seed Company (PNOS – Państwowe Przedsiębiorstwo Nasiennictwa Ogrodniczego i Szkółkarstwa) in Ożarów Mazowiecki, Poland. After surface sterilization for 8 min in 0.1% HgCl₂, the seeds were placed in Petri dishes (10 cm in

diameter) containing solid MS medium at pH 5.8 [Murashige and Skoog 1962] without growth regulators. After seedling development, shoots were cut into fragments approximately 0.5 cm in length under sterile conditions and transferred to 200 mL glass jars with heat-resistant Magenta B-caps (Figure 1B). Each jar contained 20 mL of MS medium supplemented with 3% sucrose, 0.8% Difco Bacto agar, 0.20 mg dm⁻³ BAP (6-benzylaminopurine), 0.25 mg dm⁻³ Kin (kinetin), 0.20 mg dm⁻³ NAA (1-naphthaleneacetic acid) and 10, 20, 30, and 40 mg dm⁻³ ZnONPs; a treatment without ZnONPs served as the control. Cultures were maintained in a growth room at 22–24°C, 60% relative humidity, and a 16 h photoperiod with a light intensity of 54 µmol m⁻² s⁻¹ provided by fluorescent lamps (Radium NL 36W/830, Germany). After four months of *in vitro* culture, the regenerated asparagus plantlets were removed from the culture vessels and their shoot and root parameters, as well as their fresh weight, were measured. The plantlets were then transferred into plastic containers filled with sterilized soil (Figure 1). Some of the plant material was used for further analyses, including the determination of metal content (K, Ca, Mg, Fe, Zn, Mn, Cu), chlorophyll a and b, total chlorophyll (a + b), carotenoids, total phenolic content (TPC), and total antioxidant capacity (TAC). Each experiment and analysis was performed in triplicate.

Preparation of plant extracts. The plant material was first homogenized, weighed and mixed with a suitable extraction solvent at a ratio of 1 : 10 (m/v); this was followed by 5 minutes of homogenization during which the samples were kept in an ice bath. After homogenization, samples were centrifuged for 5 min at 5000 rpm. The obtained supernatant was frozen and used for further biochemical analyses. Depending on the parameter analyzed, different extraction solvents were used: 70% methanol in water for phenolic compounds, 80% acetone in water for photosynthetic pigments, and 70% ethanol in water for antioxidant activity (DPPH assay).

Determination of total phenolic content. TPC was determined spectrophotometrically using the Folin–Ciocâlteu reagent. This method is based on the ability of polyphenols to reduce phosphomolybdate–phosphotungstate complexes present in the reagent, resulting in the formation of blue-coloured products. The intensity of the colour is directly proportional to the concentration of phenolic compounds in the sample. The reaction mixture consisted of 0.05 cm³ of methanolic extract from asparagus plantlets, 0.15 cm³ of Folin–Ciocâlteu reagent, 0.3 cm³ of 7.5% sodium carbonate solution, and 2.525 cm³ of distilled water. After incubation for 1 h, absorbance was measured at 765 nm using a Shimadzu spectrophotometer (Japan), model UV-1280. Results were expressed as gallic acid equivalents (GAE) per 1 g of fresh weight (FW), according to the method described by Singleton et al. [1999].

Determination of total antioxidant capacity. TAC was determined using the DPPH method. This method employs the stable radical DPPH• (2,2-diphenyl-1-picrylhydrazyl), which exhibits an intense violet color in ethanol solution. In the presence of antioxidants, the DPPH• radical is reduced, leading to a gradual discoloration of the solution. The decrease in colour intensity, measured as a reduction in absorbance, is proportional to the antioxidant activity of the sample – the stronger the antioxidant properties, the greater the decrease in absorbance. Measurements were taken at 517 nm after 30 min of reaction using the Shimadzu UV-1280 spectrophotometer, according to the method described by Addai et al. [2013]. The reaction mixture consisted of a DPPH• solution prepared in ethanol and 0.05 cm³ of the ethanol extract from asparagus plantlets. TAC was expressed as Trolox equivalents per 1 g fresh weight.

Determination of mineral composition. After harvesting, the plantlets were thoroughly rinsed in distilled water and then dried in a forced-air oven at 70°C for 72 h until constant weight was reached. The dried plant material was then mineralized using a mixture of concentrated nitric acid (V) and perchloric acid (VII) (HNO₃ + HClO₄). After digestion and appropriate dilution, the content of macro- and microelements – potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) – was determined. Mineral content was expressed in mg 100 g⁻¹ DW (dry weight). The Zn content was used as the reference value and set to 1.00. Relative mineral content = mineral content / Zn content.

Statistical analysis. The results were subjected to statistical analysis using analysis of variance (ANOVA). The significance of differences between means was evaluated using Tukey's post hoc test at *p* < 0.05. Additionally, Pearson's correlation coefficients (*r*) were calculated to determine relationships between the analyzed parameters. Statistical analyses were performed using Statistica v.13.1.

RESULTS

The germination rate of garden asparagus seeds was 76% after 14 days and 86% after 21 days of culture on MS medium. The shoots of the seedling were then cut into fragments and transferred to vessels containing solid MS medium, which had been enriched with growth regulators and zinc oxide nanoparticles. The *in vitro* culture was conducted over two subcultures lasting a total of four months. During the first subculture, it was observed that the percentage of explants regenerating organs was slightly lower in the treatments with ZnONPs than in the control. In the control group, 75.6% of the explants developed both shoots and roots. When treated with 10 mg dm⁻³ ZnONPs, 68.0% of the explants developed both shoots and roots; at 20 mg dm⁻³ ZnONPs, 62.2% showed the same response; at 30 mg dm⁻³ ZnONPs, 71.4% of the explants developed both shoots and roots; and at 40 mg dm⁻³ ZnONPs, the corresponding percentage was 70.0%.

Analysis of morphological traits. After the second subculture, plantlets were removed from the culture vessels and morphological traits were analyzed (Figure 1B, 1C).

Table 1. The effect of increasing concentrations of zinc oxide nanoparticles in Murashige and Skoog solid medium on the biometric characteristics of asparagus plantlets (*Asparagus officinalis* L.) after 4 months of *in vitro* culture (mean value of the characteristic per explant)

Biometrical parameter	Concentration of ZnONPs (mg dm ⁻³)					LSDp < 0.05
	0 (control)	10	20	30	40	
Number of shoots	15.93a	14.07ab	11.97b	10.86b	9.83c	2.495
Shoot length (cm)	10.47a	10.02a	9.42ab	7.98b	5.89c	2.150
Number of roots	4.77a	3.73ab	3.57ab	2.63b	1.97c	1.395
Root length (cm)	3.98a	2.71b	2.22c	1.96d	0.71e	1.271
Fresh weight of plant (g)	0.595a	0.515a	0.376b	0.343bc	0.164c	0.132
(Increment, g)	(0.572)a	(0.496)a	(0.355)b	(0.321)bc	(0.145)c	(0.110)

Different letters within a line indicate a significant difference according to the Tukey's test ($p < 0.05$).

Overall, the highest number and length of shoots and roots, as well as the highest fresh weight, were observed in the control and at the lowest ZnONPs concentration (10 mg dm⁻³) (Table 1, Figure 1C). Increasing ZnONPs concentrations generally had a negative effect on plantlet growth and development. At concentrations of 20–40 mg dm⁻³, the number and length of shoots decreased compared with the control, with the most pronounced reductions observed at 40 mg dm⁻³. The number and length of roots were also significantly reduced at the higher ZnONPs concentrations, particularly at 30 and 40 mg dm⁻³. Similarly, fresh weight decreased progressively with increasing ZnONPs concentration, with the lowest value recorded at 40 mg dm⁻³ (Table 1). Negative Pearson correlation coefficients were observed between ZnONPs concentration and all analyzed morphological traits, including the number and length of shoots and roots and plantlet fresh weight.

Mineral content in *Asparagus officinalis* plantlets. ZnONPs influenced the mineral composition of *A. officinalis* (Table 2). The zinc content in plantlets increased as the concentration of ZnONPs in the culture medium increased. At a concentration of 10 mg dm⁻³, the Zn content was four times higher than in the control, while at 40 mg dm⁻³ it was up to fourteen times higher. Plant material from the control treatment most often differed significantly from that obtained from ZnONPs treatments in terms of the content of the analyzed minerals. In plantlets from treatments containing 10–40 mg dm⁻³ ZnONPs, the content of K, Zn, and Cu increased, whereas the content of Ca and Mn decreased compared to the control. The content of Mg and Fe was higher than in the control in plant material from the 10 and 20 mg dm⁻³ ZnONPs treatments; however, in the 30 and 40 mg dm⁻³ treatments, their levels were lower (Table 2).

Figure 1. Effect of different concentrations of ZnONPs on the growth and development of asparagus (*Asparagus officinalis* L.): A) germinating seeds in Petri dishes without ZnONPs, B) developing explants in sterile vessels, C) regenerated plantlets after 4 months (from the left to right: control, 10, 20, 30 and 40 mg dm⁻³), D) regenerated plantlets in plastic cups filled with sterile soil

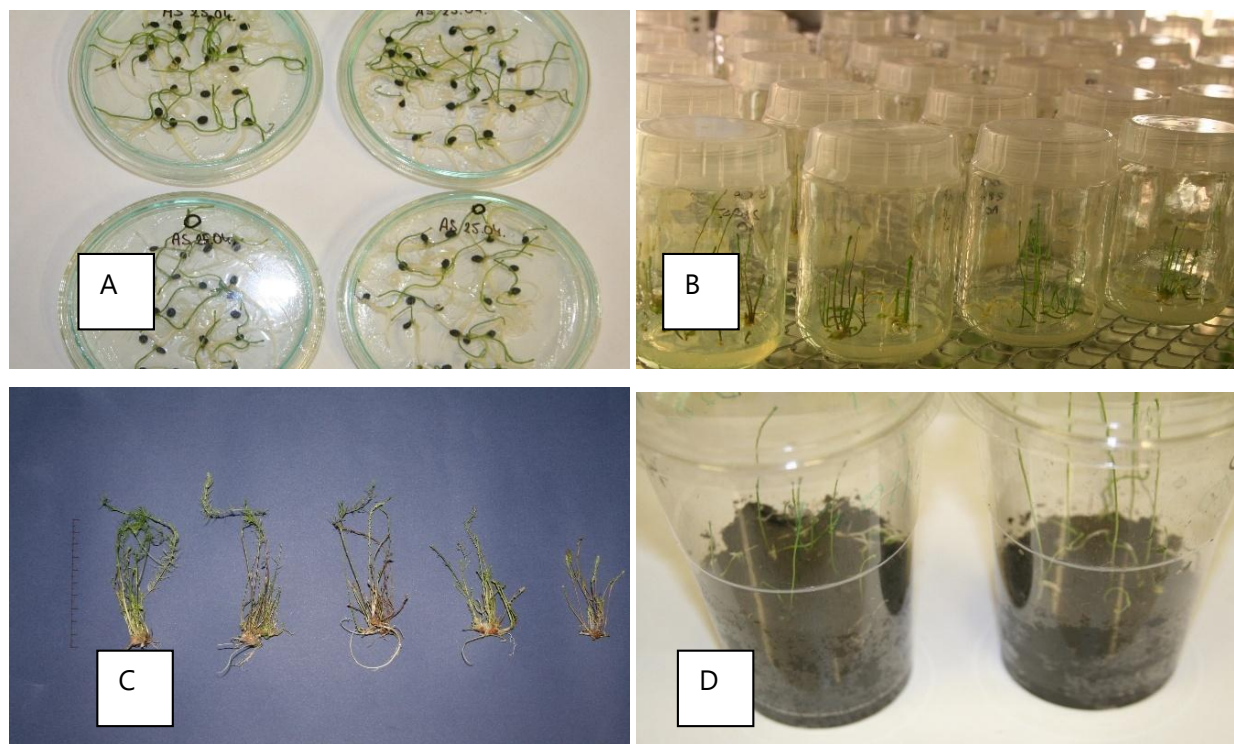


Table 2. The influence of ZnONPs on mineral content in plantlets of asparagus (*Asparagus officinalis* L.) after 4 months of *in vitro* culture

ZnONPs (mg dm ⁻³)	Mineral content (mg 100 g ⁻¹ DW)						
	K	Ca	Mg	Fe	Mn	Zn	Cu
0	2340 ^e	195 ^a	84.0 ^b	17.9 ^b	10.30 ^a	9.29 ^e	0.48 ^d
10	2530 ^d	169 ^b	85.4 ^a	18.4 ^b	9.47 ^b	37.70 ^d	0.57 ^b
20	2740 ^b	158 ^c	85.9 ^a	21.0 ^a	9.31 ^b	82.00 ^c	0.60 ^a
30	2820 ^a	143 ^d	82.4 ^c	17.8 ^b	8.73 ^c	112.00 ^b	0.52 ^c
40	2580 ^c	139 ^e	73.8 ^d	16.2 ^c	8.28 ^c	128.00 ^a	0.49 ^d
LSD _{p < 0.05}	14.50	1.87	1.39	1.30	0.48	1.70	0.03

Mean values assigned the same letter within a column do not differ significantly according to Tukey's test ($p < 0.05$).

Positive Pearson correlation between ZnONPs treatments and the content of elements in *A. officinalis* plant material were observed for potassium ($r = 0.637$) and zinc ($r = 0.989$). In contrast, negative correlations were recorded for calcium ($r = -0.967$), manganese ($r = -0.937$), and magnesium ($r = -0.798$). No correlations were found for copper and iron. In the control treatment without ZnONP, the order of elements based on their content in plantlets was as follows: K > Ca > Mg > Fe > Mn > Zn > Cu. In plantlets treated with 10–40 mg dm⁻³ ZnONPs, zinc content exceeded that of iron and manganese, and in the 30 and 40 mg dm⁻³ treatments, zinc content was higher than that of magnesium (Table 2). The ratio of Cu, Mn, Fe, Mg, Ca, K, and Zn content in the control plantlets was 0.052 : 1.11 : 1.93 : 9.04 : 20.99 : 251.88 : 1.00. The addition of ZnONPs

markedly reduced the relative values of K, Ca, Mg, Fe, Mn, and Cu in relation to Zn content. The most pronounced changes were observed for K and Ca. The decrease in the mineral-to-Zn ratios resulted mainly from the substantial increase in Zn accumulation in plantlets exposed to ZnONPs (Table 3).

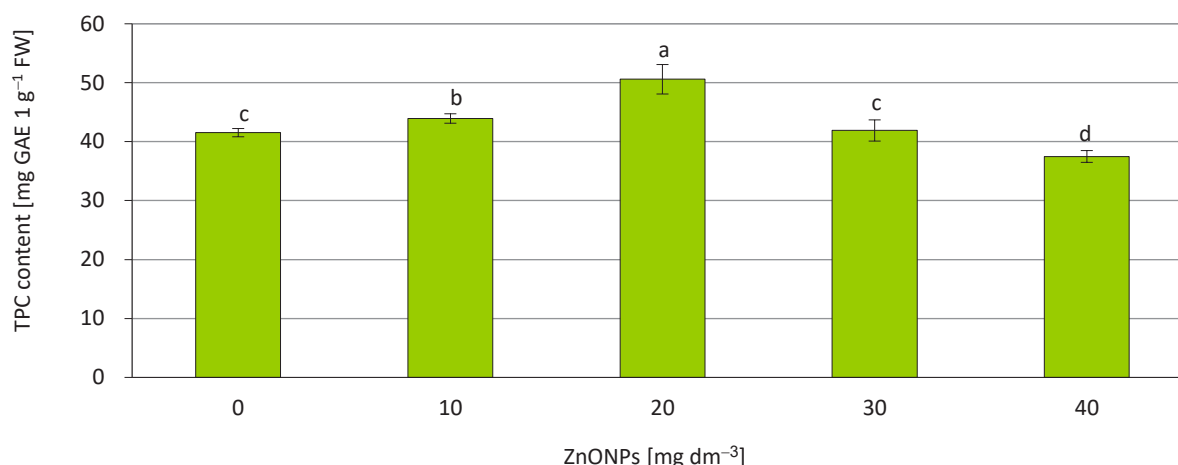
Table 3. The influence of ZnONPs on the relative mineral content in plantlets of asparagus (*Asparagus officinalis* L.) after 4 months *in vitro* culture

ZnONPs (mg dm ⁻³)	The ratio of the mineral content*						
	K	Ca	Mg	Fe	Mn	Zn	Cu
0	251.88	20.99	9.04	1.93	1.11	1.00	0.052
10	67.11	4.48	2.27	0.49	0.25	1.00	0.015
20	33.41	1.93	1.05	0.26	0.11	1.00	0.007
30	25.18	1.28	0.74	0.16	0.08	1.00	0.005
40	20.16	1.09	0.58	0.13	0.06	1.00	0.004

* Zn content was assumed as 1.

Evaluation of TPC in extracts. TPC in methanolic extracts from the fresh weight of asparagus plantlets grown in media supplemented with zinc oxide nanoparticles ranged from 37.49 mg GAE g⁻¹ FW (for 40 mg dm⁻³ ZnONPs) to 50.59 mg GAE g⁻¹ FW (for 20 mg dm⁻³ ZnONPs), as in Figure 2. TPC values of 43.93 and 50.59 mg GAE g⁻¹ FW were higher in the 10 and 20 mg dm⁻³ ZnONPs treatments, respectively, than in the control (41.53 mg GAE g⁻¹ FW). In contrast, samples from the 40 mg dm⁻³ ZnONPs treatment showed significantly lower TPC values compared to the control (Figure 2). No correlation was found between TPC and ZnONPs concentrations 0 (control), 10, 20, 30, and 40 mg dm⁻³.

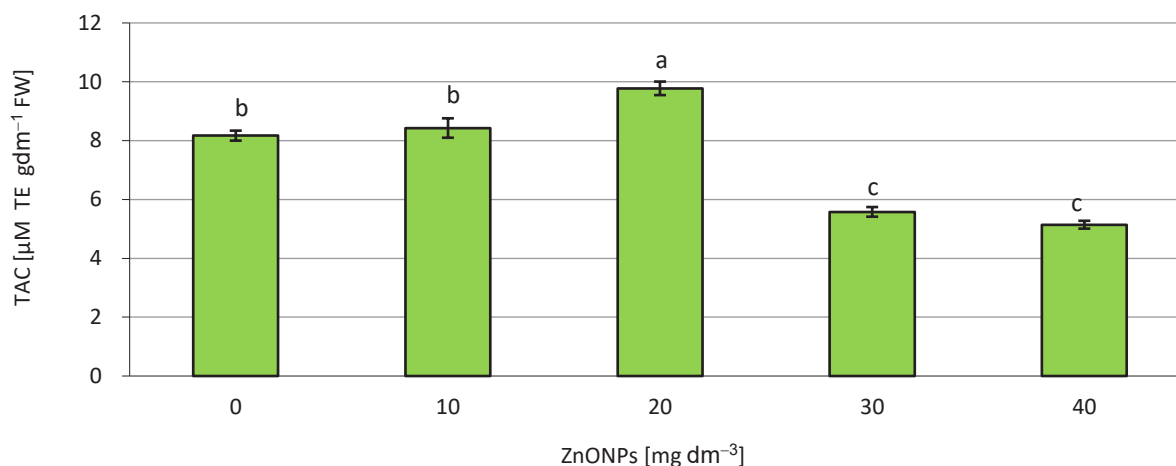
Figure 2. Total phenolic content (TPC) in methanolic extract of fresh asparagus (*Asparagus officinalis* L.) plantlets. Treatments of zinc oxide nanoparticles ZnONPs: 0 (control), 10, 20, 30, 40 mg dm⁻³. Error bars represent ±SD. Different letters indicate significant differences according to Tukey's test ($p < 0.05$)



Determination of total antioxidant capacity (TAC). TAC was measured in extracts from asparagus plantlets using a method based on the reduction of the DPPH radical and expressed as Trolox equivalents [Addai et al. 2013]. In treatments with ZnONPs at concentrations of 10–40 mg dm⁻³ TAC, ranged from 5.14 μM TE g⁻¹ FW (40 mg dm⁻³ ZnONPs) to 9.78 μM TE g⁻¹ FW (20 mg dm⁻³ ZnONPs), as in Figure 3. The Pearson correlation coefficient between ZnONPs concentrations and TAC data was negative ($r = -0.708$), as in Table 4. The antioxidant capacity showed different responses depending on the concentration of ZnONPs. In the

control sample TAC was $8.17 \mu\text{M TE g}^{-1} \text{FW}$ (Figure 3). A concentration of 10 mg dm^{-3} ZnONPs caused a slight increase in antioxidant activity ($8.43 \mu\text{M TE g}^{-1} \text{FW}$). The highest TAC value was recorded at 20 mg dm^{-3} ZnONPs ($9.78 \mu\text{M TE g}^{-1} \text{FW}$), corresponding to 119.71% of the control value. ZnONPs concentrations of 30 and 40 mg dm^{-3} led to a decrease of TAC values by 32% and 37%, respectively (Figure 3).

Figure 3. Total antioxidant capacity (TAC) in extracts of fresh asparagus (*Asparagus officinalis* L.) plantlets determined by DPPH method. Treatments of ZnONPs: 0 (control), 10, 20, 30, 40 mg dm^{-3} . Error bars represent + SD. Different lowercase letters indicate significant differences according to Tukey's test ($p < 0.05$)



Determination of photosynthetic pigment content. The content of photosynthetic pigments in asparagus plantlets was significantly affected by ZnONPs concentration (Figure 4). In the treatments with 10 and 20 mg dm^{-3} ZnONPs, the contents of chl a (12.0% and 28.4% respectively) and chl b (22.4% and 49.5%, respectively) increased compared with the control (Figure 4). The strongest reduction in total chlorophyll was observed in 40 mg dm^{-3} ZnONPs treatment corresponding to a 19.5% decrease compared with the control. The chlorophyll a : b ratio was 2.54 in the control and ranged from 2.13 to 2.33 in the treatments with ZnONPs. Carotenoid content (Figure 4) increased by 2.6% and 23.1% at 10 and 20 mg dm^{-3} ZnONPs, respectively, but decreased by 1.5% and 26.2% at 30 and 40 mg dm^{-3} , respectively, compared to the control ($29.67 \mu\text{g g}^{-1}$).

Figure 4. Content of chlorophylls a, b, a + b and carotenoids in fresh weight of asparagus (*Asparagus officinalis* L.) plantlets. Treatments of ZnONPs: 0 (control), 10, 20, 30, 40 mg dm^{-3} . Error bars represent + SD. The letters refer only to the respective photosynthetic pigment. Different letters indicate significant differences according to Tukey's test ($p < 0.05$)

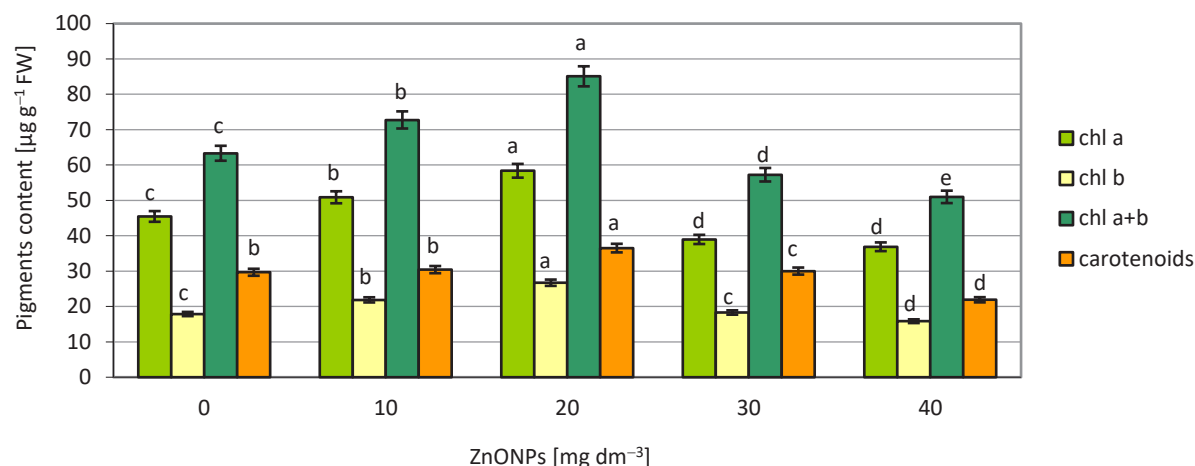


Table 4. Pearson correlation coefficient between the measured parameters

Measurements	ZnONPs treatments	TPC	TAC	chl a	chl b	chl a + b	carotenoids
ZnONPs treatments	1	–	–	–	–	–	–
TPC	–0.331	1	–	–	–	–	–
TAC	–0.708*	0.843*	1	–	–	–	–
chl a	–0.515	0.907	0.952	1	–	–	–
chl b	–0.256	0.852	0.740	0.810	1	–	–
chl a + b	–0.444	0.929	0.918	0.976	0.918	1	–
carotenoids	–0.490	0.940	0.824	0.846	0.770	0.857	1

* correlation coefficients are significant at $p < 0.05$

Pearson correlation analysis revealed negative correlations between ZnONPs concentration and all parameters presented in Table 4, with the strongest negative correlations observed for TAC ($r = -0.708$) and chl a content ($r = -0.515$). The remaining correlation coefficients were positive and high between photosynthetic pigments (ranging from $r = 0.770$ to $r = 0.976$), between photosynthetic pigments and TPC (ranging from $r = 0.852$ to $r = 0.940$), and between photosynthetic pigments and total antioxidant capacity (ranging from $r = 0.740$ to $r = 0.952$) (Table 4). A strong positive correlation was also observed between TPC and antioxidant activity measured by the DPPH method ($r = 0.843$), as in Table 4.

DISCUSSION

To date, asparagus has been propagated *in vitro* using various types of explants (from seedlings, stems, buds, nodes, and internodes) on MS medium supplemented with different growth regulators, employing both direct and indirect organogenesis methods involving callus tissue, for species such as *Asparagus officinalis* [Murashige et al. 1972, Pontaroli and Camadro 2005, Sarabi and Almasi 2010, Štajner 2013, Esmaeili et al. 2016, Rasad et al. 2019, Minh et al. 2022], *Asparagus racemosus* [Singh et al. 2023, Sharma et al. 2025], *Asparagus maritimus* [Štajner et al. 2002], and *Asparagus cochinchinensis* [Wang et al. 2022].

In the present study, seedling explants of the cultivar Argenteuil were used for the direct regeneration of asparagus shoots and roots on MS medium supplemented with 0.20 mg dm^{-3} BAP, 0.20 mg dm^{-3} NAA, and 0.25 mg dm^{-3} Kin, as well as with additional amounts of 10, 20, 30, and 40 mg dm^{-3} ZnONPs. The applied combination of plant growth regulators supported efficient direct regeneration and vigorous growth of asparagus plantlets in the control treatment (Table 1, Figure 1). This response is consistent with findings reported for different asparagus genotypes and species: *A. officinalis* [Štajner 2013], *A. racemosus* [Singh et al. 2023]. Rasad et al. [2019] also used seedling fragments of *A. officinalis* as explants for direct organogenesis. They observed the onset of germination after 2 weeks and obtained fully developed seedlings after 4 weeks. In their study, the highest number of shoots and roots regenerated from seedling fragments on MS medium supplemented with BAP and NAA. Appropriate combinations of plant growth regulators can effectively support direct organogenesis in different asparagus species and cultivars, including *A. officinalis* [Pontaroli and Camadro 2005, Sarabi and Almasi 2010, Esmaeili et al. 2016, Minh et al. 2022], as well as *A. maritimus* [Štajner et al. 2002] and *A. cochinchinensis* [Wang et al. 2022].

A positive effect of ZnONPs has been observed in plants grown *in vitro*. The influence of ZnONPs on plant propagation in tissue culture depends on the species and the applied concentration. In most cases, low and moderate concentrations stimulate growth, regeneration, and the development of callus, shoots, roots, and fresh biomass of plantlets [Singh et al. 2023]. In the present study, ZnONPs at concentrations of 10 and 20 mg dm^{-3} did not stimulate the growth and development of asparagus plantlets compared with the control (Table 1). This finding indicates that the response to ZnONPs may vary considerably among plant species and experimental conditions. However, ZnONPs treatment increased the contents

of several minerals, particularly Zn and K, while low to moderate ZnONPs concentrations also enhanced phenolic content, antioxidant activity, and photosynthetic pigment content compared with the control (Tables 2–3, Figures 2–4). Zinc oxide nanoparticles stimulated the growth and development of *Olea europaea* L. cv. Moraiolo explants *in vitro* [Regni et al. 2022]. Improved shoot regeneration in *Stevia rebaudiana* *in vitro* cultures in the presence of ZnONPs was also reported [Javed et al. 2018]. In *Brassica nigra* *in vitro* culture, ZnONPs stimulated root and shoot regeneration [Zafar et al. 2016]. *Panicum virgatum*, *Thymus kotschyanus*, and *Zataria multiflora* responded to ZnONPs with increased callus induction [Nair et al. 2010, Mosavat et al. 2019]. In blackberry cv. Nawaho and stevia cultivars Morita and Candy, lower concentrations of ZnONPs promoted shoot regeneration [Krzepiłko et al. 2024a, Krzepiłko et al. 2024b].

In the present study, an inhibitory effect of increasing ZnONPs concentrations on shoot number, shoot length, root number, root length, and fresh weight of plantlets was observed. Other authors have also confirmed that zinc oxide nanoparticles may negatively affect plant regeneration processes *in vitro*. Zinc oxide nanoparticles have also been applied in asparagus micropropagation. Desoukey et al. [2019] observed callus formation in all explants of *A. officinalis* in the presence of ZnONPs and 2 mg dm⁻³ BAP. The best results were obtained with 10 mg dm⁻³ ZnONPs, where callus fresh weight increased by 352.5% compared to the control. As ZnONPs concentration increased up to 10 mg dm⁻³, callus fresh weight gradually increased. However, above this concentration, a systematic decrease in callus fresh weight was observed up to 50 mg dm⁻³. The highest concentration (100 mg dm⁻³ ZnONPs) caused a marked reduction in callus fresh weight by 48.8% compared to 50 mg dm⁻³ [Desoukey et al. 2019]. In *Brassica napus* cultures, callus biomass decreased with increasing ZnONPs concentration; however, low concentrations stimulated callus growth and organogenesis [Mousavi Kouhi and Lahouti 2018]. In pot experiments, ZnO nanoparticles at a concentration of 500 ppm provided the best results in terms of morphological traits of *A. officinalis*, including germination capacity, seedling length, vigor index, plant height, number of internodes of the main shoot, number of lateral shoots, fresh and dry weight, and dry-to-fresh weight ratio (DW/FW) compared to the control. One possible reason for growth inhibition is oxidative stress induced by ZnONPs. Biochemical analyses showed increased catalase activity in asparagus plants treated with ZnONPs, indicating activation of antioxidant defense mechanisms and leading to increased production of secondary metabolites [Desoukey et al. 2020].

The use of ZnONPs for zinc biofortification of crops may improve the nutritional value of asparagus. Traditionally grown asparagus plants are rich in trace elements; however, different plant organs accumulate minerals to varying extents. In *in vitro* cultures, the addition of ZnONPs to the medium significantly increased Zn content in plants (Table 2). In the present study, Zn content in plantlets increased with increasing ZnONPs concentration, reaching up to 14 times higher levels than in the control at 40 mg dm⁻³. Moreover, significantly higher levels of K, Cu, Fe, and Mg were recorded in plantlets from ZnONPs treatments compared to the control (Tables 2, 3). Appropriately selected concentrations of ZnONPs may act as environmentally friendly growth stimulators and elicitors of secondary metabolites. In the present study, asparagus plants grown on medium supplemented with 20 mg dm⁻³ ZnONPs showed the highest levels of phenolic compounds, photosynthetic pigments, and antioxidant capacity (Figure 2–4). The phenolic content in asparagus depends on species, cultivation conditions, and extraction methods. In *in vitro* culture, ZnONPs at a concentration of 50 mg dm⁻³ increased total flavonoid content, including rutin [Desoukey et al. 2020]. Increased antioxidant properties and phytochemical content were observed in *A. racemosus* propagated *in vitro* compared to *in vivo* plants [Kumar et al. 2020]. In traditionally cultivated asparagus, TPC varies among green, purple, and white varieties. Purple and green varieties contain the highest levels of phenolics, with rutin being the predominant compound, whereas white varieties contain the lowest levels and lack rutin [Maeda et al. 2005]. The total phenolic content in asparagus extracts also depends on cultivation methods and extraction solvents [Esmaeili et al. 2016]. DPPH radical scavenging is a widely used method for evaluating the antioxidant potential of plant extracts. In the present study, a strong positive correlation between TPC and antioxidant activity measured by the DPPH method was also observed (Table 4). Significant differences in DPPH scavenging capacity have been reported among traditionally cultivated asparagus varieties, with purple varieties showing the highest antioxidant activity, green vari-

eties intermediate activity, and white varieties the lowest. A strong relationship between TPC and DPPH scavenging activity has been demonstrated in edible parts of asparagus from different cultivars [Maeda et al. 2005]. In asparagus plants derived from *in vitro* cultures, *in vivo* plants, and callus tissue, a positive correlation between TPC and DPPH scavenging activity has also been reported [Esmaili et al. 2016]. Extracts from *in vitro*-grown *A. racemosus* plants showed higher levels of phenolics, flavonoids, and carotenoids compared to *in vivo* plants [Kumar et al. 2020]. In asparagus plants grown *in vitro* with ZnONPs, high positive correlations were also observed between photosynthetic pigment content and TPC and TAC (Table 4). Although chlorophyll content in *in vitro*-grown asparagus plants was lower than in mature plants reported by other authors, ZnONPs at concentrations of 10 and 20 mg dm⁻³ increased chlorophyll content compared to the control (Figure 4). Mature asparagus plants grown under field conditions exhibited total chlorophyll content ranging from 0.20 to 0.38 mg g⁻¹ FW, a chlorophyll a : b ratio of 1.5–2.86, and carotenoid content of 0.059–0.123 mg g⁻¹ FW [López-Moreno et al. 2025]. In greenhouse-grown young shoots of *A. officinalis*, chlorophyll content was reported as 1.27 mg g⁻¹ FW for chlorophyll a, 0.95 mg g⁻¹ FW for chlorophyll b, and 2.23 mg g⁻¹ FW for total chlorophyll (a + b) [Liu et al. 2025]. Overall, ZnONPs may be useful as elicitors and biofortifying agents rather than as growth-promoting additives in asparagus micropropagation. Further studies are needed to optimize their concentrations and assess the persistence of these effects after acclimatization and under *ex vitro* conditions.

CONCLUSION

In vitro culture methods may serve as an effective approach for asparagus propagation, which can be highly beneficial not only for the efficient production of plantlets but also for the acquisition of metabolites. In the present study, successful micropropagation of *A. officinalis* was achieved using seedling explants. Higher concentrations of ZnONPs had a negative effect on the organogenesis of asparagus explants. A negative correlation was observed between ZnONPs concentration and morphological trait values. Additionally, high concentrations of ZnONPs adversely affected the morphological characteristics of *A. officinalis* under *in vitro* conditions. At the same time, it was demonstrated that ZnONPs in *in vitro* cultures may enhance the content of minerals, phenolic compounds, antioxidants, and photosynthetic pigments in asparagus plantlets. Compared with control plantlets, those regenerated on media supplemented with low and moderate ZnONPs exhibited higher levels of metals (K, Mg, Fe, Zn, Cu), phenolic compounds, antioxidants, and photosynthetic pigments. Strong positive correlations were recorded between TPC and TAC, and between these parameters and photosynthetic pigments content. The presented results may be useful for optimizing ZnONPs concentrations in the culture medium in order to improve the growth and development of *A. officinalis* under *in vitro* conditions. Moreover, there is still limited information regarding the content of bioactive compounds and their antioxidant activity in asparagus derived from *in vitro* cultures. The findings presented in this study may be of interest both for expanding scientific knowledge and for potential practical applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF THE USE OF GENERATIVE AI TOOLS IN THE PROCESS OF PREPARING THE PAPER

The authors declare that no generative AI tools were used in the preparation of this manuscript.

AUTHORS CONTRIBUTION

A.K., R.P.: methodology, conceptualization, project administration, data curation, visualization, writing – original draft preparation; A.K., R.P., A.Ś.: formal analysis, investigation, resources, supervision, writing – review and editing. All authors have read and agreed to the published version of the manuscript

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